HAMSTRINGS MUSCLE ANATOMY AND FUNCTION, AND IMPLICATIONS FOR STRAIN INJURY

by

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ABSTRACT

The main aim of this thesis was to examine hamstrings anatomy and its influence on knee flexor muscle function in healthy young men. A secondary aim was to better understand the implications of hamstrings anatomy and function, and their variability, in relation to the risk of strain injury.

The functional and conventional H:Q ratios (examined up to high angular velocities) as well as the knee joint angle-specific isometric H:Q ratio exhibited good test-retest reliability at joint positions that closely replicated the conditions of high injury risk.

Football players did not exhibit any differences in angle-specific or peak torque H:Q ratios compared to recreationally active controls. Knee extensor and flexor strength, relative to body mass, of footballers and controls was similar for all velocities, except concentric knee flexor strength at 400° s^{-1} (footballers +40%; $P < 0.01$).

Muscle volume explained 30-71% and 38-58% of the differences between individuals in knee extensors and flexors torque respectively across a range of velocities. A moderate correlation was also found between the volume of these antagonistic muscle groups ($R^2 = 0.41$). The relative volume of the knee extensors and flexors explained ~20% of the variance in the isometric H:Q ratio and ~31% in the high velocity functional H:Q ratio.

Biceps femoris long head exhibited a balanced myosin heavy chain isoform distribution (47.1% type I and 52.9 % total type II) in young healthy men, while BFlh muscle composition was not related to any measure of knee flexor maximal or explosive strength.

Biceps femoris long head proximal aponeurosis area varied considerably between participants (>4-fold) and was not related to biceps femoris long head maximal anatomical cross-sectional area ($r = 0.04$, $P = 0.83$). Consequently, the aponeurosis:muscle area ratio exhibited 6-fold variability (range, 0.53 to 3.09; CV= 32.5%). Aponeurosis size was not related to isometric or eccentric knee flexion strength.

The findings of this thesis suggest that the main anatomical factor that contributes to knee flexors function *in vivo* is hamstrings muscle size, while muscle composition and aponeurosis size do not seem to have a significant influence. The high inter-individual variability of the biceps femoris long head proximal aponeurosis size suggests that a disproportionately small aponeurosis may be a risk factor for strain injury. In contrast, biceps femoris long head muscle composition does not seem to explain the high incidence of strain injuries in this muscle. Quadriceps and hamstrings muscle size imbalances contribute to functional imbalances that may predispose to strain injury and correction of any size imbalance may be a useful injury prevention tool. Finally, regular exposure to football training and match-play does not seem to influence the balance of muscle strength around the knee joint.

Keywords: Hamstrings, anatomy, muscle size, muscle balance, aponeurosis size, muscle composition, hamstrings-to-quadriceps ratio, maximal strength, explosive strength, MRI

Publications

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General Introduction

1 CHAPTER 1 - GENERAL INTRODUCTION

High-speed running and jumping are integral to human locomotion and sports participation. The hamstrings muscle group, as the primary knee flexor and a major hip extensor, plays a leading role in these activities (Schache et al., 2014; Novacheck, 1998; Baratta et al., 1988). Furthermore, an active hamstrings muscle group provides dynamic knee joint control and stability, and thus it is necessary for maintaining joint integrity. Despite these important roles of the hamstrings there is limited knowledge about precise details and inter-individual differences in hamstrings anatomy and how these influence function *in vivo*. Hamstrings exhibit a notorious susceptibility to strain injuries, which are consistently reported as the most prevalent injury in sports that involve high-speed running or sprinting (12-17% of all injuries: Alonso et al., 2012; Ekstrand et al., 2011a, 2011b; Orchard and Seward, 2002). The impact of hamstrings strains on the affected athletes is further emphasized by the 12-40% recurrence rate (Alonso et al., 2012; Ekstrand et al., 2011a, 2011b; Elliot et al., 2011; Verrall et al., 2006; Woods et al., 2004; Orchard and Seward, 2002). These alarming epidemiological data expose the limited current understanding of the aetiology, prevention and treatment of hamstrings strain injuries, which require fundamental knowledge about hamstrings anatomy and function.

Whilst the exact time at which non-contact hamstrings strain injury occurs remains debatable (Chumanov et al., 2012; Orchard, 2012), it is believed that strains occur during the late swing phase of sprinting (Chumanov et al., 2012; Schache et al., 2012), when the biarticular hamstrings are at their peak stretch and exert high forces eccentrically to decelerate the forward movement of the shank prior to ground contact. The muscle most often injured is the biceps femoris long head (BFlh), often at its proximal myotendinous junction (MTJ) (Koulouris and Connell, 2003; De Smet and Best, 2000).

Over recent decades, a large number of investigations have strived to determine the risk factors that predispose people to hamstrings strains. Although a plethora of risk factors have been suggested, only two are supported by substantial scientific evidence; the history of hamstrings injury (Hagglund et al., 2013; Gabbe et al., 2006; Orchard, 2001) and age (Arnason et al., 2004; Orchard, 2001). Strong evidence also exists for the muscle strength imbalances (unilateral and bilateral) as a risk factor (Croisier et al., 2008) yet there is still some controversy (Bennell et al., 1998). Other proposed risk factors include reduced flexibility, hamstrings anatomy, fatigue and ethnicity (Opar et al., 2012); however, the existing evidence for these is inconclusive. It is commonly speculated that hamstrings anatomy contributes to their susceptibility to injury, yet there is a surprising lack of experimental data to substantiate these speculations. In addition, the structure-function relationship for the hamstrings working *in vivo* has received relatively little attention. Abnormalities in hamstrings morphology subsequent to injury, such as atrophy and persistent scar tissue (Silder et al., 2008), may preclude the valid investigation of structure and function relationships in previously injured individuals. Therefore, a first step would be to investigate the interrelations between hamstrings anatomy and function in a normal, uninjured population. The main aim of this thesis was to examine hamstrings anatomy and its influence on knee flexor muscle function *in vivo* within normal, young individuals. A secondary aim was to better understand the implications of hamstrings anatomy and function, and their variability, in relation to the risk of strain injury. A particular focus of this thesis was the BFlh muscle-tendon unit (MTU), due to its vulnerability to strain injuries.

There is a long-standing belief that individuals with weak knee flexors relative to extensors are at an increased risk for hamstrings strains (Croisier et al., 2008; Heiser et al., 1984). The reciprocal strength balance of the muscles around the knee joint is routinely monitored with the hamstrings-to-quadriceps (H:Q) ratio derived from the peak isometric or dynamic torque values of joint extensors and flexors. Despite the wide use of the H:Q ratio as a potential risk factor for strain injury, it is usually obtained in conditions that ignore the biomechanical conditions related to strain injuries. During the late swing phase of sprinting, the hip joint is flexed at ~120-140° (Guex et al., 2012; Novacheck, 1998), while the knee joint angular velocity is very high (>1200° s^{-1}, Higashihara et al., 2010). Simulating these conditions, to the greatest possible extent, in the assessment of the H:Q ratio would provide a more meaningful measure of the reciprocal strength balance at the knee joint. In addition, no study to date has accounted for the discrepancy between the knee joint angle and crank angle that occurs during isometric testing. It has been shown that this discrepancy can be up to 20° for knee extension (Tsaopoulos et al., 2011; Arampatzis et al., 2004), whilst a similarly large difference may be present for knee flexion resulting in a total offset in the assessed knee joint angle between knee extensors and flexors of up to 40°. Finally, even though the H:Q ratio is often calculated over a range of velocities, its reliability at high velocities has yet to be examined. The development of a testing protocol that addresses the aforementioned limitations would be expected to improve the ecological validity of the H:Q ratio. However, it is important to first establish the reliability of such a protocol. The aim of the first study was to evaluate the inter-session reliability of the isometric (angle-specific) and isovelocity (functional and conventional) H:Q ratios using a protocol that included muscle function measurements with high angular velocities, and joint positions and muscle actions that closely replicate those of high injury risk. This involved first the assessment of the reliability of the knee flexors and extensors torque measurements across the torque-velocity relationship (Chapter 3).

Whilst footballers are particularly affected by hamstrings strain injuries (Ekstrand et al., 2011a; Woods et al., 2004), the findings in the literature are inconclusive regarding the influence of football participation on H:Q ratio, although there is some evidence to suggest a disproportionate development of either the knee extensors (Iga et al., 2009) or flexors (Fousekis et al., 2010; Cometti et al., 2001). To date all studies in footballers have examined the H:Q ratio using the peak torque of the reciprocal muscle groups for its calculation. This approach ignores the fact that knee extensors and flexors exert their peak torque at different knee joint angles (~115° and ~150° respectively, Knapik et al., 1983), which may reduce the validity of the H:Q ratio to assess the antagonistic muscle function at the more extended knee joint angles where hamstrings strains are thought to occur. It is possible that a hazardous muscle strength imbalance may be angle-specific and more pronounced at the extended knee joint positions. The aim of the second study was to compare the angle-specific H:Q ratios between football players and recreationally active controls up to high angular velocities (Chapter 4).

Despite the extensive use of the H:Q ratio, there is limited knowledge of the factors that influence this ratio. Muscle size is a primary determinant of maximal strength (Fukunaga et al., 2001), and it would be expected that the relative size of antagonistic muscles, such as quadriceps and hamstrings, would directly influence their respective strength balance. However, to date the association between quadriceps and hamstrings muscle size has not been directly examined and the only two studies that have examined the influence of the H:Q muscle size ratio on their strength ratio did not find any relationship (Akagi et al., 2014, 2012). However, they only examined the isometric H:Q ratio which may not reflect the distinct function of the reciprocal muscles during late swing phase in sprinting. The aim of the third study was to examine the relationship between knee extensors (quadriceps) and flexors (hamstrings) muscle size, the association of each muscle's size with its strength, and investigate if the muscle size ratio was related to the isometric and functional strength ratios (Chapter 5).

An aspect of the hamstrings anatomy that has often been speculated to contribute to strain injuries is muscle composition. However, the only existing data on BFlh muscle composition are derived solely from cadavers (Dahmane et al., 2006; Garrett et al., 1984; Johnson et al., 1973). In a much-cited study, Garret et al. (1984) reported that the hamstrings of a small number of cadavers had a 'high proportion' of type II fibres in the hamstrings (54.5%) compared to other leg muscles (quadriceps, 51.9%; adductor magnus, 44.8%) and suggested that this may contribute to their susceptibility to injury. However, vastus lateralis, an antagonist muscle to BFlh, has been found to contain a greater proportion of type II fibres within a large cohort of physically active, young men (66.1%; Staron et al., 2000); yet it does not exhibit a high frequency of strain injuries. In addition, as hamstrings muscle composition has only been determined within cadavers, its influence on knee flexor maximal and explosive strength remains unknown. The aim of the fourth study was to determine the BFlh myosin heavy chain (MHC) isoform distribution and to examine the association of hamstrings muscle size and BFlh MHC composition with knee flexor strength, including maximal strength measurements across the torque-velocity relationship (concentric, isometric and eccentric) as well as explosive isometric strength (Chapter 6).

Recently, two studies calculated higher localised tissue strains for individuals with a narrow BFlh proximal aponeurosis using computational modelling and dynamic MR imaging and suggested that individuals with a narrow aponeurosis are at an increased risk of strain injury due to the increased strains near the proximal BFlh MTJ (Fiorentino et al., 2012; Rehorn and Blemker, 2010). Further, a preliminary report from the same research group (Handfield et al., 2010) suggested that the width of the BFlh proximal aponeurosis was highly variable between individuals and unrelated to the size of the BFlh muscle. These results suggest, counterintuitively, that within the BFlh MTU, the size of the force generator (muscle) is not proportional to the size of the force transmitter (aponeurosis). The aim of the fifth study was to examine the relationship of BFlh proximal aponeurosis area with muscle size (maximal anatomical cross-sectional area and volume) and knee flexor strength (isometric and eccentric) (Chapter 7).

Chapter 2

Literature Review

2 CHAPTER 2 – LITERATURE REVIEW

2.1 INTRODUCTION

This review is divided into four main parts; the first part gives a brief description of the muscle apparatus and its fundamental properties, as expressed at the sarcomere- and *in vivo* level. The following part presents the morphological and neural factors that determine muscle function. The third part gives a description of the anatomy of the hamstrings muscle group, while it also describes the function of hamstrings during sprinting, the activity during which the majority of strain injuries occur. The final part of this review describes and discusses the problem of the hamstrings strain injuries.

2.2 PART I – BASIC MUSCLE STRUCTURE AND FUNCTION

2.2.1 Overview of muscle structure

Skeletal muscles are designed to produce force for human locomotion and skeletal support. Their structure exhibits a high level of organisation from the molecular to the whole-muscle level. The smallest functional unit of muscle is the sarcomere which is composed primarily of myosin and actin proteins and the interaction of these proteins is responsible for the production of force. Chains of sarcomeres form the myofibrils which are grouped into muscle fibres by the endomysium. Each muscle fibre contains thousands of sarcomeres in series (a hamstrings' muscle fibre contains ~43,000 sarcomeres in series; Enoka, 2002), while the number of fibres contained within a muscle varies from a few hundreds up to >1,000,000 (Enoka, 2002). Bundles of muscle fibres are surrounded by the perimysium and form the muscle fascicles. In turn, muscle fascicles are grouped together with the epimysium to form the muscle. Endomysium, perimysium and epimysium are layers of non-contractile connective tissue composed of collagen that also assist in force transmission (Huijing, 1999). Finally, muscles are attached through their aponeuroses and tendons onto the skeleton. Similar to the layers of connective tissue that envelop the muscle, tendons and aponeuroses also consist of collagen. In the examination of muscle function *in vivo*, the smallest functional unit is a muscle along with its tendons, collectively described as the muscle-tendon unit (MTU).

2.2.2 Muscle contraction (Excitation-contraction coupling)

Muscle contraction is initiated upon arrival of a propagating neural impulse (action potential) from the motor neurons and through the neuromuscular junction (via the neurotransmitter acetylcholine) onto the muscle fibre membrane (sarcolemma). The action potential causes depolarisation of the sarcolemma and propagates longitudinally along the fibre length and transversely into the muscle cell, via the transverse tubules (T-tubules). As the action potential travels through the T-tubules, it causes Ca^{2+} to be released from the sarcoplasmic reticulum into the muscle cell. The released Ca^{2+} then binds onto the specialised area of the troponin and causes the troponin-tropomyosin complex to move and reveal the active sites on the actin filaments. Then, the already energised myosin heads - through hydrolysis of their adenosine triphosphate (ATP) molecule - bind to the exposed active sites and form the cross-bridges. The stored energy from the ATP hydrolysis is then released causing a rotation of the myosin heads, called power stroke. The power stroke generates force causing the actin filament to slide towards the centre of the sarcomere and the sarcomere to shorten (sliding filament theory). Following the power stroke, a new ATP molecule binds onto the myosin heads which then detach from the initial active sites and are ready to attach on new ones further along the fibre, forming new cross-bridges. This cycle continues as long as the concentration of the intracellular Ca^{2+} remains elevated. When the action potential generation ceases, the intracellular Ca^{2+} returns back to the sarcoplasmic reticulum and the contraction ends.

2.2.3 Muscle composition

Muscle fibres can be classified according to their structural and functional properties. A commonly used histochemical method classifies fibres according to their myofibrillar ATPase activity into three main (I, IIA, IIX) and four intermediate (IC, IIC, IIAC, IIAX) types (Staron and Hikida, 1992). The more recent electrophoretic method separates and quantifies the different myosin heavy chain (MHC) isoforms. In human muscles, three main (MHC-I, MHC-IIA, MHC-IIX) and two hybrid (MHC-I-IIA, MHC-IIA-IIX) isoform types have been identified (Bottinelli and Reggiani, 2000). *In vitro* examination has shown that the maximum shortening velocity is ~4-fold greater in fibres expressing MHC-IIX isoform compared to fibres with MHC-I, while the maximum shortening velocities of the intermediate fibres fall within this range (Bottinelli et al., 1999). However, there is a degree of overlap in shortening velocities within the continuum of MHC isoforms. Similarly, fibres with MHC-

IIX isoforms produce ~1.5-fold greater specific tension and >8-fold greater power than type I fibres (Bottinelli et al., 1996), with the MHC-IIA fibres being intermediate. Further, MHC-II fibres exhibit also ~6-fold greater rate of force development than MHC-I (Harridge et al., 1996). Although MHC isoform content is a main determinant of function *in vitro*, its influence on muscle function *in vivo* is unclear (discussed further in 2.3.1.5 & 2.3.2.3).

2.2.4 Muscle architecture

Architecture of a muscle is the internal arrangement of its fibres. Muscle fibres can be arranged in series, in parallel and at an angle relative to the muscle's line of action. The arrangement of sarcomeres in series and, thus longer fibre length, facilitates maximal excursion and shortening velocity, while the arrangement of sarcomeres in parallel (i.e. increased number of cross-bridges in parallel) is optimal for greater force production. Finally, the arrangement of the muscle fibres at an angle relative to the muscle's line of action (pennation angle) is a trade-off between the force that can effectively be transmitted to the line of action (as a function of the cosine of the pennation angle) and the number of fibres that can be accommodated within a given muscle volume. It has been shown that the optimum pennation angle is 45° (Alexander and Vernon, 1975), however the majority of the human muscles exhibit a pennation angle that does not exceed 30° at rest.

2.2.5 Fundamental muscle mechanics

2.2.5.1 Force-length relationship

The magnitude of force produced by a sarcomere is determined by the number of cross-bridges formed and power strokes performed at any particular moment in time. The number of cross-bridges that can be formed is dictated by the degree of overlap between myosin and actin filaments (Gordon et al., 1966). The highest amount of force can be generated when this overlap is maximal and corresponds to a relatively narrow range of optimal sarcomere lengths (plateau region, Fig. 2.1). If the sarcomere is stretched beyond the plateau region (descending limb), then the myosin-actin overlap is reduced, fewer cross-bridges can be formed and, consequently, less force can be produced. Similarly, if the sarcomere length is shorter than optimal (ascending limb), the interaction between myosin and actin filaments becomes less efficient as the actin filaments move across the centre of the sarcomere and overlap with the actin filaments of the opposite side.

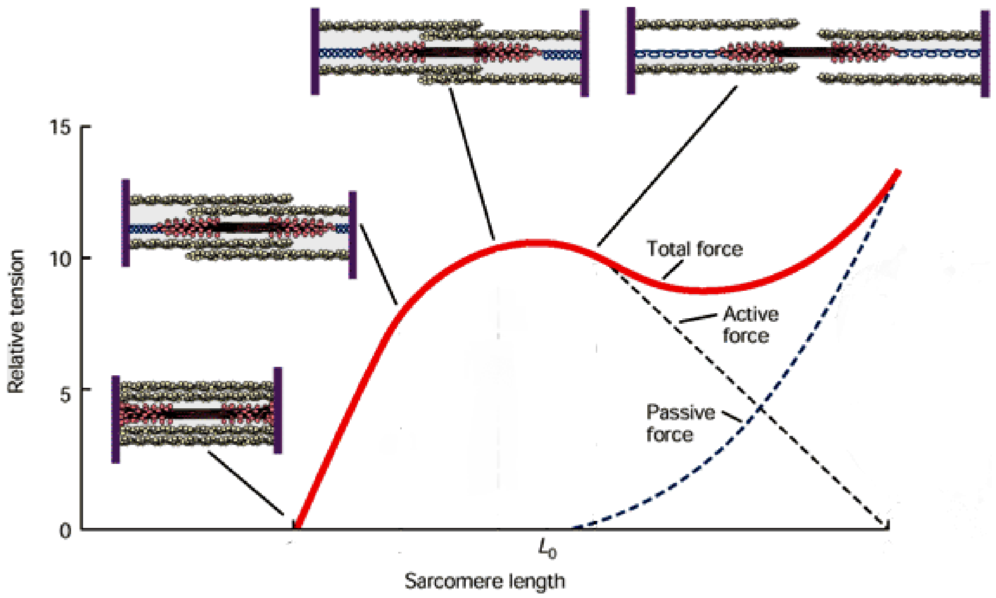

Figure 2.1. Force-length relationship. L_0 corresponds to sarcomere optimal length where maximal actin-myosin overlap occurs and maximal tension is produced. Total force is the sum of active and passive force components (adapted from Kandel et al., 2012).

The force-length (F-L) relationship of a whole muscle is smoother than that obtained from single fibres and sarcomeres due to the non-uniform sarcomere lengths. Similarly, at the *in vivo* level, the F-L relationship (reflected in the torque-length relationship, T-L) has a broader shape compared to the F-L. In addition to the non-uniform sarcomere lengths, the difference in the shape of T-L is due to the geometry of the tendons with which the muscle attaches onto the skeleton, the resultant moment arms and the contribution of other muscles. At muscle lengths longer than the optimal, the force produced is not solely due to the interaction of myosin and actin filaments (active force); rather, large structural proteins within the sarcomeres (titin), as well as the connective tissue (i.e. epimysium, perimysium, endomysium) and the cytoskeleton provide tension upon stretching (passive force) which increases at longer muscle lengths. The T-L relationship is obtained through maximal voluntary isometric contractions at different angles over the range of motion of the joint in examination.

2.2.5.2 Force-velocity relationship

Whilst the F-L relationship describes how changes in muscle length influence force generation, the force-velocity (F-V) relationship considers the influence of contraction velocity on muscle force (Fig. 2.2). The F-V relationship examined in isolated muscle preparations dictates that the maximal force generation increases with decreasing shortening velocity (concentric contraction) in a hyperbolic manner (Hill, 1938). The highest point of this hyperbola is reached when the velocity is zero (isometric contraction). When a muscle is lengthened (eccentric contraction), the force that can be produced is approximately 1.5-1.9 times the isometric force and remains relatively constant with increasing velocity (Katz, 1939). A similar shape can be seen for the *in vivo* F-V relationship (reflected in the torque-velocity relationship, T-V) when it is examined using evoked contractions. However, when voluntary contractions are examined, deviations from this pattern can be seen. While the concentric part of the T-V relationship is similar to that obtained during *in vitro* or evoked contractions, the eccentric force generating capacity increases only at a maximum of 1.1-1.2 times above isometric force or even shows a depression below isometric force level (Pain et al., 2013; Kellis and Baltzopoulos, 1998; Dudley et al., 1990; Westing, 1988). This reduced capacity in eccentric force in voluntary contractions is attributed to neural inhibition (Westing et al., 1991).

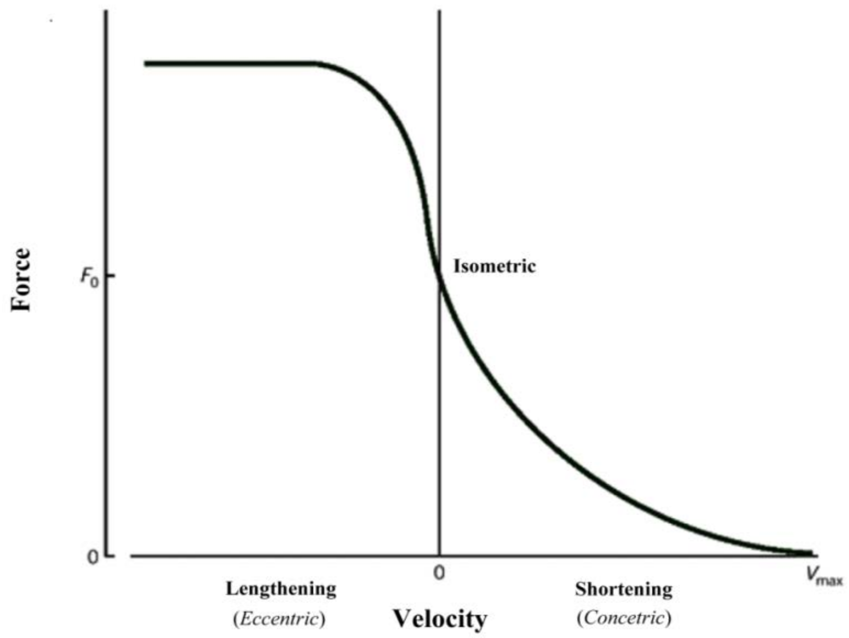

Figure 2.2. Force-velocity relationship (adapted from Kandel et al., 2012).

2.3 PART II – DETERMINANTS OF MUSCLE FUNCTION

2.3.1 Determinants of maximum strength

Maximum strength is the capacity of the muscles to produce maximal force (or torque). Maximal strength is influenced by a number of structural and neural factors. This section will give an overview of the main factors that determine maximal strength.

2.3.1.1 Muscle size and architecture

As muscle force *in vitro* is directly related to the number of contracting sarcomeres in parallel, it can be assumed that muscle size is the primary determinant of maximal strength *in vivo*. Examination of this relationship has shown that various indices of muscle size (specifically anatomical cross-sectional area (ACSA), physiological cross-sectional area (PCSA) or volume), explain a substantial proportion of the inter-individual variability in maximal isometric (elbox flexors, R^2=0.58, Akagi et al., 2009; plantar flexors, R^2= 0.42, Bamman et al., 2000; quadriceps, R^2= 0.35, Maughan et al., 1983), concentric (plantar flexors, R^2= 0.22-0.24, Baxter and Piazza, 2014; quadriceps R^2= 0.39-0.74, Blazevich et al., 2009; hamstrings, R^2= 0.31-0.41, Masuda et al., 2003) and eccentric (knee extensors R^2= 0.35-0.46, knee flexors, R^2= 0.47-0.48, Carvalho et al., 2012) strength of various muscles.

Nevertheless, there is no consensus to which index of muscle size is a better predictor of maximal strength. The effective PCSA, which represents the total cross sectional area of all muscle fibres and also accounts for any angulation between the fibres and muscle's line of action, is considered as the most theoretically appropriate measure of muscle size, as it best accounts for the muscle architecture. Yet, muscle volume and anatomical cross-sectional area have been found to be better determinants of maximal strength *in vivo* (Blazevich et al., 2009; Fukunaga et al., 2001; Bamman et al., 2000). This may be due to the difficulty of accurately measuring the architectural parameters needed for the calculation of PCSA as the measurement of muscle volume, pennation angle and fascicle length are required.

Despite the relationship between muscle size and maximal strength, current data show a large variation in the strength of this relationship, while a substantial portion (26-78%) of the inter-individual differences in strength remains unexplained. Therefore, other variables are likely to contribute to differences in maximal strength, including muscle architecture (Aagaard et al., 2001), moment arm (Baxter and Plazza, 2014; Blazevich et al., 2009), agonist neural activation (Westing et al., 1990) and antagonist co-activation (Kellis and Baltzopoulos, 1998).

Pennation angle (PA) is related to muscle size (Ikegawa et al., 2008; Kawakami et al., 2006; Aagaard et al., 2001; Kawakami et al., 1993). For example, Kawakami et al. (2006) examined the relationship between PA and muscle thickness for triceps brachii, vastus lateralis and gastrocnemius muscles in 711 men and women (age: 3-94 years) and found that PA inter-individual differences explained 31-66% of the differences in muscle thickness. This positive correlation between PA and muscle size reflects the fact that a greater PA allows for more muscle fibres to be accommodated within the same muscle volume (Kawakami et al., 1993), even though some of the force of the fibres is not resolved along the line of action of the aponeurosis/tendon (according to the cosine of the PA). This beneficial effect of increasing PA on isometric strength is thought to only exist up to a PA of 45º after which the loss of force resolved to the tendon exceeds any gains in force production from the fibres (Alexander & Vernon, 1975). Also, muscle contraction causes the muscle fibres to rotate and for a given muscle shortening the fibres shorten less and thus they operate at a length closer to their optimal. Finally, as the muscle fibres shorten less due to the fibre rotation (relative to their insertion point), they also shorten at a lower velocity relative to the total muscle shortening velocity and according to the F-V relationship, this facilitates the production of near-maximal forces.

Fascicle length (FL) also influences the muscle's force production capacity. A muscle with longer fascicles has more sarcomeres in-series and, therefore, has a higher maximal shortening velocity, while it can produce near-maximal forces over a greater muscle length range (Lieber and Friden, 2000). Experimental data showed that FL was related with sprint performance (Abe et al., 2001, 2000; Kumagai et al., 2000). In a study by Abe et al. (2000), sprinters exhibited greater FL compared to distance runners and controls. In another study, Kumagai et al. (2000) showed that longer FL of vastus lateralis and gastrocnemius muscles were significantly related to 100-m sprint performance ($r = -0.40$ to -0.57, $P < 0.05$). These authors suggested two possible mechanisms by which FL increases power and consequently sprint performance. First, longer FL increases shortening velocity and as power is the product of muscle force exerted by shortening velocity, longer FL would increase power. Second, at a given shortening velocity the sarcomeres of longer FL would shorten less over a range of motion and, therefore, they would operate closer to their optimum length. This would result in increased force production at that velocity which, in turn, would result in increased power production. These findings suggest that FL may facilitate higher force production at higher velocities. However, further research is needed to elucidate the influence of FL on maximal isometric and dynamic strength.

2.3.1.2 Moment arm

The torque produced by a muscle is the product of the muscle force applied and the perpendicular distance between the joint centre of rotation and the line of muscle action. Therefore, it can be assumed that moment arm is a determinant factor of maximal strength. However, the existing data are mixed as Blazevich et al. (2009) reported a significant correlation between moment arm and maximal isometric (r= 0.50) but not concentric knee extensor torque (r= 0.43-0.44) in a mixed cohort of young men and women (n= 19). Also, when muscle volume was introduced into a regression analysis with moment arm, it did not improve the prediction of maximal isometric torque (Blazevich et al., 2009). In contrast, Baxter and Piazza (2014) found that, within 20 young men, plantar flexor moment arm was significantly related with isometric (r= 0.56) and concentric torque at various velocities (r= 0.66–0.69) and these correlations were similar or stronger than the correlations between plantar flexor volume and torque (r= 0.47–0.57). Based on these results, the authors argued that moment arm was at least as important a determinant of maximal strength as muscle volume.

2.3.1.3 Agonist activation

Electromyography (EMG) and electrical stimulation studies have shown that even during maximal voluntary contractions, individuals cannot activate fully their agonist musculature (Tillin et al., 2011; Kooistra et al., 2007; de Ruiter et al., 2004). This is most pronounced in eccentric contractions during which peak torque has consistently been found to be lower in voluntary compared to evoked or superimposed contractions (Pain et al., 2013; Westing et al., 1990; Dudley et al., 1990). Westing et al. (1990) reported an increased torque production by 21-24% on average during eccentric contractions (60-360° s^{-1}) with superimposed electrical stimulation compared to maximal voluntary contractions. In a different study, the same authors reported a decreased EMG activity of the superficial knee extensors during eccentric compared to concentric contractions at various velocities (45-360° s^{-1}), suggesting the presence of neural inhibition for the agonist muscles (Westing et al., 1991). Interestingly, a difference between voluntary and superimposed eccentric contractions was evident in sedentary but not in elite high-jumpers, suggesting that any neural inhibition may be attenuated by training (Amiridis et al., 1996). While the exact mechanism(s) remains unknown, it is believed that neural mechanisms at spinal and supraspinal levels inhibit neuromuscular activation during maximal eccentric efforts of untrained individuals. This neural inhibition is thought to protect the joint from potentially injurious high levels of force that can be produced during eccentric contractions (Duchateau and Baudry, 2014).

2.3.1.4 Antagonist co-activation

Upon activation, the net joint torque exerted is the result of the torque produced by the agonist muscle(s) and any opposing torque produced by the antagonist muscle(s). Therefore, it is clear that the level of antagonist co-activation has the potential to influence the resultant net joint torque. Aagaard et al. (2000) reported a 15-35% of hamstrings antagonist co-activation during slow isokinetic knee extensions (30° s^{-1}) compared to a 10% antagonist co-activation of the quadriceps. The same authors reported that the level of hamstrings co-activation was higher at the more extended knee joint angles compared to the mid-range joint positions. Yet, other studies did not confirm this effect of angular position in antagonistic co-activation (Kellis and Baltzopoulos, 1997). Kellis and Baltzopoulos (1996) found that hamstrings antagonist co-activation during isokinetic knee extensions increased by 31% from 30 to 150° s^{-1} i.e. it is velocity dependent, while antagonistic activity of both quadriceps and hamstrings was higher during concentric contractions than eccentric. Overall, it seems that the level of antagonist co-activation is muscle-specific, and depends on the type and velocity of contraction.

The antagonistic co-activation is thought to be an injury preventing mechanism that reduces the net joint torque and also increases the stiffness of the joint (Kellis and Baltzopoulos, 1998; Hagood et al., 1990; Baratta et al., 1988). For example, hamstrings co-activation during knee extension has been suggested to reduce the anterior shear of the tibia and thus the stress on the anterior cruciate ligament (ACL) (Aagaard et al., 2000; Baratta et al., 1988). Furthermore, the lower antagonistic co-activation during eccentric compared to concentric contractions may also partly explain the higher torques seen in eccentric (compared to concentric) contractions (Kellis and Baltzopoulos, 1996).

2.3.1.5 Muscle composition

As discussed in 2.2.3, type II fibres (i.e. fibres that express MHC-IIA and IIX isoforms) exhibit greater specific tension than type I *in vitro* (Bottinelli et al., 1996). As hamstrings muscle composition has only been determined within cadavers, its influence on muscle function remains unknown. However, the influence of muscle composition on maximal strength *in vivo* has been examined in the vastus lateralis muscle in relation to knee extensors strength and a significant correlation has been reported in some of these studies (Gür et al., 2003; Aagaard & Andersen 1998; Viitasalo & Komi, 1978; Thorstensson et al., 1976) while others did not confirm such a relationship (Maughan and Nimmo, 1984; Schantz et al., 1983; Viitasalo et al., 1981; Inbar et al., 1981). However, within the studies that reported a significant correlation several limitations may have confounded their results. For example, in order to ensure a large variability in the muscle composition of their examined cohorts, some investigators included highly diverse, athletic populations (Gür et al., 2003) where numerous other variables (e.g. hypertrophy) could be acting as confounding factors. Other confounding factors included examination of small cohorts (Aagaard & Andersen, 1998) or no consideration of gravitational effects or acceleration artefacts (Schantz et al., 1983; Thorstensson et al., 1976). In contrast to the above mentioned studies, Maughan and Nimmo (1984) did not find any relationship between knee extensors maximal strength and vastus lateralis muscle composition within physically active men. Overall, the existing data on the relationship between muscle composition and *in vivo* maximal strength are mixed and confounded by methodological limitations.

2.3.1.6 Muscle-tendon unit stiffness

Muscle force is transferred by the tendinous tissues (aponeuroses and tendons) to the skeleton for any action to occur at the joint level. Therefore, the interaction of the muscle-tendon unit has important effects on the *in vivo* function. This interaction is influenced by the mechanical properties of the aponeurosis and tendon tissues. Stiffness is an important mechanical property of these structures and can be defined as the resistance of a material to deformation. Therefore, a stiff tendon would resist any stretching while a more compliant tendon would change its length more for a given force. The implication of this property on muscle function is that a more compliant tendon will shift the T-L curve to the right and the optimum muscle length will be at a slightly longer position.

In a computational modelling study, Lemos et al. (2008) measured the influence of tendon and aponeurosis compliance on F-L relationship and found that increased compliance resulted in a rightward shift of the F-L and the peak isometric force occurred at longer muscle-tendon unit lengths. They also found that increased compliance resulted in reduced peak isometric force. The authors discussed that whilst isometric contractions suggest a static muscle-tendon unit length, this is not the case for the contractile elements. Therefore, from rest to contraction fibres shortened more (on average 4.59 mm) when the compliance of the tendinous tissues was increased compared to a muscle-tendon unit with stiffer tendon and aponeurosis (on average 2.74 mm). These findings were in accordance to the results of an *in vivo* study by Kubo et al. (2006) who found that both elongation and strain in vastus lateralis tendon and aponeurosis exhibited a weak but significant correlation with the knee extensor peak isometric torque exerted at 100° relative to 50° (i.e. an index of an individual's optimal angle) (elongation, $r = 0.48$, $P < 0.05$; strain, $r = 0.42$, $P < 0.05$). This result suggested that increased MTU compliance is related to a greater force production at longer muscle lengths.

Finally, significant positive relationships have been found between MTU stiffness and maximal isometric ($r = 0.57$-0.67, Hannah and Folland, 2014; $r = 0.58$, Stenroth et al., 2012) and dynamic strength ($r = 0.54$-0.60, Bojsen-Moller et al., 2005) suggesting that the MTU stiffness scales with muscle strength and the functional capacity of the muscle. If this is the case, the fibre shortening afforded by the tendon and aponeurosis compliance during the transition from rest to maximum torque would be similar irrespective of strength.

2.3.2 Determinants of explosive strength

Explosive strength can be defined as the 'capability to increase contractile force from a low or resting level as quickly as possible' (Folland et al., 2013) and is typically assessed during isometric contractions. Determinants of explosive strength include maximal strength (Folland et al., 2013; Andersen and Aagaard, 2006), neural activation (Folland et al, 2013; Tillin et al., 2010), fibre type composition (Harridge et al., 1996) and muscle-tendon unit stiffness (Bojsen-Moller et al., 2005).

2.3.2.1 Maximal strength

Maximal strength has been found to correlate well with explosive strength, especially during the later stages of an explosive contraction (Folland et al., 2013; Andersen and Aagaard, 2006). Folland et al. (2013) showed that maximal voluntary strength explained 35-90% of the variance in explosive strength (measured as force at specific time points after contraction onset). The same authors also showed that the influence of maximal strength on explosive strength increased as the explosive contraction progressed and the highest correlation was seen at 150 ms (r= 0.95). Similar findings were presented by Andersen and Aagaard (2006) who also reported an increasing contribution of maximal strength to explained variance in explosive strength (measured as rate of force development in time epochs from contraction onset up to 250 ms). The lowest (yet significant) correlation was reported at 0-50 ms time epoch ($r = 0.40$, $P < 0.05$) and the highest at time intervals >150 ms ($r > 0.80$, $P < 0.001$). Overall, maximal strength seems to be a primary determinant of explosive strength during the later stages of an explosive contraction. This is probably expected, as maximal strength represents the maximal capacity of voluntary force production and it can be achieved in >400 ms from contraction onset (Thorstensson et al., 1976). Therefore, as the explosive contraction progresses over time, the force levels achieved are increasingly influenced by the maximal voluntary force (Folland et al., 2014).

2.3.2.2 Agonist activation

Both cross-sectional and training studies have shown that explosive strength is influenced by agonist activation (Folland et al., 2013; Tillin et al., 2011, 2010; de Ruiter et al., 2007, 2006, 2004; Aagaard et al., 2002). In a cohort of forty untrained individuals, agonist activation was found to be a significant determinant of explosive strength, particularly at the initial part of an explosive contraction (<75 ms) explaining 17-37% of the differences in absolute and 21-51% in relative (normalised to maximal strength) explosive strength (Folland et al., 2013).

Furthermore, the same authors reported large inter-individual variability in the agonist activation at the early stages of contraction (50 ms, CV= 38%) These results confirmed previous findings in smaller cohorts (n≤ 11, de Ruiter et al., 2007, 2006, 2004). In a comparison study between power athletes and untrained individuals, Tillin et al. (2010) reported that the greater normalised rate of force development exhibited by the athletes was explained by their higher neural activation (greater synchrony in the activation onset of the agonist muscles and greater EMG amplitude in the first 50 ms after contraction onset). Training studies have also provided some evidence of the relationship between agonist activation and explosive strength (Tillin and Folland, 2104; Tillin et al., 2011; Aagaard et al., 2002). For example, Aagaard et al. (2002) found a concurrent increase in EMG amplitude and explosive strength after 14 weeks of heavy-resistance training. Overall, the level of agonist activation seems to be a significant determinant of explosive strength during the early stages of contraction.

2.3.2.3 Muscle composition

At the single-fibre level, rate of force development was found to be slower in MHC-I fibres compared to MHC-II fibres (Harridge et al., 1996). However, when these authors examined evoked contractions *in vivo*, there was no relationship between time to peak torque and muscle composition in three different muscles (vastus lateralis, triceps brachii and soleus). Only when the data from the individual muscles were pooled together, a significant relationship arose (r= 0.99) (Harridge et al., 1996). Other investigators have reported significant association between muscle composition and voluntary explosive force *in vivo* (Viitasalo et al., 1981; Viitasalo and Komi, 1978). For example, Viitasalo and Komi (1978) reported a significant correlation between % type I fibres and time to reach 30% maximal force in a double-leg press exercise (r= 0.48, P< 0.01). However, the inclusion of athletes from different training modalities and the testing procedures used (double-leg press) may have confounded their results. From the existing data, it remains unclear whether muscle composition influences explosive strength *in vivo*.

2.3.2.4 Muscle-tendon unit stiffness

Theoretically, a stiffer muscle-tendon unit would facilitate a more effective force transmission from muscle fibres to the skeleton. Therefore - ceteris paribus - individuals with a stiffer muscle-tendon unit would be expected to exhibit greater explosive strength. This hypothesis was confirmed by Bojsen-Moller et al. (2005) who found a moderate positive correlation between knee extensor explosive isometric strength and vastus lateralis tendon-aponeurosis stiffness ($r = 0.55$, $P < 0.05$). However, in that study there was no control for the influence of maximal strength in the examined relationship. As both explosive strength and muscle-tendon unit stiffness are influenced by maximal strength, their results may merely reflect the effect of maximal strength on these variables. Hannah and Folland (2014) also reported significant correlations of knee extensors voluntary and evoked explosive strength (measured as time to achieve specific levels of force) and vastus lateralis muscle-tendon unit stiffness (voluntary, $r = -0.35$ to -0.54, $P < 0.05$; evoked, $r = -0.41$ to -0.64, $P < 0.05$), but these relationships became non-significant when maximal strength was taken into account. These findings suggest that, when the influence of maximal strength is taken into account, muscle-tendon stiffness is not an important determinant of explosive strength.

2.4 PART III – HAMSTRINGS ANATOMY AND FUNCTION DURING SPRINTING

2.4.1 Hamstrings anatomy

The term 'hamstrings' refers to three muscles located in the posterior thigh; the semitendinosus (ST) and the semimembranosus (SM) are located at the medial side, and the biceps femoris at the lateral side (Fig. 2.3). The biceps femoris has two anatomically and functionally distinct heads, the long (BFlh) and the short (BFsh) head. The BFlh, ST and SM cross the hip and the knee joint (biarticular muscles) and due to this configuration they are primary knee flexors and major hip extensors. The BFsh crosses only the knee joint (mono-articular muscle) and, therefore, contributes only to knee flexion. The medial and lateral hamstrings also assist in knee and hip internal and external rotation respectively.

Figure 2.3. Illustration of the hamstrings muscle group in the right leg (posterior view) (adapted from Schuenke et al., 2010).

The proximal region of the hamstrings presents a complex morphology. The BFlh and ST are closely related sharing a common (conjoint) proximal tendon (van der Made et al., 2013; Battermann et al., 2011; Miller et al., 2007). The conjoint tendon arises from the medial facet of the ischial tuberosity (Battermann et al., 2011), while connections with the sarcotuberous ligament have also been reported (Sato et al., 2013; Woodley and Mercer, 2005; Martin, 1968). Also, some ST muscle fibres originate directly from the ischial tuberosity (Woodley and Mercer, 2005). The two muscles separate from their common tendon at ~9 cm distally from the ischium (Battermann et al., 2011; Miller et al., 2007). The origin of the SM is also located at the ischial tuberosity (lateral facet), with a lateral (Miller et al., 2007) or anterolateral (van der Made et al., 2013) position relative to the BFlh/ST conjoint tendon. Some studies described that the most proximal site of the SM tendon is also directly connected with the BFlh/ST conjoint tendon (van der Made et al., 2013) or by means of fibrous adhesions, however other studies did not report such connections (Sato et al., 2012; Woodley and Mercer, 2005). As the SM tendon extends distally, it twists from anterolateral to posteromedial position relative to the BFlh/ST tendon. Distally, the BFlh exhibits three insertion sites; the head of the fibula, the lateral condyle and the fascia of the leg (Koulouris and Connel, 2005). The BFsh originates from the linea aspera of the femur and shares the distal tendon of the BFlh for its attachment. The ST inserts via a long tendon onto the anteromedial part of the tibia while the SM inserts onto the posterior surface of the medial tibial condyle.

The BFlh and SM have long proximal and distal tendons (including their aponeuroses) extending up to or more than half the total muscle length (Woodley and Mercer, 2005) so that they overlap, while the ST has a long distal but short proximal tendon with no overlap between the two (van der Made et al., 2013). Preliminary reports found the size of the proximal BFlh aponeurosis to be highly variable between individuals (Handsfield et al, 2010), while computational modelling studies reported that a small BFlh aponeurosis concentrates high strains (Fig. 2.4, Fiorentino et al., 2014a, 2012; Rehorn and Blemker, 2010). These findings suggest that the size of the proximal BFlh aponeurosis may be a risk factor for strain injury (discussed further in 2.5.3.3). Concerning the ST, it has a distinct V-shaped tendinous inscription (or raphe) that divides the muscle into two regions (van der Made et al., 2013; Woodley and Mercer, 2005).

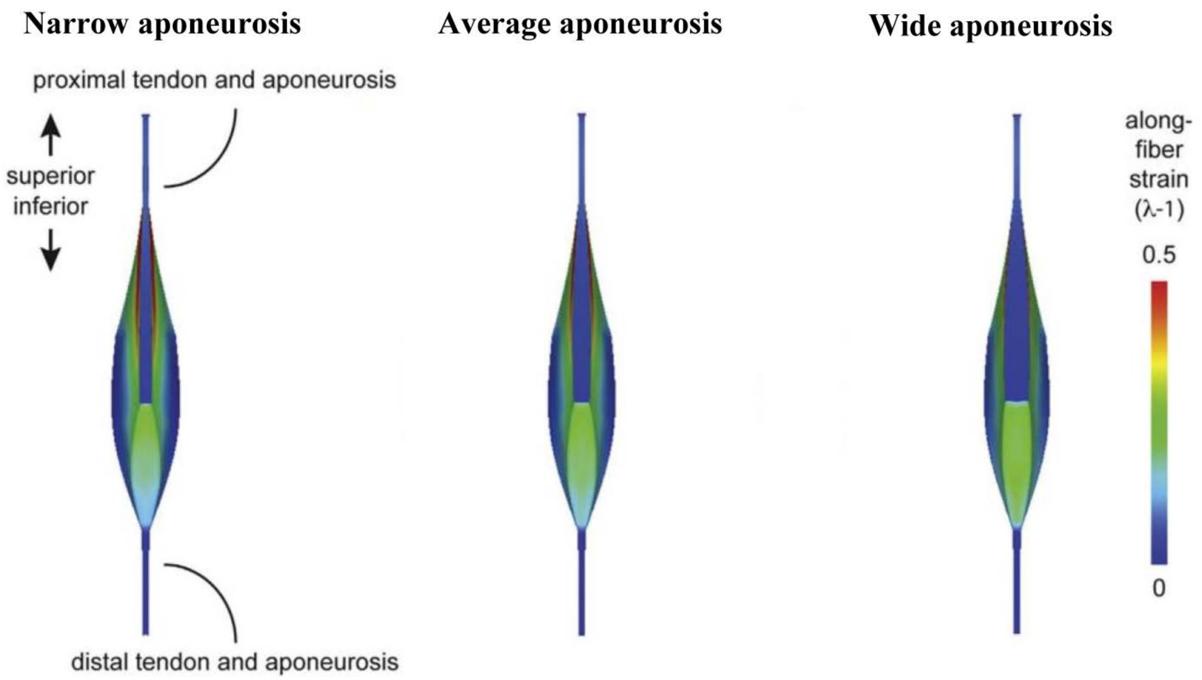

Figure 2.4. Individuals with narrow proximal BFlh aponeurosis experience higher strains (red colour) near the aponeurosis during active lengthening compared to individuals with wider aponeurosis (adapted from Fiorentino et al., 2014a).

Innervation of the two heads of the biceps femoris comes from different branches of the sciatic nerve with the tibial division supplying the BFlh, and the peroneal division supplying the BFsh (Koulouris and Connel, 2005). This dual innervation has been speculated to contribute to hamstrings strain injuries via fatigue-induced altered coordination and asynchronous activation (Croisier et al., 2004; Woods et al., 2004; Sutton, 1984). Whilst this possibility has not been examined directly, supporting evidence has been provided recently by Timmins et al., (2014a), who reported a 10% reduction in BFlh EMG activity (no reduction in medial hamstrings activity), with a concomitant 15% reduction in knee flexor eccentric strength after overground repeated sprints in uninjured individuals. Finally, the ST and SM are both innervated by the tibial division of the sciatic nerve (Koulouris and Connel, 2005).

Hamstrings architectural data are derived mainly from cadavers (Kellis et al., 2012, 2010, 2009; Ward et al., 2009; Woodley and Mercer, 2005), although comparable data have been obtained from in vivo measurements of the BFlh architecture (Timmins et al., 2014b; Potier et al., 2009; Chleboun et al., 2001). Despite the consideration of hamstrings as a functional group, its constituent muscles exhibit significant architectural differences (Table 2.1). In general, the SM is the largest of the hamstrings muscles and has the highest PA. In contrast, the ST appears to have the longest fascicle lengths with the smallest pennation angle (van der Made et al., 2013; Kellis et al., 2012, 2010; Ward et al., 2009). In an effort to compare the muscle architecture within the hamstrings group, Kellis et al. (2012) calculated a difference index (δ, Blazevich et al., 2006; Lieber et al., 1992) based on muscle thickness, PA and FL measured in cadavers. The highest similarity was observed between BFlh and SM and the lowest between BFlh and ST. Kellis et al. (2012) also suggested that each of the lateral (BFlh-BFsh) and medial (ST-SM) hamstrings pairs is composed of one muscle designed for force production (short fascicle length and high pennation angle) and one for excursion (long fascicle length and small pennation angle). In addition to the differences between the hamstrings muscles, significant intramuscular variations have been found for the BFlh and ST architecture (Kellis et al., 2010). Namely, the BFlh exhibited 35% higher pennation angle (23.96° vs. 17.78°, $P < 0.05$) and 12% longer fascicle length (7.12 cm vs. 6.35 cm, $P < 0.05$) at its most proximal site (initial 20% of MTU length) compared to the most distal site (last 25% of the MTU length). In contrast, the ST exhibited 67% higher pennation angle (14.69° vs. 8.81°, $P < 0.05$) and 18% longer fascicle length (15.49 cm vs. 13.10 cm, $P < 0.05$) distally compared to the most proximal site (Kellis et al., 2010).

Table 2.1. Hamstrings muscles architectural data derived from cadaveric studies (Kellis et al., 2012, 2010, 2009; Ward et al., 2009; Woodley and Mercer, 2005).

	Muscle wet mass (g)	**Muscle length (cm)**	**FL (cm)**	**PA (°)**	**PCSA (cm^2)**
BFlh	96.3 - 113.4	29.6 – 34.7	6.5 - 9.8	11.6 - 20.7	11.3 - 12.7
BFsh	55.9 - 59.8	21.2 – 22.4	10.4 - 12.4	12.3 - 13.2	5.0 – 5.1
ST	84.7 - 99.7	27.7 – 29.7	13.8 - 19.3	9.14 - 12.9	4.8 - 5.4
SM	109.3 - 134.3	25.8 – 29.3	5.0 - 6.9	15.1 – 16.0	18.2 – 18.4

Currently, the limited data on the hamstrings muscle composition are derived solely from cadavers and the biceps femoris has been reported to contain 33.1-54.5% type II fibres (Dahmane et al., 2006; Garret et al., 1984; Johnson et al., 1973). In the only study that has examined the muscle composition of all hamstrings muscles, Garret et al. (1984) found an average of 54.5%, 58.2%, 57.5% and 50.5% type II fibres for the BFlh, BFsh, ST and SM respectively. In that study, hamstrings were reported to contain a higher proportion of type II fibres than other thigh muscles (quadriceps, 51.9% and adductor magnus, 44.8%) and the authors suggested that this muscle composition may contribute to the high susceptibility of the hamstrings to strain injuries. Yet, in vivo studies have shown that the VL muscle, an antagonist to BFlh muscle function, contains a greater proportion of MHC-II isoform (66.1% total MHC-II in 95 physically active young men; Staron et al., 2000). The limited cadaver data on hamstrings composition do not provide any evidence on whether hamstrings composition is a risk factor for strain injuries. To elucidate whether such an association exists, the hamstrings muscle composition in healthy young adults needs to be determined first.

2.4.2 Hamstrings function during sprinting

Hamstrings strain injuries are suggested to occur primarily during high-speed running or sprinting at maximal or near maximal speed (Askling et al., 2013, 2007). Therefore, it is important to understand the function of hamstrings during this high-risk activity. The hamstrings muscle group is composed of three biarticular muscles (biceps femoris long head (BFlh), semitendinosus (ST) and semimembanosus (SM)) that cross the hip and knee joint and one mono-articular muscle (biceps femoris short head, BFsh) which crosses the knee joint. This configuration allows hamstrings to act as both hip extensors and knee flexors. During running, hamstrings activation starts at the mid-swing phase and continues through the late swing to the stance or early swing phase (Schache et al., 2012; Chumanov et al., 2011; Higashihara et al., 2010; Yu et al., 2008; Kyrolaien et al., 1999). During the late swing phase, hamstrings undergo eccentric loading to decelerate the forward movement of both the thigh and the shank (Yu et al., 2008; Thelen et al., 2005). After the successful control of the knee extension, and prior to the subsequent foot contact, a transition of the hamstrings action from eccentric to concentric occurs as knee flexion commences (stretch-shortening cycle). This concentric activity continues throughout the stance phase (Schache et al., 2012; Chumanov et al., 2011) contributing to the hip extension as the body moves forwards. However, some eccentric activity has also been reported during the late stance phase for the BFlh and ST (Yu et al., 2008) or the BFsh (Schache et al., 2012). Yu et al. (2008) suggested that this discrepancy may be due to the differences in lower extremity kinematics between treadmill and overground sprinting used in these studies. This suggestion was based on results from Frishberg (1983), who found that during take-off the knee joint was at a more extended position in overground sprinting compared to treadmill sprinting, implying that the hamstrings MTU were at a longer length in the former case.

Modelling studies have shown that muscle-tendon unit stretch increases with increasing running velocity up to ~80% of maximum, but remains relatively constant at faster velocities (Schache et al., 2013; Thelen et al., 2005). However, the magnitude of strain differs between the hamstrings muscles. Musculoskeletal modelling studies have shown that, during the late swing phase, it is the BFlh muscle-tendon unit that exhibits the greatest extension (9.8-13.0% change in length relative to upright standing length) compared to SM (7.7-11.0%) and ST (8.4-11%) (Schache et al., 2013, 2012; Chumanov et al., 2011; Thelen et al., 2005). Recently, Fiorentino et al. (2014b) were the first to quantify the along-fibre strains in the BFlh during sprinting (at 70%, 85% and 100% of maximum speed) using computational modelling and predicted that, while the strain of the MTU and the whole-fibre remain relatively constant, the peak local fibre stains near the proximal MTJ increase at higher speeds. In addition, the local fibre strains were found to be increasingly non-uniform as the speed increased (Fiorentino et al., 2014a).

Riley et al. (2010) calculated that, during low speed running (3.16 m s^{-1}), the peak iliacus MTU length occurred simultaneously with the peak biceps femoris MTU length of the contralateral limb, and the authors suggested that the hip flexors of one limb may influence the hamstrings stretch of the contralateral limb. The implication of this timing is that tight hip flexors may cause increased anterior pelvis tilt, which would in turn stretch further the contralateral BFlh MTU and potentially increase the risk for strain injury in that muscle. The role of hip flexors in BFlh MTU stretch during running was also highlighted in another modelling study (Chumanov et al., 2007), which reported that hip flexors induced >20 mm increase in biceps femoris stretch of the contralateral limb at near maximal and maximal running speeds.

In conclusion, current data show that hamstrings activation is greatest during the late swing and early stance phases. The former involves a stretch-shortening cycle with a large eccentric action of the hamstrings that imposes different loading on each of the different hamstrings muscles, while the latter involves a high concentric loading. The high biomechanical load imposed on this muscle group highlights their significance during high-speed running activity.

2.5 PART IV – HAMSTRINGS STRAIN INJURIES

Hamstrings strain injuries are the most prevalent injuries in sprint-based sports (e.g. different codes of football and track sprinting; Alonso et al., 2012; Ekstrand et al., 2011; Orchard et al., 2001) accounting for 12-17% of all injuries, while they also exhibit a high re-injury rate (12-40%; Alonso et al., 2012; Ekstrand et al., 2011a, 2011b; Elliot et al., 2011; Verrall et al., 2006; Woods et al., 2004; Orchard and Seward, 2002). A significant amount of research has been conducted in order to improve our understanding on hamstrings strain injuries; however their high rates of incidence and persistent nature highlight that our knowledge on the mechanisms, the risk factors and the rehabilitation process of strain injuries remains limited.

2.5.1 Site of injury

Hamstrings strain injuries affect predominantly the BFlh muscle (Malliaropoulos et al., 2010; Askling et al., 2013, 2007; Koulouris and Connell, 2003; Slavotinek et al., 2002; De Smet and Best, 2000; Garrett et al., 1989). In a carefully selected cohort (over a 3-year period) of 18 sprinters who sustained a first-time hamstrings strain injury (verified by magnetic resonance imaging, MRI), Askling et al. (2007) reported that the BFlh was the primary site of injury for all individuals. Other studies that have used MRI to confirm the location of the injury, have reported that the BFlh was affected in 60-83% of the total hamstrings strains (Hallen and Ekstrand, 2014; Askling et al., 2013; Koulouris and Connell, 2003; Slavotinek et al., 2002; De Smet and Best, 2000). Despite the general agreement on the muscle that is mostly injured, controversy exists on the second most injured muscle with some studies reporting the ST (Askling et al., 2007; Slavotinek et al., 2002; De Smet and Best, 2000) while others the SM (Hallen and Ekstrand, 2014; Koulouris and Connell, 2003; Malliaropoulos et al., 2011). While it is unclear why this discrepancy in injury patterns exists, it is possible that the type of activity may determine the muscle involved (Askling et al., 2013, 2006, 2000). Interestingly, in a prospective randomised controlled trial Askling et al. (2013) reported that, within 75 football players that sustained an acute hamstrings strain injury, the BFlh was primarily affected in sprinting-type strains (94%) while the SM in stretching-type injuries (76%). However, currently there is no clear understanding why these muscles are injured at different conditions. Concerning the exact location of strain injuries, Garret et al. (1987) reported that all experimentally-induced strains examined in animal models occurred in the muscle tissue adjacent to the MTJ, even though other *in vitro* studies reported damage at the MTJ itself (Tidball and Chan, 1989). *In vivo* examinations in humans have shown that the hamstrings strains are commonly located near a MTJ (proximal or distal) (Malliaropoulos et al., 2010; Koulouris and Connell, 2003; Slavotinek et al., 2002; De Smet and Best, 2000). Despite the fact that some studies specifically identified the BFlh proximal MTJ to be mostly affected (Askling et al., 2007; De Smet and Best, 2000), others provide only a general description of the 'proximal' BFlh (with no further details) as the most common injury site (Koulouris and Connell, 2003; Slavotinek et al., 2002; Garrett et al., 1989). It is unclear why strain injuries occur near the MTJ, however it has been reported that sarcomeres near the MTJ (within ~1 mm from the MTJ into the muscle) are stiffer compared to the central sarcomeres of the muscle fibre and therefore stretch less as a response to an applied force (Noonan, 1992). In addition, emerging evidence suggest that the morphology of the BFlh MTU may contribute to increased localised strains along the proximal MTJ and therefore increase the risk of a strain injury (discussed further in 2.5.3.3).

In conclusion, current data suggest that the proximal BFlh is the primary site of hamstrings strain injuries, while the MTJ (proximal or distal) is typically involved irrespective of the muscle affected.

2.5.2 The inciting mechanism of hamstrings strain injury

The majority of the hamstrings strain injuries occur during high-speed running or sprinting (Askling et al., 2013, 2007; Brooks et al., 2006; Woods et al., 2004). However, the exact time of injury has yet to be clearly identified. Muscle strain injuries are the result of excessive stretch, either passive or more commonly active (i.e. eccentric contraction, Lieber and Fridén, 1993; Garret et al., 1987). In sprinting, hamstrings undergo eccentric contraction during the mid- and late swing phases (Schache et al., 2012; Chumanov et al., 2011; Thelen et al., 2005) as well as during the late stance phase (Schache et al., 2012; Yu et al., 2008) (see 2.4.2). Modelling studies have calculated that all hamstrings muscles reach their peak stretch during the late swing phase, with the BFlh experiencing the highest strain of all hamstrings muscles. (Schache et al., 2012). Moreover, the BFlh highest peak local strains are located near the proximal MTJ (Fig. 2.5B, Fiorentino et al. 2014b), which is the site where strain injuries typically occur (see 2.5.1). Therefore, the late swing phase is believed to correspond to the time of injury (Higashihara et al., 2014; Schache et al., 2013, 2012; Chumanov et al., 2012, 2011; Thelen et al., 2005). This belief is also supported by two case studies of hamstrings strain injuries that occurred during data collection in biomechanical studies (Schache et al., 2009; Heiderscheit et al., 2005). In both studies, the authors using kinematic data concluded that the inciting event for the injury occurred during the late swing phase.

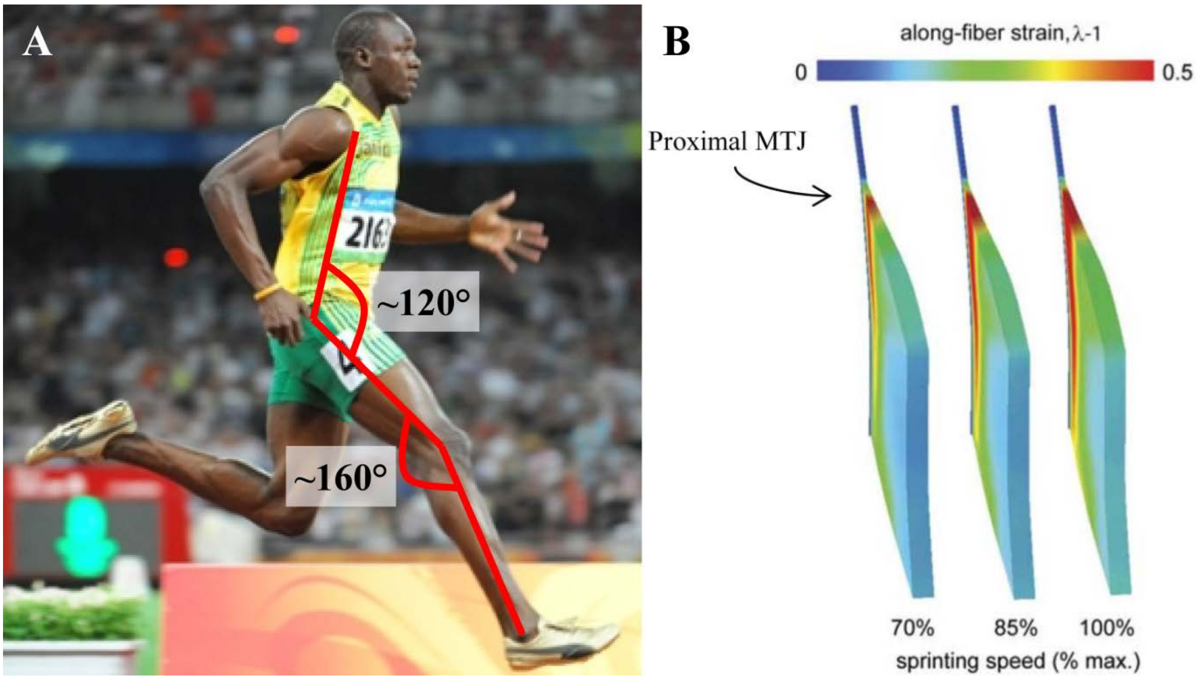

Figure 2.5. Example of the late swing phase during sprinting (A, photo adapted from AFP/Joe Klamar). Note that, towards the end of the late swing phase, the leading leg is slightly flexed at the hip (120-140°, 180°= full extension) and nearly fully extended at the knee joint. During this phase, all hamstrings muscles reach their peak strains, with BFlh experiencing the greatest strain (Schache et al., 2012). In addition, computational model simulations predict that, during the late swing phase, the BFlh peak local strains are located near the proximal MTJ and increase with running speed (B, picture adapted from Fiorentino et al., 2014b). These biomechanical conditions are believed to lead the predisposed athlete to a hamstrings strain injury (Fiorentino et al., 2014b, Chumanov et al., 2012; Schache et al., 2012).

Whilst the main body of the literature seems to agree, based on indirect evidence, that the late swing phase is the most probable time of injury, Orchard (2012) suggests that hamstrings are most susceptible during the early stance phase. Orchard (2012) argues that at the early stance phase, hamstrings have to counteract high hip flexion and knee extension moments, resulting from the large ground reaction forces (>300% of body weight). In contrast, there is no ground reaction force during the late swing phase, and hamstrings eccentric action is to control the forward movement of the shank (Orchard, 2012). Yet, animal studies have shown that muscle damage is a result of the muscle fibre strain that occurs during active lengthening rather than the level of force per se (Lieber and Fridén, 1993).

2.5.3 Risk factors

Hamstrings strain injuries are a persistent problem for the athletes, teams and physicians involved, and the identification of the factors that can lead to these injuries is of high importance. The risk factors can be described as intrinsic and extrinsic. The intrinsic risk factors are those that relate to the individual/athlete (e.g. muscle strength and flexibility), while the extrinsic risk factors are related to the environment (e.g. the game conditions, other athletes, climate etc.). The multifactorial nature of the hamstrings strain injuries suggests that it is not the sole existence of a single risk factor that leads to injury; rather, an injury is the result of the accumulation of a number of risk factors in combination with exposure to high risk conditions and ultimately an inciting event. According to a model proposed by Meeuwisse et al. (2007), the risk factors are dynamic and change the susceptibility of an individual. For example, sprint training may provide adaptations that protect an individual from hamstrings strain injuries, and therefore decrease the risk of injury. On the other hand, residual fatigue, due to excessive training with inadequate rest, can cause reduced hamstrings eccentric strength that increases the risk for strain injury. Meeuwisse et al. (2007) suggest that an athlete is susceptible when 'the intrinsic and extrinsic risk factors and the interactions between all of the risks accumulate'.

Despite the significant research efforts over the last decades, there is a surprisingly limited knowledge of what constitutes a risk factor for hamstrings strain injury. Numerous potential risk factors have been suggested, however only a few of them are supported by robust scientific evidence. More high-quality studies (e.g. large, randomised controlled studies) are needed to improve our understanding of the risk factors for hamstrings strain injuries. This section presents an overview of the main risk factors proposed in the literature.

2.5.3.1 Previous injury

A previous hamstrings injury has been consistently reported as a significant risk factor for a subsequent strain injury, and athletes with a history of hamstrings strains have 2-5 times increased risk for a future injury (Hagglund et al., 2013; 2006; Engebretsen et al., 2010; Gabbe et al., 2006; Arnason et al., 2004; Orchard, 2001; Verrall et al., 2001). Silder et al. (2008) found evidence of scar tissue adjacent to the injury site up to 23 months after the injury, and they suggested that it may increase the stiffness of the tissue. The implication is that, due to the presence of inelastic scar tissue, the muscle fibres would need to lengthen more for a given change in MTU length than before the injury. Using CINE phase contrast imaging, it was calculated that individuals with a prior proximal BFlh strain injury exhibited greater strains near the proximal BFlh MTJ under eccentric loading compared to healthy individuals (Silder et al., 2010). The greater localised strains may reflect the limited stretch capacity of the scar tissue present in the injured individuals, yet it cannot be precluded that these individuals exhibited stiffer aponeurosis-tendon complex before the injury (Silder et al., 2010).

Silder et al. (2008) reported that the previously injured athletes exhibited BFlh muscle atrophy (-10%) compared to their uninjured leg, while no atrophy was present in control individuals. Notably, all injured athletes had followed a supervised rehabilitation programme and had resumed their normal athletic activities for at least one month before taking part in that study. While strength was not assessed, the BFlh muscle atrophy would be expected to result in decreased knee flexor strength and H:Q strength imbalances which, in turn, are considered as risk factors for hamstrings strains (discussed below). Interestingly, some of the athletes with BFlh atrophy exhibited a hypertrophy in BFsh, suggesting an adaptive response to compensate for the lower BFlh strength capacity. This hypertrophic response may also suggest an underlying BFlh neuromuscular inhibition, despite the increased knee flexor loading that typically occurs during rehabilitation (Fyfe et al., 2013).

Other investigations have reported that knee flexors angle of peak concentric (Brocket et al., 2004) and eccentric (Proske et al., 2004; Croisier and Crielaard, 2000) torque shifted towards more flexed knee joint angles following a hamstrings strain injury, implying a shorter optimum muscle length. A shorter optimum length suggests that at more extended knee joint angles, hamstrings will operate at their descending part of their F-L curve. According to the ‘popping sarcomere’ hypothesis (Morgan, 1990), at the descending part of the F-L curve some sarcomeres are stretched beyond their acto-myosin filament overlap. As these sarcomeres would be the weakest along the muscle fibre, further stretch during an eccentric contraction would result in an uncontrolled lengthening of these sarcomeres leading to microscopic muscle fibre damage. Brockett et al. (2001) suggested that accumulation of such microscopic damage may eventually lead to strain injury. Nevertheless, it remains unclear if the shift in angle of peak torque seen in previously injured individuals pre-existed or was a result of the injury.

Some interesting data emerged from a recent study reporting 18-20% reduced EMG activity during eccentric contractions for the previously injured BFlh but not for the medial hamstrings compared to the uninjured leg in recreational athletes (Opar et al., 2013b). This neural inhibition was accompanied with 10-11% lower eccentric strength compared to the uninjured leg. Again, all participants had undergone rehabilitation and were permitted to return to competition at the time of testing which was conducted at least 2 months after the injury. The authors discussed that previously injured BFlh may be less responsive to eccentric training (a widely recommended tool in hamstrings strains pre- and rehabilitation, Heiderscheit et al., 2010) and therefore more susceptible to a future injury. In another study by the same authors (Opar et al., 2013a), similar reductions in BFlh neural activation (but not the medial hamstrings) were reported along with lower knee flexor rate of torque development and reduced impulse at 50 ms and 100 ms after the contraction onset in slow eccentric contractions. However, these retrospective studies cannot elucidate whether the reduced BFlh neural activation was the cause or the result of the hamstrings injury and further prospective studies are needed.

In summary, hamstrings injuries result in neuromuscular and functional alterations that may be present long after the injury occurrence and even when athletes are cleared to return to their usual athletic activities, while these changes may predispose them to re-current strain injuries.

2.5.3.2 Strength imbalances

Knee joint muscle strength imbalances are typically assessed by comparing the knee extensors or flexors strength between the two sides (bilateral imbalances) and/or by calculating the relative strength of the knee extensors and flexors (H:Q ratio) unilaterally. Originally, the H:Q ratio was calculated from the concentric peak torque of the knee extensors and flexors, known as the conventional ratio. Later, the dynamic strength ratio (Dvir et al., 1989) or functional ratio (Aagaard et al., 1998, 1995) was introduced, which calculates the ratio of hamstrings peak eccentric to quadriceps peak concentric torque, and it is thought to better reflect the reciprocal antagonistic function of these muscles during athletic activities such as sprinting and kicking. Despite the widespread use of the H:Q ratio, there are no objective cut-off ratio limits due to differences in isokinetic dynamometers and exercise protocols used (Croisier, 2002).

Strength imbalances, either bilateral for knee flexors or between knee extensors and flexors of the same leg, have been long considered a risk factor for strain injuries (Fousekis et al., 2010; Yeung et al., 2009; Croisier et al., 2008, 2002, 2000; Orchard et al., 1997; Heiser et al., 1984). Croisier et al. (2002) found that the most affected functional parameters in previously injured individuals were the hamstrings eccentric bilateral strength and the hamstrings eccentric to quadriceps concentric strength ratio (functional H:Q ratio). These authors highlighted the discriminating character of the eccentric strength deficits and discussed that had they only examined the concentric strength, 23% of the individuals with a history of strain injury would have not been identified with strength imbalances. In a large prospective study (n= 462) that examined the relationship between strength imbalances and injury risk, Croisier et al. (2008) recorded 35 hamstrings injuries and found that professional footballers with preseason strength imbalances that were left untreated, either bilateral hamstrings strength deficits >15% and/or a low conventional (<0.47-0.49) or functional (<0.80-0.89) H:Q strength ratio), had >4-fold increased risk of strain injury during the subsequent season compared to players with no strength imbalances. In addition, players with initial imbalances that were restored (according to statistically defined cut-off criteria) reduced their risk of injury to levels comparable to players with no imbalances. In a smaller prospective study, Yeung et al. (2009) examined forty-four sprinters over 1 year and recorded a total of 12 hamstrings strain injuries in 8 athletes. Using Cox regression analysis, it was found that athletes with a conventional H:Q ratio <0.60 exhibit a 17-fold greater risk for a hamstrings strain injury. Another small prospective study (n= 37) has also reported that a conventional H:Q ratio <0.61 at 60° s^{-1} increases the risk of strain injury in American football players. In contrast to the aforementioned studies, Bennell et al. (1998) did not find any association between low bilateral hamstrings strength ratio (<0.90) or low H:Q strength ratio (<0.60) and increased risk of strain injury. However, they only recorded 9 injuries over a season in a cohort of 102 athletes. It is important to highlight that most of the above studies have used relatively small sample sizes and recorded a low number of hamstrings injuries. A number of 20-50 injury cases are needed to detect a moderate to strong association with a potential risk factor (Bahr and Holme, 2003). Considering the multifactorial nature of the hamstrings strain injuries, it is unlikely that any single factor will exhibit a strong relationship with this type of injury. In that case, >200 injury cases are needed to detect small to moderate associations (Bahr and Holme, 2003). It is clear that further large-scale prospective studies are needed to better understand the relationship between strength imbalances and hamstrings strains.

Most studies that examined the knee joint muscle strength imbalances have focused on the H:Q peak torque ratio. However, the time needed to achieve peak isometric torque can be >400 ms (Thorstensson et al., 1976), which is substantially longer than the time available for the knee flexors to decelerate the shank during the late swing phase (<100 ms, Schache et al., 2013). Therefore, examination of the explosive H:Q ratio could provide valuable information that would otherwise be undetected by a maximal strength ratio. Indeed, Hannah et al. (2014) examined the explosive H:Q ratio of the reciprocal muscle groups and found that at the initial 50 ms from the activation onset, the explosive H:Q ratio was significantly lower compared to the maximal strength H:Q ratio (0.17 vs. 0.56, $P< 0.001$), suggesting that the knee joint is particularly vulnerable to injury at that time. Interestingly, this large difference was mainly attributed to the 2 times longer hamstrings electromechanical delay compared to quadriceps (44.0 vs. 22.6 ms, $P< 0.001$). It must be noted however that in that study the explosive H:Q ratio was examined isometrically, while hamstrings act eccentrically during the late swing phase. The type of contraction has been found to exhibit a differential influence on explosive torque production capacity (Tillin et al., 2012).

2.5.3.3 Hamstrings anatomy

Despite the common speculation that the anatomy of the hamstrings might influence injury risk, this has received surprisingly little attention. Only recently, two studies using computational modelling and dynamic MR imaging, calculated higher localised tissue strains for individuals with a narrow proximal BFlh aponeurosis and they suggested that a disproportionately small BFlh proximal aponeurosis may be a potential risk factor for strain injury (Fiorentino et al., 2012; Rehorn and Blemker, 2010). Initially, Rehorn and Blemker (2010) examined finite element models of BFlh based on MR images and examined the influence of proximal and distal aponeurosis dimensions on stretch distribution in the muscle during a simulated eccentric contraction and found that a decrease in proximal aponeurosis width by 80% resulted in 60% increase in peak stretches along the proximal MTJ. The findings of that study were confirmed by an *in vivo* study from the same laboratory that used CINE dynamic MR imaging to measure the BFlh strains during active and passive lengthening in 13 individuals (Fiorentino et al., 2012). Specifically, they found that individuals with a narrow BFlh proximal aponeurosis experienced the highest strains near the aponeurosis during active lengthening compared to individuals with a wider aponeurosis. These two studies provided the first evidence that aponeurosis size may contribute to hamstrings strain injuries and that individuals with a narrow aponeurosis may be at an increased risk. However, to date the inter-individual variability of the BFlh proximal aponeurosis size has not been examined. Some preliminary data suggested that the width of the BFlh proximal aponeurosis is highly variable between individuals and unrelated to the size of the BFlh muscle (Handsfield et al., 2010), suggesting that within the BFlh MTU the force transmitter may not be proportional to the force generator. If this is the case a disproportionately small BFlh proximal aponeurosis may concentrate mechanical strain on the surrounding muscle tissue (Fiorentino et al., 2014a, 2012; Rehorn and Blemker, 2010) and be a risk factor for hamstrings strain injury. However, in that preliminary report aponeurosis width was measured at a single arbitrary point along the muscle, which may be a poor reflection of the size of the aponeurosis. In contrast, measuring the whole contact interface between the muscle and aponeurosis may better reflect the concentration of mechanical strain at this interface. Further research is needed to elucidate the variability of the proximal BFlh aponeurosis size and its relationship with muscle size. Hamstrings muscle composition and innervation has also been speculated as risk factors for strain injuries (see 2.4.1), however to date no studies have examined these possibilities. Future prospective studies are needed to elucidate whether hamstrings anatomy is related to increased risk for strain injury.

2.5.3.4 Fatigue

Nearly half (47%) of the hamstrings strains sustained during a football match occur towards the end of each half period (Woods et al., 2004). This suggests that fatigue may induce changes in muscle strength and sprint mechanics that could contribute to the hamstrings injury susceptibility. Knee flexor maximal strength was significantly reduced in professional and amateur footballers after the completion of laboratory and field-based football-specific exercise (Greco et al., 2013; Delextrat et al., 2010; Small et al., 2010; Greig et al., 2008). Interestingly, some studies reported that only eccentric strength was affected (Small et al., 2010; Greig et al., 2008), while other studies reported a decrease in isometric, concentric and eccentric knee flexor strength (Greco et al., 2013; Delextrat et al., 2010). Also, knee flexor rate of force development (RFD) at 0-50 and 0-100 ms from contraction onset was reduced after a laboratory-based soccer-specific exercise (Greco et al., 2013). In general agreement, the functional H:Q ratio was significantly reduced after exercise that simulated a soccer match (Greco et al., 2013; Delextrat et al., 2010; Small et al., 2010; Greig et al., 2008). In contrast, an unchanged (Small et al., 2010; Greig et al., 2008) or decreased (Greco et al., 2013; Delextrat et al., 2010) conventional H:Q ratio has been reported after soccer-specific exercise protocols. Despite the discrepancy in the results of the above studies, overall these results suggest that at the later stages of a football match, the knee flexors have a decreased capacity to absorb energy during the late swing phase of sprinting which may increase the risk of a strain injury (Schache et al., 2012).

Changes in sprinting mechanics have also been observed due to fatigue (Small et al., 2009; Pinniger et al., 2000). Pinniger et al. (2000) reported a reduced hip and knee flexion, and reduced thigh and leg angular displacement during swing phase after a fatiguing protocol involving isolated knee flexion and 40-m repeated maximal sprints. These changes were accompanied with changes in neural activation patterns with the rectus femoris activation ceasing earlier while the hamstrings were activated earlier during the swing phase. The authors suggested that the observed kinematic changes may be protective mechanisms to reduce the fast eccentric action of the fatigued hamstrings during the late swing phase and, therefore, the stress and strains within the hamstrings. Similarly, the earlier activation of the hamstrings and their increased duration of activation may compensate for their reduced force production capacity, providing more time to the weaker hamstrings to successfully decelerate the shank before ground contact. In contrast to Pinniger et al. (2000), Small et al. (2009) found a reduced hip flexion but increased knee flexion and lower limb velocity after a football-specific field protocol also. Small et al. (2009) also reported an increased anterior pelvis tilt and suggested that these changes in sprint kinematics may predispose the hamstrings to strain injuries, as an increased anterior pelvis tilt would increase the hamstrings stretch and strain. The increased knee flexion may reflect the shift in angle of peak torque towards shorter muscle lengths after exercise-induced muscle damage (Proske and Morgan, 2001). Forced lengthening of hamstrings to greater lengths, combined with the reduced eccentric capacity of hamstrings due to fatigue, could potentially result in a strain injury. Also, an increased anterior pelvis tilt suggests an increase in the hamstrings MTU length which again may predispose to a strain injury.

2.5.3.5 Age

Increasing age has been frequently reported to be related to higher risk of hamstrings strain injury (Henderson et al., 2010; Gabbe et al., 2006, 2005; Arnason et al., 2004; Orchard, 2001 Verrall et al., 2001), even though this relationship was not always confirmed (Hagglund et al., 2013, 2006). Interestingly, older age remained a significant risk factor even when other confounding factors were controlled (e.g. previous injury, Arnason et al., 2004; Orchard et al., 2001). Further, every additional year of age increases the risk of injury by 1.4-1.8 times in professional athletes such that a 30-year old athlete has 14-18 times greater risk than a 20-year old (Henderson et al., 2010; Arnason et al., 2004). However, it remains unclear why older athletes are predisposed to strain injuries. In a prospective study, Gabbe et al. (2006) found that older athletes (≥25 years) had increased body mass and reduced hip flexibility compared to younger athletes (≤20 years) and multivariate analysis showed that these were independent risk factors for strain injury in the older athletes. Finally, Orchard et al. (2001) speculated that lumber degeneration leading to L5/S1 nerve impingement may result in hamstrings denervation and loss of muscle strength in older individuals. However, these suggestions are not supported by any scientific evidence and therefore it remains unclear why increasing age predisposes to hamstrings strains.

2.5.3.6 Flexibility

Current findings concerning the relationship between hamstrings flexibility and risk of strain injury are conflicting. Three prospective studies in professional footballers have found that decreased flexibility of hip and knee flexors increases the risk of hamstrings strain injury (Henderson et al., 2010; Bradley and Portas, 2007; Witvrouw et al., 2003), while other studies did not find any association (Engebretsen et al., 2010; Yeung et al., 2009; Gabbe et al., 2006, 2005; Arnason et al, 2004). While it is unclear why this discrepancy in the results exists, it may be partly due to the different methods used and the difficulty in differentiating hamstrings flexibility from flexibility in the lumbar spine and pelvis (Opar et al., 2013; Dallinga et al., 2012; Prior et al., 2009).

Chapter 3

Reliability of isometric and isovelocity hamstrings-to-quadriceps ratio and strength measures for the knee extensors and flexors

3 CHAPTER 3 – RELIABILITY OF ISOMETRIC AND ISOVELOCITY HAMSTRINGS-TO-QUADRICEPS RATIO AND STRENGTH MEASURES OF THE KNEE EXTENSORS AND FLEXORS

3.1 INTRODUCTION

Strength imbalance between the knee extensors and flexors has been suggested as a risk factor for hamstrings strains and anterior cruciate ligament injuries (Croisier et al., 2008, 2002; Sugiura et al., 2008; Griffin et al., 2006). Typically, the strength balance around the knee joint is examined with the hamstrings-to-quadriceps peak torque ratio (H:Q) using isokinetic dynamometry. However, the assessment of the H:Q ratio is commonly performed in conditions that do not reflect the biomechanics of the activities where injuries occur (e.g. late swing phase of sprinting for strain injuries, Chumanov et al., 2012). Accounting for these conditions would be expected to improve the validity of the measurements; yet, the reliability of such protocol should be established before its employment. In addition, the ‘ideal’ protocol should require the minimum amount of time and involvement from the athletes, in order to minimise any disruption of their training schedule and maximise the frequency of performance monitoring. Therefore, the development of an ecologically valid, reliable protocol that assesses the strength balance of the knee extensors and flexors in the least time spent in the laboratory is critical.

Three main variations of the H:Q ratio can be calculated: isometric, conventional and functional ratios. The conventional ratio is defined as the knee flexors to extensors concentric peak torque, whilst the functional ratio as the knee flexors eccentric to knee extensors concentric peak torque. Whilst the functional ratio is considered to reflect better the antagonistic function of these muscle groups (Aagaard et al., 1998, 1995), the ecological validity of the H:Q ratio remains limited. In most studies, knee extensor and flexor strength is assessed at a seated, upright position with the hip joint at 90-100° (180°= full extension). However, this position is far from the ~120-140° hip flexion during the late swing phase when strain injuries occur (Guex et al., 2012; Novacheck, 1998). Another limitation is the large discrepancy in the angular velocity of the knee joint during athletic activities (e.g. >1200° s^{-1} during kicking or sprinting, Higashihara et al., 2010; Kellis and Katis, 2007) and that attainable in isokinetic dynamometry (≤500° s^{-1}). Clearly, this limitation cannot be overcome with current dynamometers; however, the H:Q ratio has been examined at high isokinetic velocities, even though the reliability of measurements >240° s^{-1} has not been established. At higher isokinetic velocities, individuals may have a difficulty to maintain maximal neuromuscular activation due to the short available time resulting in greater torque variability at those velocities (Caruso et al., 2012; Iga et al., 2006). Therefore, it is important to ensure that muscle strength testing at velocities >240° s^{-1} produces reliable measurements. The adoption of a testing position that resembles the hip angle during the late swing phase along with the examination of H:Q ratio at the highest attainable velocities would improve the ecological validity of the reciprocal strength assessment. However it is essential to establish the reliability of these measurements.

The isovelocity ratios are based on the peak torque of the reciprocal muscles, irrespective of the angle at which peak torque occurs. However, the isometric H:Q ratio is calculated at a specific angle that is the same for both muscle groups (Kong and Burns, 2010). A significant limitation of the isometric ratio is that during dynamometry measurements of 'isometric' knee flexion and extension contractions, movement occurs at the knee joint resulting in a discrepancy between the crank angle and the actual knee-joint angle (Tsaopoulos et al., 2011). This movement is mainly due to the deformation of the soft tissue of the leg and the compliance of the dynamometer, and despite a fixed crank angle it has been found to afford up to 20° of discrepancy between crank angle and knee angle for knee extension (Tsaopoulos et al., 2011; Arampatzis et al., 2004). Similar differences would be expected for knee flexion, when the knee joint moves forwards and up relative to the dynamometer rotational axis. As knee flexion and extension contractions are in opposite directions, the discrepancy in actual knee joint angles between knee extensor and flexor peak torque could be as large as 40°. Therefore, valid isometric ratios that are genuinely angle specific require measurement of knee joint angle that is independent of crank angle. However, to date no studies have accounted for the discrepancy in the examination of the isometric H:Q ratio and thus the reliability of the isometric ratio obtained at true knee joint angles remains to be examined.

Therefore, the aim of this study was to evaluate the inter-session reliability of the isometric (knee-joint angle-specific) and isovelocity (functional and conventional) H:Q ratios using a short protocol that included muscle function measurements up to high angular velocities and joint positions that closely replicate the conditions of high injury risk. This involved the assessment of the reliability of the knee flexors and extensors torque measurements across the torque-velocity relationship.

3.2 METHODS

3.2.1 Participants

Nine healthy, recreationally active males (age 24 ± 3 years, height 178 ± 6 cm and body mass 69.9 ± 8 kg, mean ± SD) volunteered to take part in this study. None of the participants were involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. Their physical activity was assessed using the International Physical Activity Questionnaire short format [www.ipaq.ki.se/downloads.htm, (Craig et. al., 2003)] and their average energy expenditure was 2244 ± 1284 MET-minutes/week. After completing the physical activity and health screen questionnaires, participants provided written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no musculo-skeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit.

3.2.2 Overview

All participants visited the laboratory on four occasions, each separated by 7 days, at a consistent time of day. All sessions involved unilateral measurements of dominant leg knee flexor and extensor strength conducted with an isokinetic dynamometer (Con-Trex MJ, CMV AG, Duebendorf, Switzerland). The first and third session involved identical isometric knee flexor and extensor assessment while the second and fourth session involved identical dynamic assessment of both muscle groups. All isometric contractions performed in the first and third sessions, were recorded with a video camera in order to assess actual knee-joint angles during these isometric contractions, and also facilitate conversion of crank angles to actual knee-joint angles during all the contractions. Participants were familiarized with the procedures of the dynamic assessment during their first visit to the laboratory. All testing sessions were conducted by the same investigator to avoid inter-examiner variability.

3.2.3 Dynamometer Procedures

Participants were seated on the dynamometer chair with a hip angle of 120° (180°= full extension). Two 3-point belts secured the torso and additional straps tightly secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint with the dynamometer rotational axis during active muscle contractions was done separately for knee extension and flexion contractions. Specifically, in each case the alignment was done during isometric contractions of >50% MVF at a knee joint angle of ~115°. The dynamometer's shin brace was placed ~2 cm above the medial malleolus, anterior to the shank for knee extension contractions and posterior for knee flexion contractions, prior to the shank being tightly secured to the dynamometer lever arm. During the knee extension contractions, an additional moulded rigid plastic shin pad, lined with 2 mm of high density foam, was tightly secured to the tibia to avoid any discomfort to the shin during maximum contractions. The range of motion was established passively and anatomical zero was set at the most extended position where participants felt comfortable and without excessive stretch of their hamstrings. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque by the dynamometer software. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardised verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen. Torque, crank angle and crank angular velocity were recorded at 512 Hz during all contractions.

3.2.4 Torque-velocity relationship assessment

3.2.4.1 Isometric strength

Measurements were recorded first with the knee flexors and then the knee extensors. Prior to the recorded contractions for each muscle group, participants completed a standardized warm-up consisting of a progressive series of submaximal contractions. For the assessment of peak isometric torque of each muscle group, participants performed two sets of five maximum contractions, one at each of five different crank angles (165°, 150°, 135°, 120° and 105° in a randomized order; 180°= full extension). Participants were instructed to "push" or "pull" as hard and as fast as possible for 3-5 s. One-minute rest was given between each contraction, with 2 min between sets and 5 min between muscle groups.

3.2.4.2 Concentric and Eccentric strength

Initially, participants performed a standardized warm-up protocol with five submaximal contractions of progressively higher intensity. Following the warm-up, first the knee extensors were tested for their concentric and eccentric torque at three velocities, and then the knee flexors were also tested at the same concentric and eccentric velocities. This involved a protocol of concentric-eccentric contractions at low (60° s^{-1}), medium (240° s^{-1}) and high (400° s^{-1}) angular velocities in this order. At each velocity participants performed 2 sets of 2 (60° s^{-1}), 3 (240° s^{-1}) or 5 (400° s^{-1}) concentric-eccentric contractions over approximately 80-85° of range of motion. A minimum of one-minute rest was given between each set, with 2 min between velocities and 5 min between muscle groups.

3.2.5 Torque data analysis

The isometric contraction with the highest torque at each crank angle was chosen for further analysis. Isometric peak torque was defined as the average over a 500 ms epoch around (250 ms either side) the instantaneous highest torque. In order to account for the differences between crank angle and knee-joint angle between the two sessions, the isometric torque-knee joint angle data for each muscle group was smoothed by performing 2nd order polynomial fitting to the raw torque values. Then the polynomial fit was used to interpolate torque values for knee joint angles at 105, 120, 135, 150 and 165°. The isometric torque for each muscle group and the isometric H:Q ratio data presented are the interpolated values. The concentric and eccentric contractions at each velocity with the highest torque and isovelocity range were chosen for further analysis. In order to control for the torque overshoot during the acceleration and deceleration phases (Schwartz et al., 2010), data during these phases were excluded and the constant isovelocity period (within ±10% of the prescribed crank angular velocity, Baltzopoulos et al., 2012) was identified. Peak torque was calculated by averaging the torque values over a 1-2° range of angles around the highest recorded torque value.

3.2.6 Knee joint angle

In order to account for the dynamometer compliance and the position change of the knee joint relative to the dynamometer crank during testing, the actual knee joint angle was determined during the isometric contractions. A video camera (Panasonic NV-GS200 mini-DV, Japan) was used to record sagittal plane images at a sampling rate of 50 Hz. The camera was positioned ~2.5 m perpendicular to the dynamometer and mounted on a tripod at a height of ~2.2 m in order to have an unobstructed view of the knee joint. Joint centres were identified with 2 cm diameter circular marks drawn on the surface of the hip (greater trochanter), knee (lateral collateral ligament just below the lateral femoral epicondyle) and ankle (lateral malleolus of the fibula) joints. The knee joint angle was measured from the coordinates of the three anatomical reference points during each participant’s best isometric contraction at each angle. The camera tilt relative to the plane of movement, introduced a systematic error to the knee joint angle measurements. To quantify this error, the horizontal and vertical sides of a right angle with known dimensions that was on the plane of movement, were digitised and used as a scaling factor. The error was found to be on average ±6° (range= 0-14°) over a 90° range of motion (90-180°, 180°= full extension). However, this systematic error was not expected to influence the reliability of the angle-specific torque measurement as the same camera position was replicated throughout the measurements. The measured knee joint angles were plotted against the respective crank angles and a quadratic equation was fitted in order to generate a knee joint angle-crank angle relationship for each muscle group. These relationships facilitated conversion of crank angles recorded during all contractions (isometric, concentric and eccentric) to actual knee joint angles. The coefficient of determination for these relationships (knee joint angle vs. crank angle), calculated for each muscle group of each participant, were very high ($0.9729 \leq R^2 \leq 1$).

3.2.7 Isometric Hamstrings-to-Quadriceps ratio

The isometric hamstrings-to-quadriceps ratio ($H{:}Q_{isom}$) was calculated by dividing the hamstrings torque at each knee-joint angle by the quadriceps torque at the same angle.

3.2.8 Functional Hamstrings-to-Quadriceps ratio

The dynamic hamstrings-to-quadriceps functional ratio ($H{:}Q_{func}$) was calculated by dividing the hamstrings eccentric peak torque at each angular velocity by the quadriceps concentric peak torque at the same velocity.

3.2.9 Conventional Hamstring-to-Quadriceps ratio

The hamstrings-to-quadriceps conventional ratio ($H{:}Q_{conv}$) was calculated by dividing the hamstrings concentric peak torque by the quadriceps concentric peak torque at the same angular velocity.

3.2.10 Statistics

Group data are presented as mean ± standard deviation (SD) between individuals. Differences between sessions for peak torque and H:Q ratios were examined with one-way repeated measures analysis of variance. Test-retest absolute reliability was assessed with the intra-individual standard deviation (SDw) and the coefficient of variation (CVw, calculated as the SDw divided by the mean of the two sessions for each individual). No established cut-off criteria exist for the interpretation of the reliability statistics. In this study, the arbitrary classification followed was: <10% high, 10-15% moderate and >15% low for the CV, and <0.60 low, 0.60-0.80 moderate and >0.80 high for the ICC. Differences in the CVw statistic were examined with repeated measures analysis of variance and post hoc comparisons were performed with paired *t*-tests with Bonferonni correction. The intra-class correlation coefficient (ICC; two-way random effects model with single measure reliability - 2,1) was used to assess the relative reliability. Differences in the ICC statistic between muscle groups were examined with paired *t*-tests. A $P < 0.05$ level of significance was used for all statistical tests.

3.3 RESULTS

3.3.1 Torque-velocity relationship

Knee extensor and flexor strength measurements did not exhibit any differences between the two sessions for isometric (Table 3.1), concentric or eccentric torque at any velocity (Table 3.2).

Overall, isometric torque measurements had a moderate to high absolute reliability (CVw= 4.3-13.8%). Knee extensor isometric torque was more consistent at the mid-range angles (120°-135°; CVw= 5.3-5.9%, Table 3.1), compared to the extremes of the range of motion (105°, CVw= 12.4%; 165°, CVw= 13.8%), however these differences were not significant ($P > 0.05$). Similarly, knee flexors isometric torque exhibited high absolute reliability at most angles (105°-150°, CVw= 4.3-5.9%), while the most extended angle had lower but not significantly different absolute reliability (165°; CVw= 11.7%, $P > 0.05$). No difference in absolute reliability was found between the two muscle groups ($P > 0.05$). The isometric torque measurements exhibited a moderate-to-high relative reliability at all knee-joint angles for both muscle groups (ICC= 0.76-0.94, Table 3.1), and the relative reliability was similar for both muscle groups when the data were collapsed across knee joint angles ($P = 0.417$).

Table 3.1. Isometric peak torque for knee extensors and flexors presented as mean ± SD with the respective reliability measures (n= 9).

	Knee-joint angle (°)	Torque (Nm)		Reliability	
		Session 1	Session 2	CVw (%)	ICC
Knee extensors	105	148 ± 50	158 ±55	12.4	0.88
	120	201 ± 45	204 ±49	5.3	0.94
	135	214 ± 51	215 ±49	5.5	0.91
	150	187 ± 49	192 ± 45	5.9	0.91
	165	120 ± 38	133 ± 50	13.8	0.76
Knee flexors	105	103 ± 14	101 ± 20	5.9	0.82
	120	114 ± 15	114 ± 21	5.3	0.83
	135	119 ± 17	120 ± 22	4.3	0.91
	150	117 ± 23	118 ± 23	4.7	0.93
	165	108 ± 34	109 ± 27	11.7	0.79

The isovelocity (concentric and eccentric) strength measurements exhibited high absolute reliability for both knee extensors and flexors (CVw= 4.0–9.7%, Table 3.2). There was no difference in absolute reliability between muscle groups, contraction type or velocities examined (*P*> 0.05). However, the knee extensors had higher relative reliability than the knee flexors when the data were collapsed across velocities (ICC= 0.90 and 0.66 respectively, *P*= 0.001).

Table 3.2. Isovelocity peak torque for knee extensors and flexors for each measurement session presented as mean ± SD with the respective reliability measures (n= 9).

	Type of contraction	Angular velocity (° s^{-1})	Torque (Nm)		Reliability	
			Session 1	Session 2	CVw (%)	ICC
Knee extensors	**Concentric**	60	160 ± 35	167 ± 36	7.2	0.83
		240	119 ± 26	124 ± 25	4.0	0.95
		400	94 ± 24	94 ± 24	4.7	0.96
	Eccentric	60	224 ± 48	219 ± 51	5.4	0.92
		240	228 ± 43	226 ± 51	5.0	0.92
		400	239 ± 44	233 ± 53	7.3	0.82
Knee flexors	**Concentric**	60	104 ± 25	102 ± 12	8.3	0.57
		240	83 ± 24	80 ± 14	9.7	0.74
		400	60 ± 18	56 ± 15	9.7	0.84
	Eccentric	60	125 ± 23	126 ± 17	9.3	0.54
		240	122 ± 22	125 ± 13	6.5	0.74
		400	118 ± 21	120 ± 12	7.6	0.52

3.3.2 Hamstring-to-quadriceps ratios

The isometric H:Q ratio was not significantly different between sessions (Table 3.3). The absolute reliability at the most extended knee-joint angle (165°; CVw= 16.8%) was significantly lower compared to 135° (CVw= 5.7%, *P*= 0.046) and 150° (CVw= 8.4%, *P*= 0.050), with a tendency to be lower than 120° (CVw= 4.8, *P*= 0.070). The relative reliability of the isometric H:Q ratio was high for 135° and 150° (ICC= 0.90) but moderate at the other knee-joint angles (ICC= 0.65-0.72, Table 3.3).

Table 3.3. Isometric H:Q ratio presented as mean ± SD for each measurement session with the respective reliability measures (n= 9). Different from 165°, * *P*< 0.05

Knee-joint angle (°)	H:Q ratio		Reliability	
	Session 1	Session 2	CVw (%)	ICC
105	0.76 ± 0.28	0.69 ± 0.19	11.1	0.65
120	0.58 ± 0.08	0.57 ± 0.06	4.8	0.70
135	0.58 ± 0.12	0.57 ± 0.11	5.8*	0.90
150	0.66 ± 0.21	0.64 ± 0.18	8.4*	0.90
165	0.96 ± 0.36	0.89 ± 0.29	16.8	0.72

The functional and conventional H:Q ratios were not different between sessions. Both ratios exhibited moderate to high absolute reliability at all velocities (CVw= 7.8-11.8%, Table 3.4), while there were no differences between the two types of the H:Q ratio or between the angular velocities examined in each ratio (*P*> 0.05). The relative reliability was only low to moderate for the functional ratio (ICC= 0.45-0.75) and low for the conventional ratio (ICC= 0.38–0.58), while the functional ratio exhibited a trend for higher relative reliability compared to the conventional ratio, when the data were collapsed across velocities (*P*= 0.064).

Table 3.4. Conventional and functional ratio at different angular velocities measured during two repeated measurement sessions together with the respective reliability measures. Data are presented as mean ± SD (n= 9).

	Angular velocity (° s^{-1})	H:Q ratio		Reliability	
		Session 1	Session 2	CVw (%)	ICC
Functional	60	0.80 ± 0.15	0.78 ± 0.15	11.8	0.45
	240	1.05 ± 0.20	1.03 ± 0.19	7.8	0.75
	400	1.29 ± 0.23	1.34 ± 0.29	8.2	0.71
Conventional	60	0.65 ± 0.11	0.63 ± 0.11	10.8	0.38
	240	0.70 ± 0.12	0.65 ± 0.12	9.4	0.56
	400	0.64 ± 0.07	0.61 ± 0.11	8.8	0.58

3.4 DISCUSSION

The main aim of this study was to examine the test-retest reliability of the isometric, functional and conventional H:Q ratio using a short protocol that replicated the hip and knee joint angles during conditions of high injury risk. We found that the functional and conventional H:Q ratio exhibited good absolute reliability but low to moderate relative reliability. Furthermore, the knee-joint angle-specific isometric H:Q ratio exhibited high absolute reliability at all mid-range angles, but it was less reliable at extreme knee joint angles. Overall, the applied protocol assessed the knee-joint strength balance with acceptable test-retest reliability similar to that reported in the literature.

Even though the H:Q ratio is extensively used to identify potentially injurious knee-joint strength imbalances, it is often obtained in conditions that ignore the biomechanics of the hamstrings strain injury. The protocol employed in this study accounted for these conditions (i.e. relatively extended hip joint position and high knee-joint angular velocities) to the greatest possible extent, and produced reliable measurements of the isovelocity and isometric H:Q ratios. Both functional and conventional ratios had moderate to high absolute reliability at all examined velocities (CVw= 7.8-11.8%). The current investigation is the first to examine the reliability of the H:Q ratio at velocities >240° s^{-1}, and found that it can be examined with acceptable reliability at velocities up to 400° s^{-1}. Concerning the lower velocities, our results are within the range of previous findings (Ayala et al., 2012; Impellizzeri et al., 2008; Sole et al., 2007). However, absolute reliability for the isovelocity H:Q ratios has been found to vary significantly (60° s^{-1}, CV= 5.1-7.1%; Impellizzeri et al., 2008; 60-240° s^{-1}, CV= 16.3-20.6%; Ayala et al., 2012). These studies thoroughly familiarised their participants using 3 testing sessions and more extensive protocols compared to the current study. However, Ayala et al. (2012) reported low reliability which may be due to the adoption of a prone testing position, as at that position it is more difficult to control for extraneous movement at the hip joint. Despite the good absolute reliability found for the H:Q ratios in our study, the relative reliability was generally low. Similar results have been reported previously (Ayala et al., 2012; Impellizzeri et al., 2008; Sole et al., 2007).

Overall, concentric and eccentric strength measurements for the knee extensors and flexors had high absolute reliability (CVw= 4.0-9.7%) suggesting that this protocol accurately assessed the individuals' torque-velocity relationship. These results are better (Ayala et al., 2013) or similar to those reported in other studies (Sole et al., 2007; Pincivero et al., 1997).

However, higher absolute reliability has been found in studies that provided more thorough familiarisation (Impellizzeri et al., 2008; Maffiuletti et al., 2007; Gleeson and Mercer, 1992). In this study, a relatively short protocol was applied in an effort to reduce the time needed for the assessment of the reciprocal strength balance at the knee joint. While extensive familiarisation would be expected to improve the reliability of the measurements (Hopkins et al., 2001), it is not always feasible when assessing athletes.

In our study, the relative reliability of isovelocity strength was higher for the knee extensors compared to knee flexors when collapsed across different contraction types and velocities (0.90 and 0.65 respectively, P= 0.001). Yet, other studies reported similar relative reliability between these muscle groups (Impellizzeri et al., 2008; Maffiuletti et al., 2007; Sole et al., 2007; Gleeson and Mercer, 1992). These studies examined more heterogeneous cohorts including males and females compared to the current investigation. As the relative reliability examines how well the individuals maintain their rank within the cohort from session to session, individuals with similar peak torque values may change rank between sessions without exhibiting large intra-individual differences. This may explain the relatively low relative reliability (low ICC) in the current study, despite high absolute reliability (low CVw).

This is the first study to examine the reliability of the measured angle-specific isometric H:Q ratio. The isometric ratio was highly consistent (CVw= 4.8-8.4%) at knee joint angles between 120° and 150°, but less reliable at the extended position of 165°. A similar pattern was noted for the angle-specific isometric torque which was consistent between sessions for the mid-range knee-joint angles (CVw= 4.3-5.9%) and more variable at the extremes, particularly for the knee extensors (CVw=11.7–13.8%). This may be partly explained by the need to extrapolate outside the range of measured angles for some individuals and therefore the predicted values may be considerably away from the real ones due to the inherent uncertainty of extrapolation (Chapra, 2008). Also, at these more extreme positions participants reported increased discomfort compared to the other angles and this may have also influenced their motivation. Similarly, there is evidence to suggest that voluntary activation of the knee extensors during maximum efforts may be inhibited at shorter muscle lengths/extended positions (Becker and Awiszus, 2001) potentially due to the shorter muscle spindle lengths which, in turn, decrease the Ia afferents discharge rate resulting in lower excitatory drive. Another possible mechanism is the increase in knee joint ligament tension that would alter the gamma drive to quadriceps spindles and finally the excitatory drive (Becker and Awiszus, 2001). The above-mentioned methodological and physiological factors may explain the reduced consistency of knee extensor peak torque measurements at the most extended position. To our knowledge, the only other study that examined the isometric H:Q ratio reported lower absolute reliability at a single crank angle (120°, CVw= 10.6%; de Carvalho Froufe Andrade et al., 2013). For knee extensors and flexors isometric torque, our findings are in accordance with previous investigations (120°, knee extensors, CVw= 4.2-5.5% knee flexors, CVw= 4.7-5.8 %, de Carvalho Froufe Andrade et al., 2013; Maffiuletti et al., 2007). In our study, the relative reliability of the isometric H:Q ratio was moderate to high suggesting that individuals maintained their position within the cohort well (ICC= 0.70-0.90). However, de Carvalho Froufe Andrade et al. (2013) reported higher reliability (ICC= 0.87). Generally, good relative reliability was found for the isometric strength assessment of the individual muscle groups (ICC= 0.76-0.94). However, Maffiuletti et al. (2007) reported very high relative reliability (ICC> 0.97) for both knee extensors and flexors. This difference is likely due to the examination of a more diverse cohort of males and females with high between-subjects variability (CV, 27.5% (extensors) and 30.7% (flexors) vs 5.3% for both muscle groups at 120° in the current study) resulting in high ICC scores.

In conclusion, the protocol used in the present study produced consistent measurements between sessions of the functional, conventional and isometric H:Q ratio and the torque-velocity relationship of the knee extensors and flexors up to high angular velocities. The lower relative reliability compared to other studies may be attributed to the small homogenous sample size and the limited familiarisation allowed. The results of this study supported the further use of the current protocol in the examination of the strength balance between footballers and normal individuals (Chapter 4).

Chapter 4

Angle-specific hamstrings-to-quadriceps ratio. A comparison of football players and recreationally active males

4 CHAPTER 4 – ANGLE-SPECIFIC HAMSTRINGS-TO-QUADRICEPS RATIO. A COMPARISON OF FOOTBALL PLAYERS AND RECREATIONALLY ACTIVE MALES

4.1 INTRODUCTION

Hamstrings strains have been reported as one of the most common injuries in a variety of football codes, accounting for 12-16% of all injuries (Woods et al., 2004). Woods et al (2004) found an average of 90 training days and 15 matches were missed per club per season due to hamstrings strains. This type of injury is thought to occur during the late swing phase of sprinting when the hamstrings are at their peak stretch and working eccentrically to decelerate the shank (Heiderscheit et al., 2005).

Muscle imbalances and particularly hamstrings-to-quadriceps imbalances, have been widely suggested as potential risk factors for non-contact knee joint injuries and hamstrings strains (Yeung et al., 2009; Croisier et al., 2008, 2002). Hamstrings-to-quadriceps imbalances can be defined as disproportionately low hamstrings strength (maximal or explosive) relative to quadriceps strength (Hannah et al., 2014; Greco et al., 2013; Zebis et al., 2011). Furthermore, it has been shown that correcting muscle strength imbalances of football players decreased the incidence of hamstrings injuries during the subsequent season (Croisier et al., 2008). Regular football training and match play could induce an imbalance in hamstrings-to-quadriceps function. For example, football participation involves running and jumping on soft turf as well as frequent kicking both of which may promote disproportionate quadriceps development. However, it remains unclear if football players present a systematic hamstrings-to-quadriceps imbalance that predisposes them to hamstrings strains.

Muscle balance at the knee joint has typically been quantified by measuring the hamstrings-to-quadriceps peak torque ratio. Originally, it was calculated from the concentric peak torque of the two muscle groups, known as the conventional ratio. Later the functional ratio was introduced (Aagaard et al., 1995), which calculates the ratio of hamstrings peak eccentric to quadriceps peak concentric torque, and it is thought to better reflect the reciprocal antagonistic function of the muscles during athletic activities such as sprinting and kicking. However, the opposing quadriceps and hamstrings muscles exert their peak torque at different knee joint angles (~115° and ~150° respectively, 180°= full extension) (Knapik et al., 1983). This joint angle discrepancy inherent within any peak torque ratio may reduce the validity of the functional ratio to assess reciprocal antagonistic muscle function. Assessment of the knee flexors to extensors strength ratio at the same knee joint angle may provide a more functionally relevant measurement. This angle-specific functional strength ratio could be calculated throughout the range of motion, although measurements at extended knee joint positions, similar to those during the late swing phase, may be most relevant for hamstrings strain injury. It is possible that a hazardous muscle strength imbalance may be angle-specific and more pronounced at the extended knee joint positions. Therefore, monitoring the angle-specific muscle strength balance over the range of motion, and particularly at extended knee joint positions where injuries usually occur, may be crucial for the detection of any strength imbalances. However, there are no data on the knee joint angle-specific strength balance over the range of motion of either footballers or healthy, recreationally active population.

From the limited findings in the literature it is unclear how football participation influences hamstrings-to-quadriceps muscle balance, with evidence for disproportionate development of the knee extensors (Tourny-Chollet & Leroy, 2002; Iga et al., 2009) and the knee flexors (Cometti et al., 2001; Fousekis et al., 2010). A lower functional H:Q ratio at a range of different velocities has been reported for footballers compared to untrained males, with footballers presenting higher quadriceps concentric strength, but similar hamstrings eccentric strength to that of controls (Tourny-Chollet & Leroy, 2002; Iga et al., 2009). Based on these results it can be hypothesized that football participation develops quadriceps strength more than the hamstrings, leading to an imbalance that may predispose to injury. Contrary to this suggestion, two other studies have found professional players to have a higher functional H:Q ratio than players at a lower standard of competition (Cometti et al., 2001; Fousekis et al., 2010). Therefore, it is currently unclear how football participation affects knee joint muscle balance, and in particular the angle-specific functional ratio at extended joint positions remains unknown.

An angle-specific H:Q ratio across the range of motion and at a range of different velocities would provide a more complete understanding of the strength balance between the knee extensors and flexors and thoroughly examine whether footballers have a different muscle balance from that of normal, recreationally active individuals. Therefore the aim of this study was to compare the angle-specific H:Q ratios between football players and recreationally active controls up to high angular velocities.

4.2 METHODS

4.2.1 Participants

Ten male football players (age 21 ± 1 years, height 180.7 ± 6 cm, body mass 78 ± 8 kg, mean ± SD) and fourteen healthy, recreationally active males (age 25 ± 3 years, height 177 ± 5 cm, body mass 69.7 ± 7 kg, mean ± SD) volunteered to take part in this study. The football players were members of Loughborough University's 1st team which competed in Midlands Football Alliance (9th tier of English football) and BUCS Premier League (3rd place for 2011-12), and had on average 8.5 ± 6 years of experience in football practice and competition. They completed 4-5 football training sessions and 1-2 matches per week. The players also performed limited strength training; 1-2 times per month during the season and up to 8 times per month during pre-season. All testing sessions for the football players took place during the season. None of the control group participants were involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. The physical activity of the control group was assessed using the International Physical Activity Questionnaire short format [www.ipaq.ki.se/downloads.htm, (Craig et. al., 2003)] and their average energy expenditure was 1850 ± 1138 MET-minutes/week. Participants completed physical activity and health screen questionnaires before providing written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no history of musculo-skeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit.

4.2.2 Overview

All testing sessions were performed in the afternoon and each participant visited the laboratory at a consistent time of day on two occasions 7 days apart. These sessions involved unilateral measurements of the dominant leg (defined as the preferred leg when kicking a ball), specifically knee flexor and extensor strength assessed with an isokinetic dynamometer (Con-Trex MJ, CMV AG, Duebendorf, Switzerland). The first session involved anthropometric measurements and isometric knee flexor and extensor assessment, as well as familiarisation with the concentric and eccentric measurements of both muscle groups. The second session involved concentric and eccentric strength measurements of both muscle groups. Video images were recorded during the isometric contractions in session 1 and used to calculate the knee joint angle-crank angle relationships during extension and flexion contractions. These relationships were used to calculate knee joint angle from crank angle during the dynamic contractions.

4.2.3 Dynamometer Procedures

The participants were seated on the dynamometer chair with a hip angle of 120° (180°= full extension). This hip angle was selected because of its relevance to high injury risk situations i.e. similar to the hip angle during late swing phase in sprinting (Guex et al., 2012) when hamstrings strains are thought to occur. Two 3-point belts secured the torso and additional straps secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint with the dynamometer rotational axis during active muscle contractions was done separately for knee extension and flexion contractions. Specifically, in each case the alignment was done during isometric contractions of >50% MVF at a knee joint angle of ~115°. The dynamometer's shin brace was placed ~2 cm above the medial malleolus, anterior to the shank for knee extension contractions and posterior for knee flexion contractions, prior to the shank being tightly secured to the dynamometer lever arm. During the knee extension contractions, an additional moulded rigid plastic shin pad, lined with 2 mm of high density foam, was tightly secured to the tibia to avoid any discomfort to the shin during maximum contractions. The range of motion was established and anatomical zero was set at the most extended position where participants felt comfortable and without excessive stretch of their hamstrings. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque by the dynamometer software. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen. Torque, crank angle and crank angular velocity were recorded at 512 Hz during all contractions.

4.2.4 Isometric Peak Torque assessment

Measurements were recorded first with the knee flexors and then the knee extensors. Prior to the recorded contractions for each muscle group, participants completed a standardized warm-up consisting of a progressive series of submaximal contractions. For the assessment of peak isometric torque of each muscle group, participants performed two sets of five maximum contractions, one at each of five different crank angles (165°, 150°, 135°, 120° and 105° in a randomized order; 180°= full extension). Participants were instructed to "push" or "pull" as hard and as fast as possible for 3-5 s. One-minute rest was given between each contraction, with 2 min between sets and 5 min between muscle groups.

4.2.5 Dynamic Peak Torque assessment

Initially, participants performed a standardized warm-up protocol with five submaximal contractions of progressively higher intensity. Following the warm-up, first the knee extensors were tested for their isovelocity torque at three velocities, and then the knee flexors were also tested at the same velocities. This involved a protocol of concentric-eccentric contractions at low (60° s^{-1}), medium (240° s^{-1}) and high (400° s^{-1}) angular velocities in this order. At each velocity participants performed 2 sets of 2 (60° s^{-1}), 3 (240° s^{-1}) or 5 (400° s^{-1}) concentric-eccentric contractions over approximately 80-85° of range of motion. A minimum of one-minute rest was given between each set, with 2 min between velocities and 5 min between muscle groups.

4.2.6 Data Analysis

4.2.6.1 Peak Torque

The isometric contraction with the highest torque at each crank angle was chosen for further analysis. Isometric peak torque was defined as the average over a 500 ms epoch around (250 ms either side) the instantaneous highest torque. The concentric and eccentric contractions at each velocity with the highest torque and isovelocity range were chosen for further analysis. In order to control for the torque overshoot during the acceleration and deceleration phases (Schwartz et al., 2010), data during these phases were excluded and the constant isovelocity period (within ±10% of the prescribed crank angular velocity, Baltzopoulos et al., 2012) was identified. Peak torque was calculated by averaging the torque values over a 1-2° range of angles around the highest recorded torque value.

4.2.6.2 Angle-specific torque

The isometric torque-knee joint angle data for each muscle group was smoothed by performing 2nd order polynomial fitting to the raw torque values. Then the polynomial fit was used to interpolate torque values for knee joint angles at 105, 120, 135, 150 and 165°. The isovelocity torque-knee joint angle data at each velocity, for each muscle group was smoothed by performing Gaussian fitting (Forrester et al., 2011) using a root mean square method to minimise the error to the raw torque values (Matlab, The Mathworks, Inc., Natick, MA, USA). Then the Gaussian fit was used to interpolate torque values for knee joint angles every 5° over the relevant isovelocity range for each angular velocity: 100-160° for 60° s^{-1}; 105-160° for 240° s^{-1}; and 115-145° for 400° s^{-1}. Data from contractions in which participants failed to maximally activate the examined muscle group throughout the range of motion were discarded.

4.2.6.3 Knee joint angle

In order to account for the dynamometer compliance and the position change of the knee joint relative to the dynamometer crank during testing, the actual knee joint angle was determined during the isometric contractions. A video camera (Panasonic NV-GS200 mini-DV, Japan) was used to record sagittal plane images at a sampling rate of 50 Hz. The camera was positioned ~2.5 m perpendicular to the dynamometer and mounted on a tripod at a height of ~2.2 m in order to have an unobstructed view of the knee joint. Joint centres were identified with 2 cm diameter circular marks drawn on the surface of the hip (greater trochanter), knee (lateral collateral ligament just below the lateral femoral epicondyle) and ankle (lateral malleolus of the fibula) joints. The knee joint angle was measured from the coordinates of the three anatomical reference points during each participant's best isometric contraction at each angle. The camera tilt relative to the plane of movement, introduced a systematic error to the knee joint angle measurements. To quantify this error, the horizontal and vertical sides of a right angle with known dimensions that was on the plane of movement, were digitised and used as a scaling factor. The error was found to be on average ±6° (range= 0-14°) over a 90° range of motion (90-180°, 180°= full extension). However, this systematic error was not expected to invalidate the comparison of the angle-specific torque and the H:Q ratios between the examined cohorts as the same camera position was replicated throughout the measurements. The measured knee joint angles were plotted against the respective crank angles and a quadratic equation was fitted in order to generate a knee joint angle-crank angle relationship for each muscle group. These relationships facilitated conversion of crank angles recorded during all contractions (isometric, concentric and eccentric) to actual knee joint angles. The coefficient of determination for these relationships (knee joint angle vs. crank angle), calculated for each muscle group of each participant, were very high ($0.9729 \leq R^2 \leq 1$), however on average the hamstrings regression line was slightly steeper than that of the quadriceps.

4.2.6.4 Isometric Hamstrings-to-Quadriceps ratio

The isometric hamstrings-to-quadriceps ratio ($H:Q_{isom}$) was calculated by dividing the hamstrings torque at each knee joint angle by the quadriceps torque at the same angle.

4.2.6.5 Functional Hamstrings-to-Quadriceps ratio

The non-angle specific dynamic hamstrings-to-quadriceps functional ratio ($H:Q_{func}$) was calculated by dividing the hamstrings eccentric peak torque (H_{ecc}) at each angular velocity by the quadriceps concentric peak torque (Q_{con}) at the same velocity. Peak torque was independent of knee joint angle, and thus the measurements of each muscle group were made at different angles.

$$H:Q_{func} = H_{ecc} / Q_{con}$$

The dynamic angle-specific hamstrings-to-quadriceps functional ratio ($H:Q_{func\theta}$) was calculated by dividing the hamstrings eccentric torque ($H_{ecc\theta}$) at each angular velocity and knee joint angle by the quadriceps concentric torque ($Q_{con\theta}$) at the same velocity and knee joint angle.

$$H:Q_{func\theta} = H_{ecc\theta} / Q_{con\theta}$$

4.2.6.6 Conventional Hamstrings-to-Quadriceps ratio

The non-angle specific hamstrings-to-quadriceps conventional ratio (H:Qconv) was calculated by dividing the hamstrings concentric peak torque (Hcon) by the quadriceps concentric peak torque (Qcon) at the same angular velocity. Peak torque was independent of knee joint angle, and therefore the measurements of each muscle group were made at different angles.

$$H:Q_{conv} = Hcon / Qcon$$

The angle-specific hamstrings-to-quadriceps conventional ratio ($H:Q_{conv\theta}$) was calculated by dividing the hamstrings concentric angle-specific torque ($H_{con\theta}$) at each angular velocity and knee joint angle by the quadriceps concentric angle-specific torque ($Q_{con\theta}$) at the same velocity and knee joint angle.

$$H:Q_{conv\theta} = H_{con\theta} / Q_{con\theta}$$

4.2.7 Statistical Analysis

Group data are presented as mean ± SD. Torque values were normalized to body mass to compare the two groups (Folland et al., 2008). A two-way repeated measures analysis of variance (ANOVA) was used to determine if there were differences between groups for angle-specific torque (groups x angle) and angle-specific H:Q ratios (groups x angle). For the non-angle-specific torque and ratios a two-way repeated measures ANOVA was used (groups x velocity). The assumption of sphericity was assessed with Mauchly's test and the Greenhouse-Geisser correction was applied when needed. When differences were found by ANOVA, independent *t*-test with Holm-Bonferonni correction of the *P* level of significance for multiple comparisons was used as a post-hoc test. A $P < 0.05$ level of significance was used for all comparisons. The effect sizes were calculated using the Cohen's *d* statistic. All statistical procedures were performed with IBM SPSS 19 (IBM Corporation, Armonk, NY).

4.3 RESULTS

4.3.1 Anthropometric characteristics

Football players had higher body mass compared to the controls (78 ± 8 vs. 69.7 ± 7 kg, t_{22}= -2.37, *P*= 0.027), but there were no differences in height between the groups, (footballers: 180.7 ± 6 cm; controls: 177 ± 5 cm, t_{22}= -0.37, *P*= 0.73).

4.3.2 H:Q ratios

The angle-specific isometric H:Q ratio was not different between groups, $F_{1,22}$= 0.71, *P*= 0.41 (Fig. 4.1; Table 4.1). Furthermore, the angle-specific functional H:Q ratio was similar for both groups at all three velocities (0.12 < *P* < 0.50) (Fig. 4.2). This was also the case for the angle-specific conventional H:Q ratio with no differences between the groups at any velocity (0.055 < *P* < 0.612) (Fig. 4.3). In addition, when non-angle-specific functional and conventional H:Q ratios were compared there were also no differences between groups ($F_{1,22}$= 0.14, *P*= 0.71 and $F_{1,22}$= 0.15, *P*= 0.71 respectively) (Fig. 4.4).

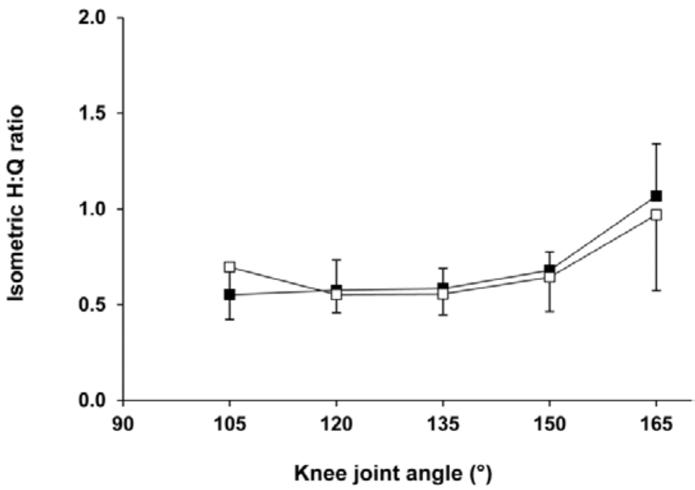

Figure 4.1. Angle-specific isometric H:Q ratio for footballers (filled squares, n=10) and controls (open squares, n= 14). Data are presented as mean ± SD.

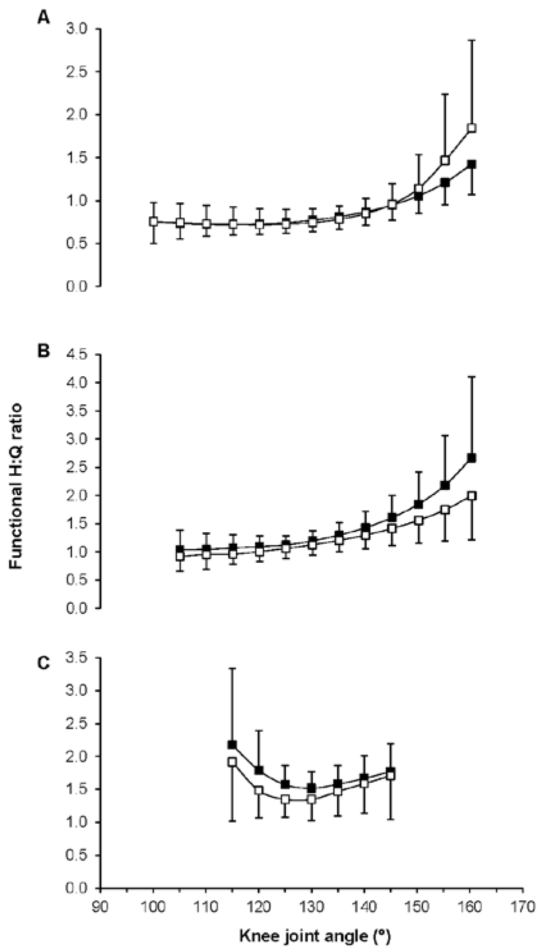

Figure 4.2. Angle-specific functional H:Q ratio for footballers (Fb, filled squares) and controls (Con, open squares) at: (A) 60° s^{-1} (Fb, n= 10, Con, n= 14), (B) 240° s^{-1} (Fb, n= 9; Con, n= 13) and (C) 400° s^{-1} (Fb, n= 10, Con, n= 14). Data are presented as mean ± SD.

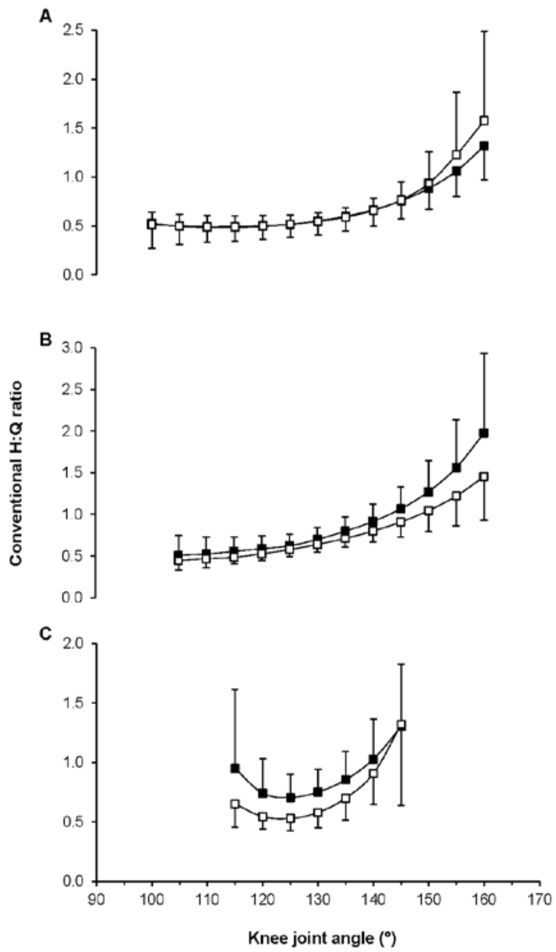

Figure 4.3. Angle-specific conventional H:Q ratio for footballers (Fb, filled squares) and controls (Con, open squares) at: (A) 60° s^{-1} (Fb, n= 10, Con, n= 14), (B) 240° s^{-1} (Fb, n= 9; Con, n= 13) and (C) 400° s^{-1} (Fb, n= 8, Con, n= 10). Data are presented as mean ± SD.

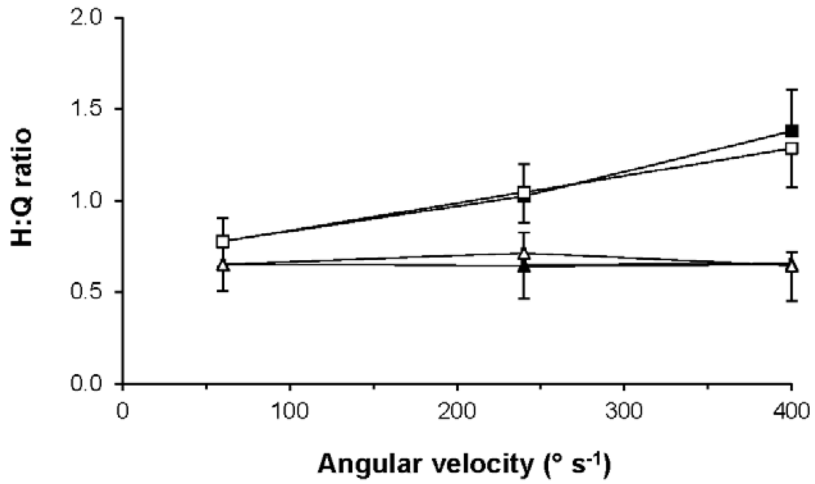

Figure 4.4. Non angle-specific functional (squares) and conventional (triangles) H:Q ratio for footballers (filled symbols, n= 10) and controls (open symbols, n= 14). Data are presented as mean ± SD.

4.3.3 Angle-specific torque

No differences were found between the groups for angle-specific knee extensors torque relative to body mass during isometric ($F_{1,22}$= 0.036, *P*= 0.85), concentric (0.29 < *P* < 0.75) (Fig. 4.5A-C) or eccentric (0.21 < *P* < 0.61) (Fig. 4.5D-F) contractions. Similarly, no differences between groups were found for knee flexors concentric and eccentric angle-specific torque relative to body mass at 60° s^{-1} and 240° s^{-1} (0.1 < *P* < 0.321) as well as for the isometric contractions ($F_{1,22}$= 0.09, *P*= 0.76). However, footballers presented on average a 1.4 fold greater knee flexors concentric torque relative to body mass at 400° s^{-1}, with higher values at all knee joint positions (*P* < 0.01), compared to the control group (Fig. 4.6C). A main effect was also found at the same velocity for the knee flexors eccentric angle-specific torque relative to body mass ($F_{1,22}$= 5.939, *P*= 0.023) but the post-hoc comparisons did not reveal any differences in this measure at specific joint positions.

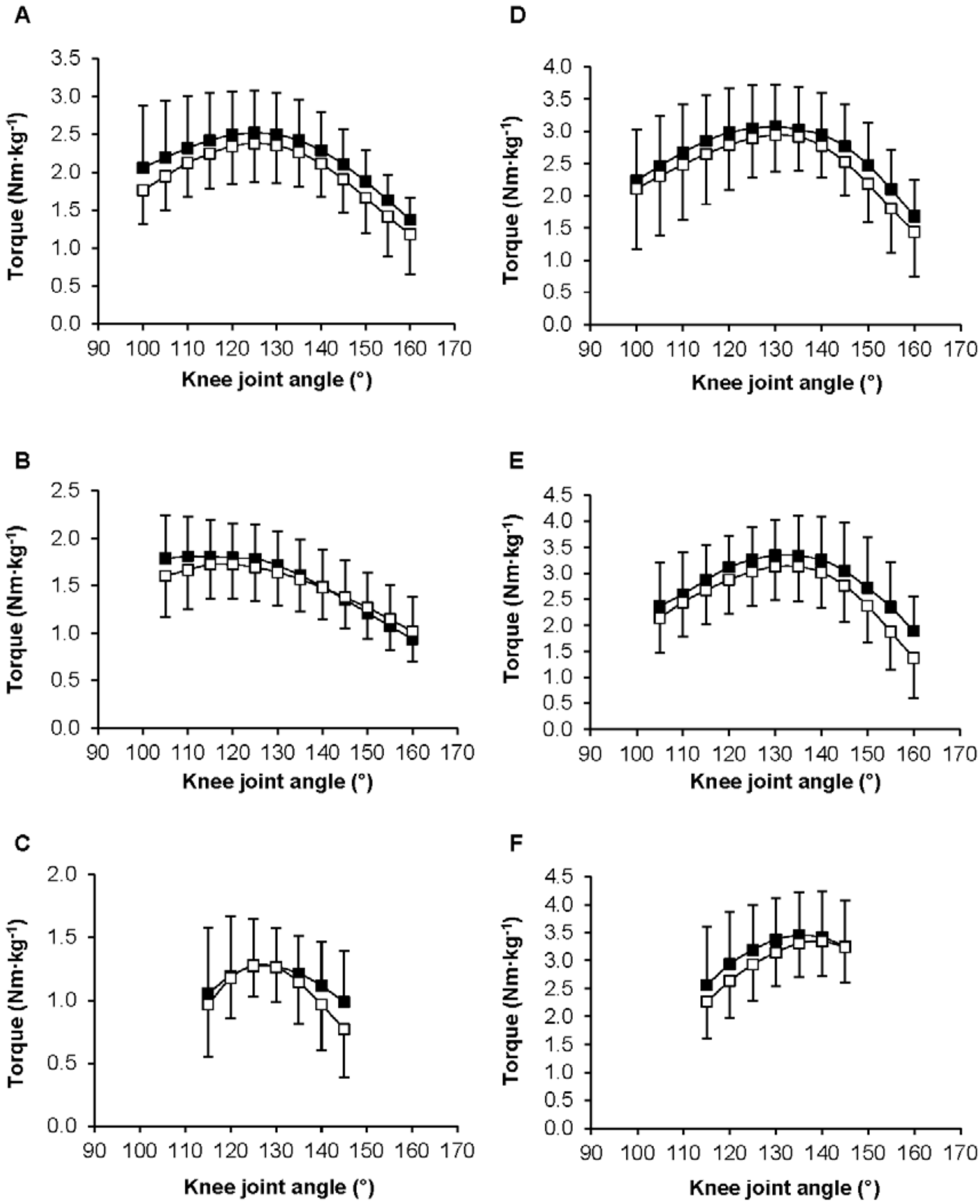

Figure 4.5. Knee extensors angle-specific torque relative to body mass for footballers (Fb, filled squares) and controls (Con, open squares) at concentric (A) 60° s^{-1} (Fb, n= 10; Con, n= 14), (B) 240° s^{-1} (Fb, n= 9; Con, n= 13), (C) 400° s^{-1} (Fb, n= 10; Con, n= 14), and eccentric (D) 60° s^{-1} (Fb, n= 10; Con, n= 14), (E) 240° s^{-1} (Fb, n= 9; Con, n= 14), (F) 400° s^{-1} (Fb, n= 10; Con, n= 14). Data are presented as mean ± SD.

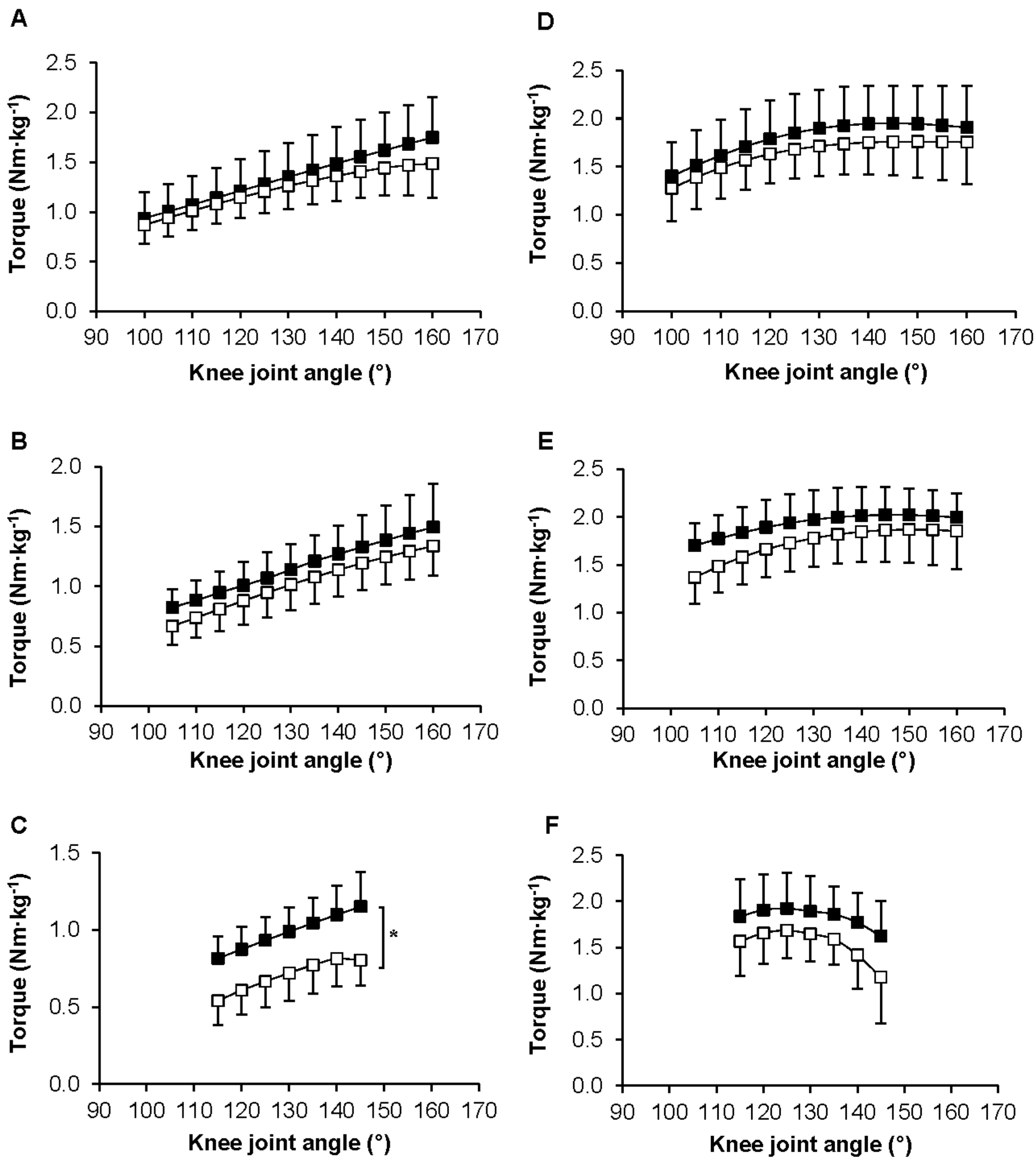

Figure 4.6. Knee flexors angle-specific torque relative to body mass for footballers (Fb, filled squares) and controls (Con, open squares) at concentric (A) 60° s^{-1} (Fb, n= 10; Con, n= 14), (B) 240° s^{-1} (Fb, n= 10; Con, n= 14), (C) 400° s^{-1} (Fb, n= 8; Con, n= 10), and eccentric (D) 60° s^{-1} (Fb, n= 10; Con, n= 14), (E) 240° s^{-1} (Fb, n= 10; Con, n= 14), (F) 400° s^{-1} (Fb, n= 10; Con, n= 14). Data are presented as mean ± SD. *: $P < 0.01$.

Table 4.1. *P*-values, confidence intervals (CI) and effect sizes for the differences between footballers (Fb) and controls (Con).

Variable			*P*-value	95% CI for difference between Fb and Con: Lower limit	Upper limit	Effect size (Cohen's *d*)
Angle-specific isometric H:Q ratio			0.410	-0.106	0.249	0.36
Angle-specific H:Q ratio	Functional	$60° s^{-1}$	0.503	-0.213	0.108	0.29
		$240° s^{-1}$	0.117	-0.053	0.446	0.75
		$400° s^{-1}$	0.313	-0.167	0.499	0.45
	Conventional	$60° s^{-1}$	0.612	-0.183	0.110	0.22
		$240° s^{-1}$	0.055	-0.004	0.305	0.93
		$400° s^{-1}$	0.159	-0.065	0.364	0.74
Non angle-specific H:Q ratio		Functional	0.713	-0.114	0.184	0.16
		Conventional	0.706	-0.123	0.085	0.17
Angle-specific isometric torque (relative to body mass)		Knee extensors	0.796	-0.564	0.438	0.11
		Knee flexors	0.153	-0.051	0.305	0.64
Angle-specific torque (relative to body mass)	Knee extensors, Concentric	$60° s^{-1}$	0.258	-0.154	0.544	0.50
		$240° s^{-1}$	0.753	-0.250	0.340	0.14
		$400° s^{-1}$	0.487	-0.157	0.320	0.31
	Knee extensors, Eccentric	$60° s^{-1}$	0.334	-0.217	0.612	0.43
		$240° s^{-1}$	0.210	-0.170	0.732	0.58
		$400° s^{-1}$	0.444	-0.318	0.702	0.34
	Knee flexors, Concentric	$60° s^{-1}$	0.321	-0.116	0.340	0.44
		$240° s^{-1}$	0.124	-0.041	0.320	0.69
		$400° s^{-1}$	0.002	0.116	0.440	1.83
	Knee flexors, Eccentric	$60° s^{-1}$	0.232	-0.112	0.438	0.53
		$240° s^{-1}$	0.100	-0.042	0.447	0.74
		$400° s^{-1}$	0.023	0.045	0.554	1.05

4.4 DISCUSSION

The main finding of this study was that the H:Q ratios were not different between healthy football players and recreationally active males. The focus of this study was on the angle-specific functional ratio, but neither this measurement nor any isometric, functional or conventional ratio showed any differences between the two groups. These similar ratios reflected the fact that the two groups exhibited similar angle-specific torque relative to body mass for both the knee extensors and flexors at all speeds and contraction types. The only exception was the higher concentric hamstrings torque exhibited by the football players at the highest velocity (400° s^{-1}).

In the present study, the functional angle-specific H:Q ratios of the footballers were similar to the controls throughout the range of motion. The current study considered angle-specific ratios, and calculated actual knee joint angles, rather than simply assuming the dynamometer crank angle reflected the knee joint angle as many previous studies have done (Pavol & Grabiner, 2000; Aagaard et al., 1998, 1995). Therefore, we consider the torque-angle relationships of each muscle group assessed in this study and the subsequent angle-specific ratios to provide a more robust comparison of knee joint muscle function for the two groups.

The functional H:Q ratio for peak torque values found in the current study was similar to previous reports. In particular, at 60° s^{-1} the footballers in the current study had a functional H:Q ratio of 0.78 ± 0.13, and previous studies have found a functional H:Q ratio of 0.79-0.85 for professional players (Fousekis et al., 2010) and 0.80 for national level amateur players (Tourny-Chollet & Leroy, 2002). For higher angular velocities, direct comparison of our results with the existing literature is difficult due to the different velocities used. Contrary to the results of the present study, it has been previously reported that football players have lower functional H:Q ratio compared to healthy untrained individuals (Tourny-Chollet & Leroy, 2002; Iga et al., 2009) and this was attributed to a disproportionally higher quadriceps concentric than hamstrings eccentric strength for the footballers compared to controls. The explanation for the contrasting findings of the current study with these previous findings is unclear. However, two other studies found professional players with a more extensive history of participation to have a higher functional H:Q ratio than players at a lower standard of competition (Fousekis et al., 2010; Cometti et al., 2001). This supports our finding that football participation does not seem to have a detrimental influence on H:Q ratio.

Besides the similar H:Q ratios between the footballers and the controls found in this study, with the exception of the knee flexors concentric torque at 400° s^{-1}, there were no differences in the actual torque values relative to body mass between the groups. This is in contrast to other studies which have reported that footballers had higher concentric torque for both knee extensors and flexors compared to controls (Tourny-Chollet & Leroy, 2002; Ergun et al., 2004). A possible explanation for the discrepancy in the results of the present study with previous findings may be the reference values to which the footballers were compared. In the present study, healthy recreationally active participants served as the reference group while in the above mentioned studies sedentary participants were used. The control group in the present study was stronger than that in the study of Tourny-Chollet & Leroy (2002) as they had higher normalized to body mass torque for knee extensors and flexors in both concentric and eccentric contractions at comparable angular velocities. Therefore, the selection of sedentary participants for the control group may have affected their results, as a sedentary lifestyle has been linked to reduced thigh muscle strength in healthy young adults (Manini et al., 2007).

The lack of differences in strength relative to body mass between the footballers and the control group in the current investigation may also be partly explained by the fact that the footballers were tested during the season but their strength training was performed mainly during the pre-season with only a session of strength training every two weeks thereafter. This frequency is below the American College of Sports Medicine's (ACSM) recommendations for strength development (ACSM position stand, 2011). However, the strength level of the footballers examined in this study was comparable to that reported for players of similar standard (Newman et al., 2004) but lower compared to the strength level of professionals (Fousekis et al., 2010). The only difference in strength between groups was found for the knee flexors concentric torque at the highest velocity (400° s^{-1}) with the footballers being stronger than the controls. Differences in muscle composition (i.e more type II fibres, Aagaard and Andersen, 1998) and/or the greater familiarity of the players with high speed movements could potentially explain this difference. However, overall it seems that football training and match play were not sufficient to increase the footballers' strength in relation to body mass when compared to controls.

The most established risk factor for hamstrings injury is a prior hamstrings injury (Orchard, 2001, Hagglund et al., 2006; Engebretsen et al., 2010) and previously injured athletes have

>2 times higher risk of sustaining a future hamstrings injury (Bennel et al., 1998; Verrall et al., 2001; Engebretsen et al., 2010). Moreover, athletes with a history of hamstrings injury often have a low functional H:Q ratio compared to their uninjured leg (Croisier et al., 2002). However, it is not clear whether the low H:Q ratio is the result or the cause of the previous injury. In the current study, in order to control for the confounding influence of previous injury on the H:Q ratio, players with a history of hamstrings injury were excluded. Consequently, if a low H:Q ratio is a risk factor for hamstrings injury, the exclusion of previously injured players may have inadvertently selected a group of players with a better muscle balance and a 'normal' H:Q ratio. Despite these issues, an extensive history of football training and competition was not associated with any differences in muscle balance within the footballers of this study.

The generalization of the results presented in this study is limited by the small sample size examined. The standard of competition of the football players could potentially have also influenced the findings. The physical and technical demands increase with the standard of play and players who are regularly exposed to such conditions may exhibit more pronounced muscle strength gains and potentially a different muscle balance. The effect of level of play was highlighted in the study of Cometti et al. (2001) where professional footballers had stronger knee flexors, especially under eccentric conditions, and higher functional and conventional H:Q ratios compared to amateur players. Interestingly, they found no differences in knee extensors concentric strength at any angular velocity between the groups. Similarly, Fousekis et al. (2010) found that players with a longer experience in professional football (≥11 years) exhibited higher functional H:Q ratio compared to players with intermediate (8-10 years) and short (5-7 years) experience. In that study, the less experienced players had lower concentric strength for the knee extensors at 60° s^{-1} and lower concentric and eccentric strength for the knee flexors at 60° $s^{-1}$and 180° s^{-1} than the more experienced players. It seems that experienced professional players at a high level exhibit a better strength balance around the knee joint and stronger knee flexors than the lower level players.

The problem of hamstrings susceptibility to strain injuries is of multi-factorial nature. Prospective studies have identified a range of modifiable and non-modifiable risk factors including thigh muscle strength imbalances, muscle fatigue, flexibility, inadequate warm-up, older age, ethnicity and previous leg injuries (in addition to previous hamstrings strains) (Hagglund et al., 2013; Henderson et al., 2010; Gabbe et al., 2006, 2005; Arnason et al., 2004; Orchard et al., 2001; Verrall et al., 2001). Since knee joint muscle imbalances do not seem to be present in previously uninjured footballers, other factors must contribute to the high hamstrings injury rates seen in football. Regular exposure to football training and matches, that involve extensive sprinting and kicking, is likely to be important in the high incidence of hamstrings strains. Muscle fatigue has been proposed as a possible factor as half of the hamstrings injuries sustained during matches occur near the end of each half (Woods et al., 2004). Recent studies using protocols that induced soccer-specific fatigue found decreased functional H:Q ratios and reduced hamstrings eccentric torque at the end of each half (Small et al., 2010; Delextrat et al., 2010; Greig et al., 2008).

In conclusion, in previously uninjured football players there was no intrinsic muscle imbalance and the high rate of hamstrings injuries seen in this sport may be due to other risk factors and/or simply regular exposure to a high risk activity (football training & match play).

Chapter 5

Quadriceps and hamstrings relative muscle size influences knee-joint strength balance

5 CHAPTER 5 – QUADRICEPS AND HAMSTRINGS RELATIVE MUSCLE SIZE INFLUENCES KNEE-JOINT STRENGTH BALANCE

5.1 INTRODUCTION

Evidence from prospective studies suggests that athletes with strength imbalances between agonists and antagonists in the upper or lower body musculature may be at an increased risk of injury (Byram et al., 2010; Croisier et al., 2008). For the knee joint, such imbalances are typically measured with the hamstrings-to-quadriceps (H:Q) maximal strength ratio, and a low ratio is thought to indicate weakness of the knee flexors relative to the knee extensors (Yeung et al., 2009; Croisier et al., 2008, 2002). The H:Q ratio appears to be important as it has been found to contribute to a substantially increased risk of hamstrings strain injury (Croisier et al., 2008). Despite the utility of the H:Q strength ratio in the examination of strength imbalances and potential prevention of strain injuries, there is little understanding of which factors influence this ratio.

Muscle size, expressed either as volume, anatomical or physiological cross-sectional area, is well established as a primary determinant of maximal strength for different muscles (Fukunaga et al., 2001; Bamman et al., 2000), and it would be expected that the relative size of antagonistic muscles, quadriceps and hamstrings, would directly influence their respective strength balance. To our knowledge how the size of the agonists relates to that of the antagonists muscles has not been documented, and it is therefore unclear how quadriceps size relates to hamstrings size. Furthermore, it is unclear whether the size ratio of these muscles influences their strength ratio. The plantarflexor:dorsiflexor volume ratio has been found to influence the isometric strength ratio of these muscles ($r = 0.61$-0.62, $P < 0.01$; Akagi et al., 2014, 2012). However, both these studies found no relationship between the H:Q muscle volume ratio and the strength ratio of these muscles (Akagi et al., 2014, 2012). These investigations examined only the isometric H:Q strength ratio of the knee extensors and flexors. While isometric strength is a convenient measure of a muscle's strength capacity, isometric measurements do not reflect the reciprocal functional activity of these muscles, where a forceful concentric contraction of one muscle is followed by a forceful eccentric contraction of the antagonist. For example, during the late swing phase of sprinting, when hamstrings strains are thought to occur (Chumanov et al., 2012), a forceful eccentric contraction of the knee flexors is required to decelerate the shank after a forceful concentric contraction of the knee extensors. Furthermore, due to the force-velocity relationship and the high knee joint velocities involved in sprinting, the knee flexor eccentric strength is expected to remain relatively constant at high velocities compared to isometric strength while concentric strength is expected to decrease significantly (Pain et al., 2013; Kellis and Baltzopoulos, 1998). Isokinetic dynamometers clearly cannot replicate the knee joint velocities experienced during sprinting, yet the use of the knee flexors eccentric to knee extensors concentric strength (functional H:Q ratio) up to high velocities seems to provide a more functionally relevant assessment of the H:Q strength balance (Aagaard et al., 1998, 1995; Dvir et al., 1989). Moreover, to date how the relative size of the knee extensors (quadriceps) and flexors (primarily hamstrings) influences their functional H:Q strength ratio has not been examined.

The aim of the present study was to examine the relationship between knee extensors (quadriceps) and flexors (hamstrings) muscle size (volume and anatomical cross-sectional area), the association of each muscle's size with its strength, and investigate if the muscle size ratio was related to the isometric and functional strength ratios. Based on previous observations of a strong relationship between the size and strength of individual muscles, we hypothesized that the H:Q muscle size ratio would be positively correlated to their functional strength ratio.

5.2 METHODS

5.2.1 Participants

Thirty-one healthy, recreationally active participants (age 20.6 ± 2.5 years; height 1.80 ± 0.07 m; body mass 71.8 ± 7.3 kg; mean ± SD) took part in this study. Participants had a low to moderate level of physical activity and were not involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. Their physical activity was assessed with the International Physical Activity Questionnaire short format (www.ipaq.ki.se/downloads.htm, Craig et. al., 2003) and their average energy expenditure was 1739 ± 814 metabolic equivalent-minutes per week. After completing the physical activity and health screen questionnaires, participants provided written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no history of musculo-skeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit.

5.2.2 Overview

Participants visited the laboratory on six separate occasions, seven days apart at a consistent time of the day (11:00-16:00 h) and all measurements were performed on the participants' dominant leg (defined as the preferred leg when kicking a ball). The first session involved the recording of the anthropometric data and familiarization with the procedures for the knee extension and flexion isometric strength testing which was conducted in the second session. During the third and fourth sessions, participants were familiarized with the isovelocity contractions and concentric and eccentric strength was measured in the fifth session. Finally, the sixth session involved magnetic resonance imaging (MRI) of the participants' thigh to assess quadriceps and hamstrings muscle size.

5.2.3 Measurements and Data analysis

5.2.3.1 Dynamometer procedures

The participants were seated on the dynamometer chair (Con-Trex MJ, CMV AG, Dübendorf, Switzerland) with a hip angle of 120° (180°= full extension). This hip angle is similar to that during late swing phase in sprinting (Guex et al., 2012) and was the most reclined position that could be obtained without participants sliding forwards during contractions. Two 3-point belts secured the torso and additional straps tightly secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint with the dynamometer rotational axis during active muscle contractions was done separately for knee extension and flexion contractions. Specifically, in each case the alignment was done during isometric contractions of >50% of isometric strength at a knee joint angle of ~115°. The dynamometer's shin brace was placed ~2 cm above the medial malleolus, anterior to the shank for knee extension contractions and posterior for knee flexion contractions, before the shank was tightly secured to the dynamometer lever arm. During the knee extension contractions, an additional moulded rigid plastic shin pad, lined with 2 mm of high density foam, was tightly secured to the tibia to avoid any discomfort to the shin during maximum contractions. The range of motion was established and anatomical zero was set at full extension of the knee joint. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque. For both isometric and isovelocity measurements, the knee flexors were assessed first and then the knee extensors.

5.2.3.2 Isometric Strength

Prior to the recorded contractions for each muscle group, participants completed a standardized warm-up consisting of a progressive series of submaximal contractions. For the assessment of peak isometric torque of each muscle group, participants performed two sets of three maximum contractions, one at each of three different crank angles (105°, 120°, 135° for knee extensors and 165°, 150°, 135° for knee flexors at this order; 180°= full extension). Participants were instructed to "push" or "pull" as hard and as fast as possible for 3-5 s. One-minute rest was given between each contraction, with 2 min between sets and 5 min between muscle groups. The contraction with the highest torque irrespective of crank angle was selected for further analysis. Isometric strength was defined as the average torque over a 0.5 s period around the highest instantaneous torque.

5.2.3.3 Concentric and eccentric strength

After the completion of a standardized warm-up protocol with five submaximal contractions of progressively higher intensity, participants performed 3 sets of 2, and 3 sets of 3, concentric-eccentric contractions at 50° s^{-1} and 350° s^{-1} respectively (in this order), over ~100° of range of motion. There was ≥1 min rest between each set and ≥2 min rest between velocities. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen.

The torque, crank angle and crank velocity signals were sampled at 2000 Hz with a PC using Spike 2 software (CED, Cambridge, UK) and smoothed with a finite impulse response filter at 15 Hz. The acceleration and deceleration phases were excluded in order to disregard torque overshoot during these phases (Schwartz et al., 2010) and the constant isovelocity period (within ±5% of the prescribed crank angular velocity) was identified. Finally, concentric and eccentric strength was defined as the highest instantaneous torque recorded within the isovelocity range of any concentric and eccentric contraction respectively.

5.2.3.4 Magnetic resonance imaging (MRI)

A 1.5 T MRI scanner (Signa HDxt, GE) was used to scan the dominant leg in the supine position with the hip and knee joints extended. T1-weighted axial plane images were acquired from the anterior superior iliac spine to the knee joint space in two blocks and oil filled capsules were placed on the lateral side of the participants' thigh to help with the alignment of the blocks during analysis. The following imaging parameters were used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm. MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland).

MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland). The hamstrings (biceps femoris long head, biceps femoris short head, semitendinosus, semimembranosus) and quadriceps (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) muscles were manually outlined in every third image starting from the most proximal image in which the muscle appeared. The largest anatomical cross-sectional area of each muscle was defined as ACSAmax and muscle volume was calculated using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). Two investigators conducted the image analysis and all manual segmentation measurements of each muscle were completed by the same investigator. To examine the reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later and the coefficient of variation (CV) was calculated. The CVs for measurements of muscle volume and ACSAmax were 0.5% and 1.2% (quadriceps), and 0.5% and 1.1% (hamstrings).

5.2.4 Statistical analysis

Data are presented as mean ± SD. Strength differences between muscle groups were examined with a two-way repeated measures ANOVA (muscle x velocity). A significant main effect was further examined with a post-hoc paired *t*-test with Holm-Bonferroni correction. Bivariate relationships were examined using Pearson product moment correlations between the dependent variables and the Holm-Bonferroni correction was used to control for multiple tests. The level of significance was set at $P < 0.05$. All statistical procedures were performed with IBM SPSS 22 (IBM Corporation, Armonk, NY).

5.3 RESULTS

5.3.1 Descriptive data for muscle size and strength

On average quadriceps had a ~2.5-fold larger muscle volume than hamstrings (1937.3 ± 265.1 cm^3 and 794.1 ± 122.2 cm^3 respectively, Table 5.1) and both muscle groups exhibited moderate variability between individuals (CV, 13.7% [Q] and 15.4% [H]). Consequently the H:Q volume ratio was 0.41 ± 0.05 (CV= 11.5%). The difference in size between the opposing muscle groups was smaller when considering ACSAmax (< 2-fold) that was reflected by a H:Q ACSAmax ratio of 0.52 ± 0.06 (Table 5.1).

Table 5.1. Descriptive data of the quadriceps and hamstrings muscle size measurements and the hamstrings-to-quadriceps muscle size ratio (H:Q) (n= 31).

	Muscle size variable	Mean ± SD	Range	CV(%)
Quadriceps	**Volume (cm^3)**	1937.3 ± 265.1	1489.6 – 2649.9	13.7
	ACSAmax (cm^2)	93.8 ± 10.4	75.3 – 118.3	11.0
Hamstrings	**Volume (cm^3)**	794.1 ± 122.2	581.1 – 1065.6	15.4
	ACSAmax (cm^2)	48.9 ± 6.6	36.3 – 63.3	13.5
H:Q	**volume ratio**	0.41 ± 0.05	0.34 – 0.51	11.5
	ACSAmax ratio	0.52 ± 0.06	0.43 – 0.65	11.4

The knee extensors were stronger than flexors across the torque-velocity relationship (P< 0.001, Fig. 5.1A). The highest torque occurred isometrically for knee extensors, but eccentrically (50° s^{-1}) for knee flexors. When normalized to isometric values the shape of the torque-velocity relationships was quite distinct for the two muscle groups, with the knee flexors achieving higher concentric and eccentric torques than the extensors (P< 0.05, Fig. 5.1B).

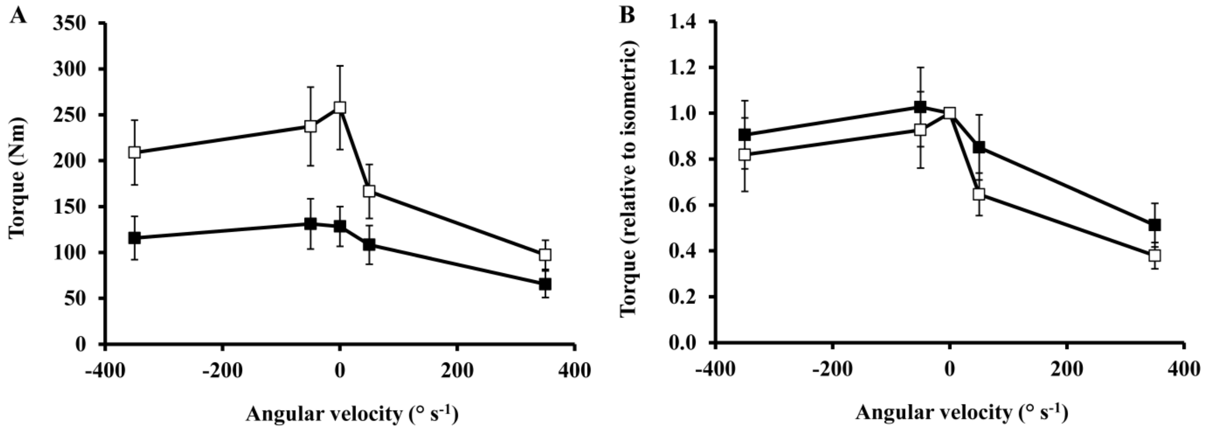

Figure 5.1. Torque-velocity relationship of the knee extensors (open squares) and flexors (filled squares) in A) absolute values and B) relative to isometric strength (n= 31). Knee extensors absolute strength was higher than knee flexors at all velocities (*P*< 0.001). In contrast, when strength was normalised to isometric values knee flexors had higher values than knee extensors at each velocity (-350° s^{-1}, *P*= 0.016; -50° s^{-1}, *P*= 0.023; 50° s^{-1}, *P*< 0.001; 350° s^{-1}, *P*< 0.001).

The greater isometric strength of knee extensors relative to flexors resulted in an isometric H:Q ratio of 0.50 ± 0.10 (CV= 19.8%). The functional H:Q ratio was greater at high contraction velocities (50° s^{-1}, 0.79 ± 0.11 (CV 13.7%); 350° s^{-1}, 1.20 ± 0.23 (CV 19.5%) *P*< 0.001) reflecting the differential effect of increasing velocity on concentric (extension) and eccentric (flexion) torque.

5.3.2 Relationships between muscle size, strength and HQ ratio

A significant but moderate correlation was found between quadriceps and hamstrings volume (r= 0.64, *P*< 0.001, Fig. 5.2A). Quadriceps volume was strongly related to isometric knee extension strength (r= 0.84, *P*< 0.001, Table 5.2), moderately associated with concentric strength (50° s^{-1}, r= 0.56, *P*= 0.004; 350° s^{-1}, r= 0.55, *P*= 0.004), but unrelated to eccentric strength (50° s^{-1}, r= 0.27, *P*= 0.149; 350° s^{-1}, r= 0.33, *P*= 0.137). In contrast, hamstrings volume exhibited moderate to strong correlations with knee flexor strength across the range of velocities (r= 0.62–0.76, *P*< 0.001, Table 5.2).

Table 5.2. Bivariate correlations coefficients between quadriceps and hamstrings muscle volume with knee extensors and flexors maximal isometric, concentric and eccentric strength (n= 31). * $P< 0.01$, ** $P< 0.001$

	Muscle volume	
Muscle strength	**Quadriceps**	**Hamstrings**
Isometric	0.84**	0.62**
Con 50° s^{-1}	0.56*	0.74**
Con 350° s^{-1}	0.55*	0.71**
Ecc 50° s^{-1}	0.27	0.76**
Ecc 350° s^{-1}	0.33	0.69**

A moderate correlation was found between H:Q volume ratio and isometric H:Q ratio (r= 0.45, P= 0.024) as well as functional H:Q ratio at 350° s^{-1} (r= 0.56, P= 0.003) (Fig. 5.3), while there was a tendency for a relationship between H:Q volume ratio and functional H:Q ratio at slow velocity (r= 0.34, P= 0.059).

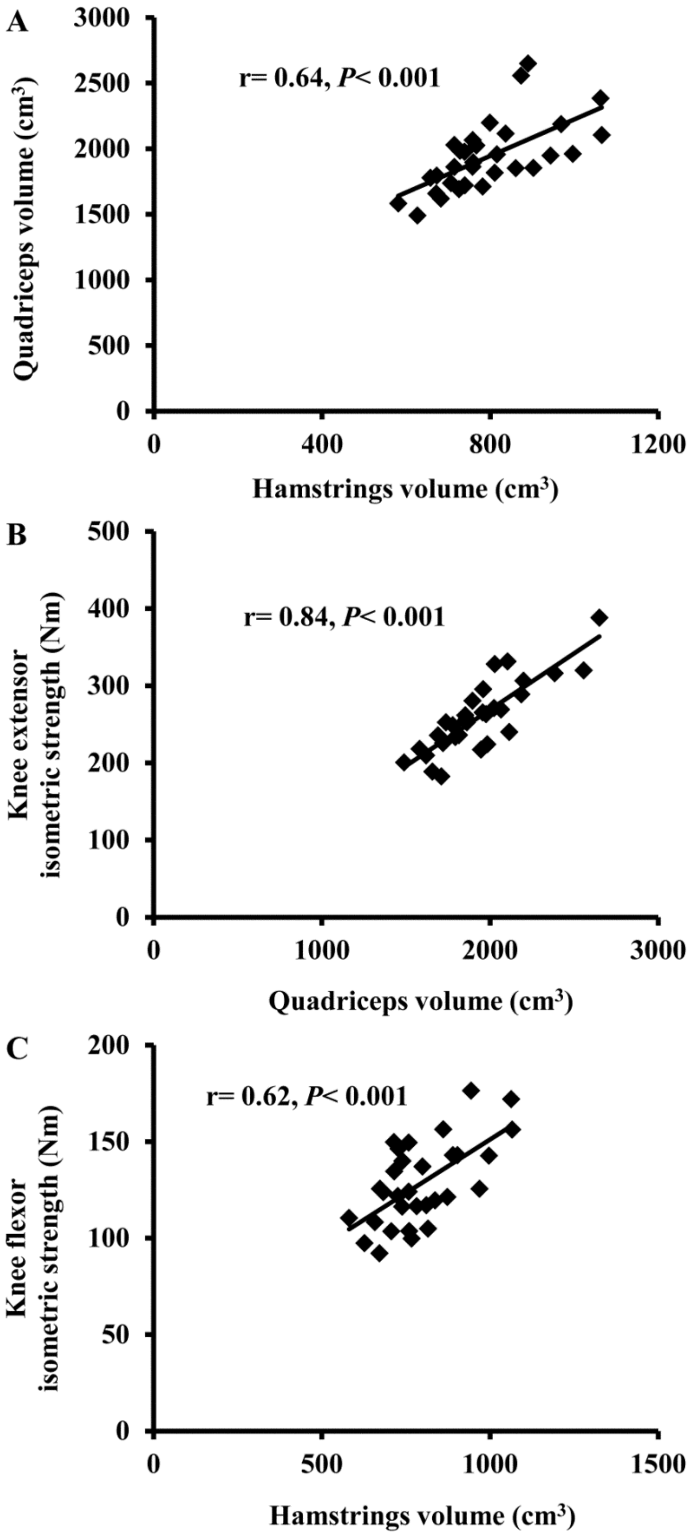

Figure 5.2. Relationships between A) quadriceps and hamstrings muscle volume, B) quadriceps muscle volume and knee extensor isometric strength and C) hamstrings muscle volume and knee flexor isometric strength (n= 31).

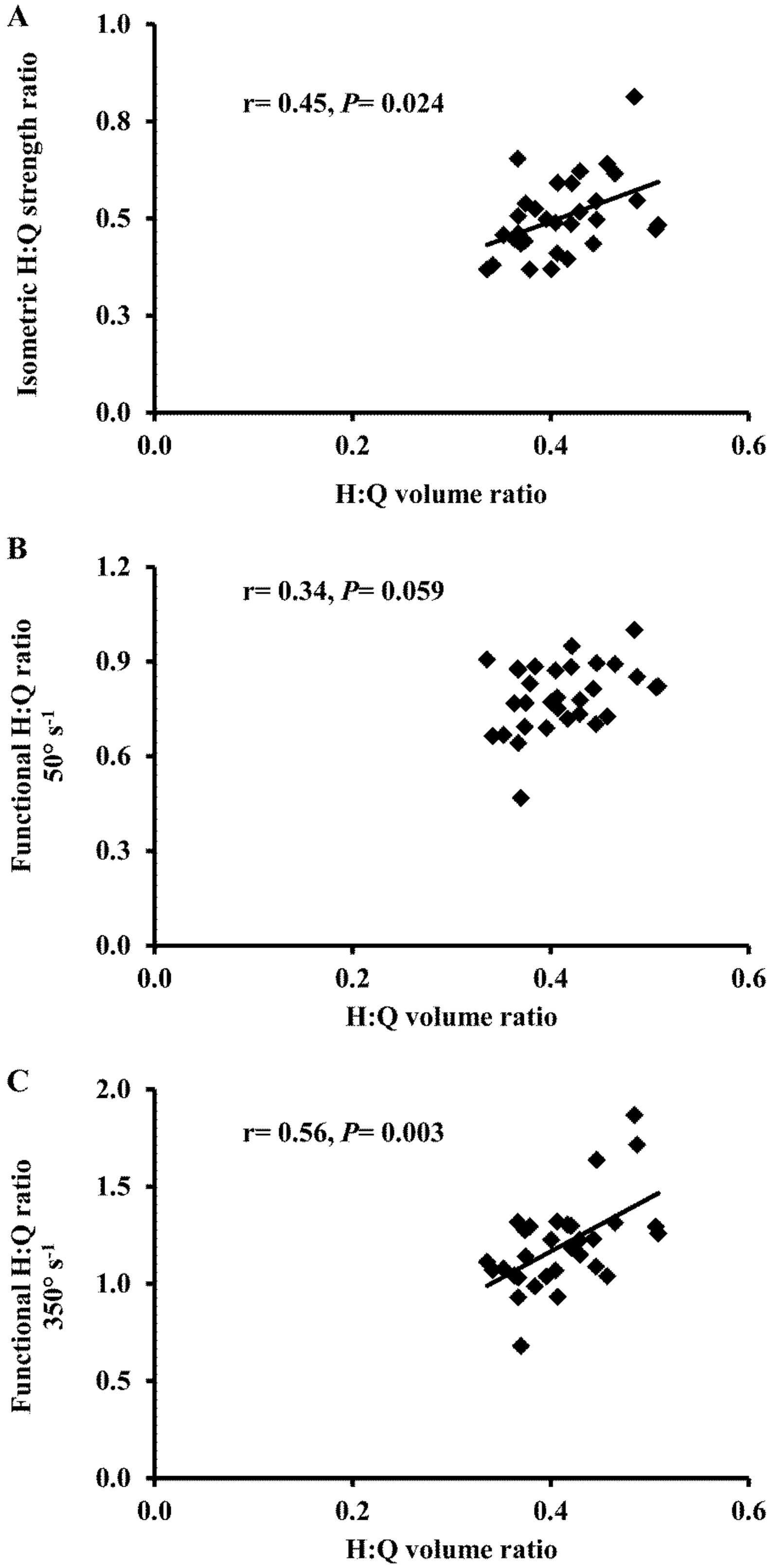

Figure 5.3. Correlations of H:Q volume ratio with A) isometric H:Q ratio, B) functional H:Q ratio at 50° s^{-1} and C) functional H:Q ratio at 350° s^{-1} (n= 31).

5.4 DISCUSSION

This study examined the association between the size of the knee extensors and flexors, how the size of these muscle groups was related to their function across the torque-velocity relationship, and whether the muscle size ratio was associated with the H:Q strength ratios. We found that muscle volume explained 38-58% of the differences between individuals in knee flexor strength (isometric 38%, concentric 50-55%, eccentric 48-58%) and up to 71% of the variation in knee extensor strength (isometric 71%, concentric 30-31%, eccentric - not significant). Further, there was a moderate correlation between the size of these antagonistic muscle groups (R^2= 0.41). Finally, in support of our hypothesis, we found that the relative size of the knee extensors and flexors explained 12-31% of the variability in H:Q strength ratios. These findings suggest that muscle size is not only an important determinant of the knee extensors and flexors' strength, but it also influences the strength balance around the knee joint.

Muscle size of the knee extensors (quadriceps) and flexors (hamstrings) exhibited strong correlations with their respective isometric strength (r= 0.62-0.84, P< 0.001). Interestingly, although hamstrings volume was strongly related to knee flexor strength at all velocities and contraction modes, quadriceps size was only moderately related to knee extensor concentric strength (r= 0.55-0.56, P< 0.01*)* and not related to eccentric strength (r= 0.27-0.33, P> 0.05*)*. In voluntary eccentric contractions, neural factors have been suggested to inhibit knee extensor strength (Amiridis et al., 1996; Westing et al., 1991; 1990; Dudley et al., 1990) and may supersede any relationship of muscle size and eccentric strength. For example, in untrained individuals maximal voluntary eccentric contractions of the knee extensors appear to be inhibited by up to 24% (Amiridis et al., 1996; Westing et al., 1990). While this inhibition is not evident in highly trained athletes (Amiridis et al., 1996), normal individuals seem to be unable to achieve complete muscle activation in spite of their 'maximal' effort. While the exact mechanism(s) remains unknown, it is believed that neural mechanisms at spinal and supraspinal levels inhibit neuromuscular activation during maximal eccentric efforts of untrained individuals and this is thought to protect the joint from potentially injurious high levels of force that can be produced during eccentric contractions (Duchateau and Baudry, 2014). Nonetheless, hamstrings volume was strongly related to knee flexor eccentric strength (r= 0.69-0.76, P< 0.001) suggesting that neural inhibition during eccentric contractions may be muscle-specific. Overall, the results of this study suggest that muscle size exhibits a differential influence on muscle strength depending on the contraction type and muscle group.

The extensors and flexors clearly had a different shape to their torque-velocity relationship as shown by the differences in relative (to isometric) concentric and eccentric torques of the two muscle groups. Architectural differences between quadriceps and hamstrings may contribute to these differences. It has been suggested that hamstrings are designed for longer excursions and faster movement (long fibre lengths, moderate physiological cross-sectional areas (PCSAs)) while quadriceps muscles are designed for higher force production (short fibre lengths, large PCSA) (Ward et al., 2009; Lieber and Friden, 2000). At a given velocity, long fibres undergo smaller change in length for a given change in total muscle length and are expected to produce higher force compared to shorter fibres. This may partly explain the ability of hamstrings to maintain a relatively high torque capacity at all examined velocities compared to the quadriceps. The pronounced isometric torque recorded during the isometric knee extensions may be partly attributed to the slight forward movement of the femur as the pelvis and torso stabilise. This movement actually results in a slow eccentric contraction, a phenomenon previously reported (Pain et al., 2013; Forrester et al., 2011).

Strength imbalance around the knee joint, defined as a low H:Q strength ratio, has been linked to increased risk for hamstrings strain injury (Yeung et al., 2009; Croisier et al., 2008). However, there has been limited consideration of the factors that determine this ratio. Due to the significant influence of a muscle's size on its strength capacity (Fukunaga et al., 2001; Bamman et al., 2000), it seems logical that the size ratio between antagonistic muscles would also determine their strength ratio. Similarly, the size of antagonist muscles would also be expected to be related. Indeed, in the present study, quadriceps size was moderately related to that of hamstrings (R^2= 0.41). In addition, the size balance of the knee extensors and flexors explained 20% of the isometric strength ratio and 12-31% of the functional H:Q ratios. However, these results are in contrast to the only previous investigations, as Akagi et al. (2012, 2014) reported no relationship between knee extensors and flexors muscle size ratio and isometric strength H:Q ratio (r= 0.13-0.14, P= 0.56-0.61). The smaller cohorts examined (n≤ 21), the prone testing position and the measurement of isometric strength at a single crank angle in the studies of Akagi et al. (2014, 2012) may have contributed to the discrepancy of their findings with our results. Overall, the results of the present study suggest that in young physically active men the relative size of the knee extensor and flexor muscles is an important determinant of their strength balance. The range of the H:Q volume ratio

(0.34-0.51) suggests that in some individuals hamstrings are 50% larger relative to quadriceps than other individuals. This would suggest that individuals with proportionately small hamstrings may be at greater risk of injury and they should be specifically targeted for prehabilitation training.

In conclusion, as hypothesised the size of quadriceps and hamstrings muscles was related (R^2= 0.41) and the relative size of the knee extensors and flexors explained the H:Q strength ratios. Therefore muscle size imbalances contribute to functional imbalances and may be an underlying risk factor for injury. For some individuals correcting an underlying muscle size imbalance through resistance training may be an appropriate injury prevention strategy.

Chapter 6

Do muscle size and composition explain knee flexor muscle function in man?

6 CHAPTER 6 – DO MUSCLE SIZE AND COMPOSITION EXPLAIN KNEE FLEXOR MUSCLE FUNCTION IN MAN?

6.1 INTRODUCTION

Understanding the determinants of muscle function is important for maintaining and improving function and consequently athletic performance and human health, including injury, illness and ageing. The hamstrings muscle group is the primary knee flexor and a major hip extensor and therefore plays a leading role in human locomotion and athletic activities such as running and jumping (Schache et al., 2014; Novacheck, 1998; Baratta et al., 1988). Hamstrings activation is also considered important for dynamic knee joint control and stability, and thus maintaining joint integrity. Furthermore, hamstrings strain injuries are the most common injury in a variety of sprint-based sports (e.g. different codes of football and track sprinting; Alonso et al., 2012; Ekstrand et al., 2011; Orchard et al., 2002), and these injuries predominantly affect the biceps femoris long head muscle (BFlh; Woodley & Mercer, 2004). Chapter 5 showed that hamstrings muscle size is an important determinant of knee flexors function. However, whether muscle composition would further explain the interindividual differences in knee flexors function remains unknown.

In fact, hamstrings myosin heavy chain (MHC) composition in young healthy individuals remains unknown as current BFlh muscle composition data are derived solely from cadavers (Dahmane et al., 2006; Garrett et al., 1984; Johnson et al., 1973). Within cadaver specimens the histochemically examined biceps femoris fibre type II composition has been reported to be 33.1-54.5% (Dahmane et al., 2006; Garret et al., 1984; Johnson et al., 1973). The small sample size (n= 6-15), the old age and unknown physical activity history of these participants may limit the relevance of these data to young healthy, active populations. Nevertheless, Garret et al. (1984) reported that hamstrings contained a higher proportion of type II fibres (55.2%) than the quadriceps (51.9%) or adductor magnus (44.8%) and suggested that this muscle composition may contribute to the high susceptibility of the hamstrings to strain injuries. However, the methodological limitations of that study (small sample size (n= 10) of elderly cadavers) highlight the need to determine hamstrings muscle composition in healthy young adults in order to understand if their composition contributes to their high incidence of strain injury.

As hamstrings muscle composition has only been determined within cadavers, its influence on muscle function remains unknown. However, within the quadriceps femoris a significant correlation between maximum isometric or isovelocity (15-240° s^{-1}) strength and composition of the vastus lateralis (VL) has often (Gür et al., 2003; Aagaard & Andersen 1998; Viitasalo & Komi, 1978; Thorstensson et al., 1976) but not always been reported (Schantz et al., 1983; Inbar et al., 1981; Viitasalo et al., 1981). Some of these studies examined this relationship within diverse athletic and training populations (e.g. Gür et al., 2003), where numerous other variables (e.g. hypertrophy) could be acting as confounding factors (Folland and Williams, 2007) while other studies examined small cohorts (n≤ 7; Aagaard & Andersen, 1998). Nevertheless, according to the balance of evidence from quadriceps studies, hamstrings muscle composition appears likely to influence maximum strength of the knee flexors. In addition, based on *in-vitro* studies fibre type composition has a more pronounced influence on function at high velocities (Bottinelli et al., 1999), however current investigations have used relatively slow velocities (≤ 240° s^{-1}). Similarly, the rate of force development has been shown to be greater in type II fibres (based on their MHC composition) in rats (Metzger and Moss, 1990) and humans (Harridge et al., 1996). Previous research suggests that a correlation between explosive isometric strength *in vivo* and muscle composition might also be expected (Viitasalo et al., 1981; Viitasalo & Komi, 1978).

Whilst the influence of hamstrings muscle composition on function *in vivo* remains to be elucidated, muscle size has been consistently found to be a substantial determinant of isometric strength in various muscles (e.g. elbox flexors, r= 0.76, Akagi et al., 2009; plantar flexors, r= 0.65, Bamman et al., 2000; knee extensors, r= 0.59, Maughan et al., 1983). Considering the hamstrings, the three studies we are aware of reported quite diverse relationships between muscle size and isometric/concentric strength measures (r= 0.41 to 0.80; Akagi et al., 2012; Kanehisa et al., 1994; Masuda et al., 2003). However, none of these studies examined eccentric or explosive strength. It is also possible that the combined influence of muscle composition and muscle size may further explain the variability in hamstrings muscle function, however the combined influence of these factors has not been investigated.

Therefore, the aim of this study was to determine the BFlh MHC isoform distribution and to examine the association of hamstrings muscle size and BFlh MHC composition with knee flexor strength, including maximal strength measurements across the torque-velocity relationship (concentric, isometric and eccentric) as well as explosive isometric strength.

6.2 METHODS

6.2.1 Participants

Thirty-one healthy, recreationally active participants (age 20.6 ± 2.5 years; height 1.79 ± 0.71 m; body mass 71.8 ± 7.3 kg; mean ± SD) took part in this study. Participants had a low to moderate level of physical activity and were not involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. Their physical activity was assessed with the International Physical Activity Questionnaire short format (www.ipaq.ki.se/downloads.htm, Craig et. al., 2003) and their average energy expenditure was 1739 ± 814 metabolic equivalent-minutes per week. After completing the physical activity and health screen questionnaires, participants provided written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no history of musculo-skeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit and to refrain from alcohol and caffeine for the last 24 h before each visit.

6.2.2 Overview

Participants visited the laboratory on seven separate occasions, seven days apart at a consistent time of the day (11:00-16:00 h). All the measurements were conducted on the participants' dominant leg (defined as their kicking leg). The first session involved recording anthropometric data and familiarization with the procedures for testing knee flexor explosive isometric strength that was measured during the second and third sessions. The third and fourth sessions involved familiarization with the isokinetic dynamometer procedures, while the knee flexor torque-velocity relationship was examined in the fifth session. The sixth session involved magnetic resonance imaging (MRI) of the participants' thigh to assess the hamstrings muscle size. In the final session, muscle tissue samples were obtained from the BFlh muscle.

6.2.3 Measurements and Data analysis

6.2.3.1 Torque-velocity relationship

The participants were seated on an isokinetic dynamometer chair (Con-Trex MJ, CMV AG, Dübendorf, Switzerland) with a hip angle of 120° (180°= full extension). This hip angle is similar to that during late swing phase during sprinting (Guex et al., 2012). Two 3-point belts secured the torso and additional straps tightly secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint centre with the dynamometer rotational axis was performed during isometric contractions of >50% of maximal isometric voluntary torque (MVT) at a knee joint angle of ~115°. The dynamometer's shin brace was placed posterior to the shank ~2 cm above the medial malleolus before the shank was tightly secured to the dynamometer lever arm. The range of motion was established and anatomical zero was set at full extension of the knee joint. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen. The torque, crank angle and crank velocity signals were sampled at 2000 Hz with a PC using Spike 2 software (CED, Cambridge, UK) and smoothed with a finite impulse response filter at 15 Hz before any further analysis.

For isometric strength measurement, participants first completed a standardized warm-up consisting of a progressive series of submaximal contractions before they performed two sets of three maximum contractions, one at each of three different crank angles (165°, 145° and 125° in a consistent order; 180°= full extension) near the angle where knee flexors exert their maximal torque (Knapik et al., 1983). Participants were instructed to flex their knee and "pull" as hard and as fast as possible for 3-5 s. One-minute rest was given between each contraction and 2 min between sets. The contraction with the highest torque irrespective of crank angle was selected for further analysis. Isometric strength was defined as the average torque over a 0.5 s period around the highest instantaneous torque.

For the concentric and eccentric strength measurement, participants first completed a standardized warm-up protocol with five submaximal concentric-eccentric contractions of progressively higher intensity. Then, they performed knee flexors maximal concentric-eccentric contractions at 50° s^{-1} (3 sets of 2 reciprocal contractions) and 350° s^{-1} (3 sets of 3 reciprocal contractions) over ~100° of range of motion. There was ≥1 min rest between each set and ≥2 min rest between velocities. For the concentric-eccentric contractions, the acceleration and deceleration phases were excluded in order to disregard torque overshoot during these phases (Schwartz et al., 2010) and the constant isovelocity period was identified (within ±5% of the prescribed crank angular velocity). Finally, concentric and eccentric strength at each velocity was defined as the highest instantaneous torque recorded within the isovelocity range of the relevant contractions.

The high velocity torque ratio was defined as the concentric strength at 350° s^{-1} divided by the isometric strength (T_{con350}/T_{isom}). A similar high-to-low velocity torque ratio has been found to correlate with muscle composition (Gür et al., 2003).

6.2.3.2 Explosive isometric strength

Participants lay in a prone position on a custom-made isometric dynamometer at fixed hip (140°, 180°= full extension) and knee (150°) joint angles selected to replicate the joint positions during the late swing phase of sprinting (Guex et al., 2012) when hamstrings strains are thought to occur. To minimize any extraneous movements, participants were fastened with two straps across the hips, a strap over the lower back and a strap over the distal thigh just above the knee joint. A metal ankle cuff with a lining of high density neoprene was placed ~4 cm above the medial malleolus and the distal leg was tightly secured to the cuff with straps. Force was measured with a calibrated strain gauge (linear response up to 500 N, Force Logic UK, UK) in series with the ankle cuff and perpendicular to the tibia. The force signal was amplified (x370) and sampled at 2000 Hz with an external analog-to-digital converter (Micro 1401-3, CED, Cambridge, UK). A PC recorded and displayed the data using the Spike 2 software (CED, Cambridge, UK). In order to remove the high-frequency oscillation in the signal (just above 500 Hz), the force signal was filtered with a 4^{th} order Butterworth filter with a low pass cut-off frequency of 500 Hz (see Appendix B). The frequencies <500 Hz were used as a reference envelope for detecting the force onset during the explosive contractions. A lower frequency filter (e.g. 15-20 Hz) would transform the signal into a gradually rising asymptotic curve, and therefore the sudden transition from rest to force production would be removed resulting in subjective and unreliable recognition of the force onset (Tillin et al., 2013). The distance between the knee joint space and the centre of the ankle cuff was measured to calculate knee flexion torque.

After a standardised warm-up, participants performed 3 maximal knee flexion contractions to establish the target torque for the subsequent explosive contractions (see below). A computer screen provided real time visual feedback by displaying the torque response. Thereafter participants completed 10 explosive contractions with 30 s rest between contractions. They were instructed to contract 'as fast and as hard as possible' for ~1 s with an emphasis on 'fast' without any countermovement or pre-tension. Real-time visual feedback was provided on the computer screen displaying the torque response, with specific performance feedback of the time from 1% to 80% of peak torque. For the detection of any countermovement or pre-tension, the resting torque was displayed on a sensitive scale. Standardized verbal encouragement was given throughout the maximal and explosive contractions.

During offline analysis the three valid explosive contractions (achieved torque ≥80% of peak torque with no discernible counter-movement or pre-tension - change of baseline signal <0.2 Nm for the 100 ms prior to the onset of contraction), with the fastest time from onset to 50% of peak torque were selected for further analysis. Analysis of these contractions consisted of measurement of the time from contraction onset to 10, 50, and 90 Nm and the time from contraction onset to 15, 45 and 75% of peak torque. Force onsets were identified manually by visual identification by a trained investigator using a systematic approach which is considered to be more valid than automated methods (Tillin et al., 2013; Tillin et al., 2010). The three analysed explosive contractions were averaged within each measurement session, before averaging across the two sessions when these measurements were made.

6.2.3.3 Magnetic resonance imaging (MRI)

A 1.5 T MRI scanner (Signa HDxt, GE) was used to scan the dominant leg in the supine position with the hip and knee joints extended. T1-weighted axial plane images were acquired from the anterior superior iliac spine to the knee joint space in two overlapping blocks and oil filled capsules were placed on the lateral side of the participants' thigh to help with the alignment of the blocks during analysis. The following imaging parameters were used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm.

MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland). The BFlh, biceps femoris short head, semitendinosus and semimembranosus muscles were manually outlined in every third image starting from the most proximal image in which the muscle appeared. All manual segmentation measurements were completed by the same investigator. Muscle volume was calculated using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). To examine reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later and the coefficient of variation (CV) was calculated. The CV for muscle volume was on average 0.6%.

6.2.3.4 Muscle sampling and myosin heavy chain composition

Muscle samples (~0.04 g) from the mid-section BFlh (~50% thigh length) of the dominant leg were obtained under local anaesthesia (1% lidocaine) using the microbiopsy technique (Pro-Mag Ultra, Angiotech, Medical Device Technologies, FL, USA). Samples were immediately frozen in liquid nitrogen and stored at -80°C for further analysis. MHC content was determined by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis using a method derived from that previously described (Fauteck & Kandarian, 1995). Electrophoresis (Mini-Protean 3, Bio-Rad) was performed on 6% (crosslinking 2.7%) polyacrylamide resolving gels with 4% (crosslinking 2.7%) stacking gels at ~4°C. The gels were electrophoresed at a constant 100 V for 1 h, and thereafter at a constant 6 mA for ~18 h. Gels were immediately silver stained (SilverQuest Silver Staining Kit, Invitrogen) and protein bands quantified by densitometry (ChemiDoc XRS+ System, Bio-Rad). Muscle samples were classified according to the relative expression of the three MHC isoforms: type I, IIA, and IIX (Fig. 6.1). The MHC analysis was run in duplicate and the mean of the 2 analyses was taken. When the first 2 analyses had a difference >10% a third analysis was run. For each individual, the representative MHC distribution was defined as the mean of all repeats in which the different MHC isoforms were within 10% between analyses. The CV for repeat samples was 3.9% for MHC-I, 5.7% for MHC-IIA and 8.4% for MHC-IIX.

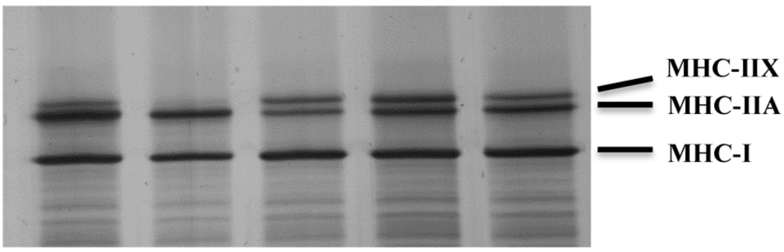

Figure 6.1. Example sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis separation of the different myosin heavy chain (MHC) isoforms in biceps femoris long head muscle sampled from 5 participants.

6.2.4 Statistical analysis

Data are presented as mean ± SD. One-way analysis of variance was used to examine for differences in muscle volume between the constituents muscles of hamstrings and in knee flexors torque at the different velocities. Bivariate relationships were examined using Pearson product moment correlations between the dependent variables and the Holm-Bonferroni correction was used to control for multiple tests. The level of significance was set at $P < 0.05$. All statistical procedures were performed with IBM SPSS 22 (IBM Corporation, Armonk, NY).

6.3 RESULTS

6.3.1 Descriptive data on BFlh MHC isoform distribution, hamstrings muscle size and knee flexor strength

On average, the BFlh muscle exhibited a balanced, mixed MHC distribution with 47.1 ± 9.1% MHC-I, 35.5 ± 8.5% MHC-IIA and 17.4 ± 9.1% MHC-IIX, but with considerable variation between individuals (Table 6.1). Total hamstrings muscle volume was on average 794.1 ± 122.2 cm^3 (CV= 15.4%), while the BFlh had smaller volume (210.0 ± 37.9 cm^3) than the other biarticular muscles (ST; 228.6 ± 45.4 cm^3, $P< 0.05$ and SM; 234.8 ± 47.7 cm^3, $P< 0.01$, Table 6.1).

Table 6.1. Descriptive data of biceps femoris muscle composition, hamstrings muscle volume, and knee flexor strength. The muscle volumes of the constituent muscles were compared to BFlh, and maximal strength measures were compared to isometric strength. * $P< 0.05$, ** $P< 0.01$

		Mean ± SD	Range	CV (%)
Muscle composition (%)	**MHC-I**	47.1 ± 9.1	32.6 – 71.0	19.3
	MHC-IIA	35.5 ± 8.5	21.5 – 60.0	23.9
	MHC-IIX	17.4 ± 9.1	0.0 – 30.9	52.4
Muscle volume (cm^3)	**BFlh**	210.0 ± 37.9	157.4 - 289.4	18.1
	BFsh	120.6 ± 22.3	76.6 - 170.4	18.5
	ST	228.6 ± 45.4*	120.5 - 342.5	19.9
	SM	234.8 ± 47.7**	125.3 - 330.2	20.3
	Total	794.1 ± 122.2	581.1 - 1065.6	15.4
Maximal strength (Nm)	**Ecc 350**° s^{-1}	115.7 ± 23.6**	58.0 - 164.5	20.4
	Ecc 50° s^{-1}	131.1 ± 27.4	66.9 - 174.2	20.9
	Isometric	128.3 ± 21.7	92.1 - 176.4	16.9
	Con 50° s^{-1}	108.3 ± 21.1**	65.9 - 154.0	19.5
	Con 350° s^{-1}	65.4 ± 14.6**	32.0 - 88.1	22.3
	$\mathbf{T_{con350}/T_{isom}}$	0.51 ± 0.10	0.29 - 0.71	18.6

The knee flexors exerted their highest torque during slow eccentric contractions (131.1 ± 27.4 Nm), and there was considerable inter-individual variability at all contraction modes and velocities (CV= 16.9 - 22.3%, Table 6.1). The high velocity torque ratio (T_{con350}/T_{isom}) was

0.51 ± 0.10 (CV= 18.6%). Knee flexor explosive strength, measured as time to specific torques, was found to vary between individuals, particularly during the later stages of the explosive contractions (Fig. 6.2).

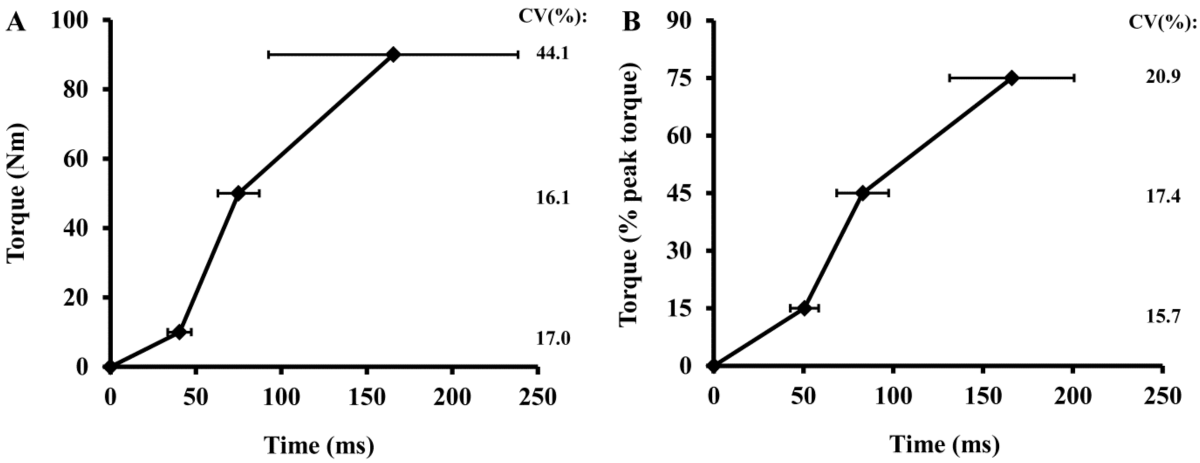

Figure 6.2. Knee flexion explosive strength expressed as time from zero to absolute (A) and relative (B) torque levels. Data are mean ± SD (n= 31) with inter-individual coefficient of variation (CV) presented at each torque level.

6.3.2 Relationships of hamstrings muscle size and BFlh MHC isoform distribution with knee flexion strength

Hamstrings muscle volume had moderate to strong correlations with knee flexor torque at all velocities (r= 0.62 – 0.76, *P*< 0.01, Table 6.2). In contrast, no relationship was found between BFlh muscle composition and maximal strength at any velocity (-0.22 < r < 0.24, *P*> 0.05, Fig. 6.3) or T_{con350}/T_{isom} (-0.16 < r < 0.24, *P*> 0.05). When torque values at all velocities were expressed relative to muscle volume there remained no association with BFlh muscle composition (-0.29 < r < 0.35, *P*> 0.05).

Table 6.2. Bivariate correlation coefficients of knee flexor maximal strength with hamstrings muscle volume and biceps femoris long head muscle composition (n= 31). MHC: myosin heavy chain, ** *P*< 0.01, † *P*< 0.001

		Hamstrings volume	BFlh muscle composition		
			MHC-I	MHC-IIA	MHC-IIX
Maximal strength	**Isometric**	0.62**	0.01	0.20	-0.20
	Con 50° s^{-1}	0.74†	0.08	0.15	-0.22
	Con 350° s^{-1}	0.71†	-0.11	0.12	0.00
	Ecc 50° s^{-1}	0.76†	0.04	0.05	-0.09
	Ecc 350° s^{-1}	0.69†	0.01	0.09	-0.10
	T_{con350}/T_{isom}	0.27	-0.16	-0.09	0.24

Hamstrings muscle volume was unrelated to explosive strength (Table 6.3), measured as time to achieve low absolute levels or relative measures of torque, however it was associated with explosive strength (time) to high absolute levels of torque (90 Nm; r= -0.53, *P*< 0.05). BFlh MHC distribution was unrelated to any measure of explosive strength (-0.20 < r < 0.24, *P*> 0.05, Table 6.3).

Table 6.3. Bivariate correlation coefficients between knee flexor explosive strength (absolute and relative) measures with hamstrings muscle volume and biceps femoris long head composition. (n= 31). MHC: myosin heavy chain, * *P*< 0.05

	Explosive strength					
	Time to absolute torque			Time to relative torque (%MVT)		
	10 Nm	**50 Nm**	**90 Nm**	**15%**	**45%**	**75%**
Hamstrings volume (cm^3)	-0.10	-0.43	-0.53*	0.01	-0.16	-0.24
MHC-I (%)	-0.09	0.14	0.06	-0.01	0.14	0.24
MHC-IIA (%)	0.01	-0.06	-0.15	0.03	-0.05	-0.05
MHC-IIX (%)	0.08	-0.09	0.08	-0.02	-0.09	-0.20

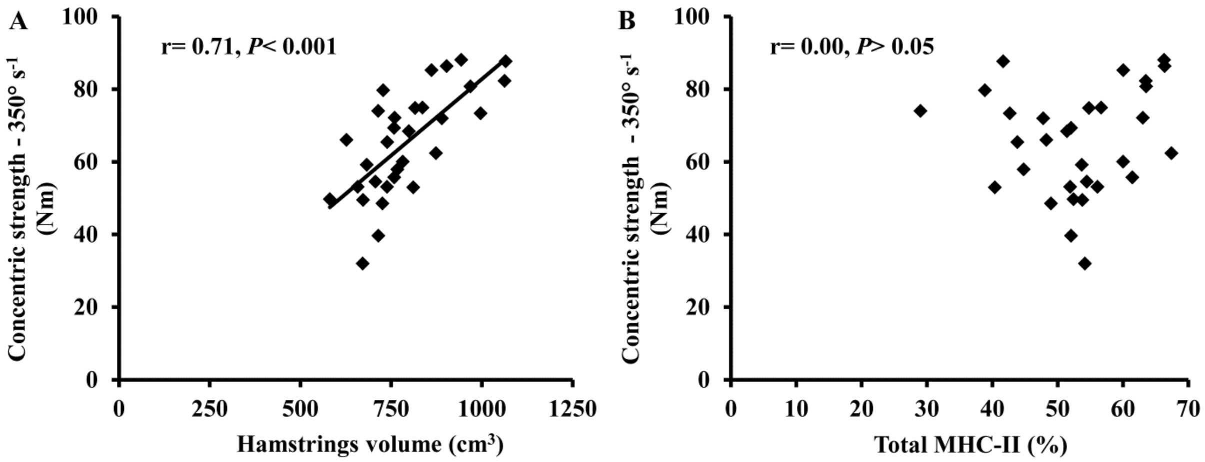

Figure 6.3. Relationships between concentric strength at 350° $s^{-1}$and (A) hamstrings volume and (B) BFlh total MHC-II isoform content (n= 31). BFlh: biceps femoris long head, MHC: myosin heavy chain.

6.4 DISCUSSION

This study examined the influence of hamstrings muscle size and BFlh muscle composition on knee flexors maximal and explosive strength. We found that within the examined cohort, the BFlh exhibited on average a balanced MHC isoform distribution that appears very similar to that of the other thigh muscles (see below), and therefore does not support the suggestion that BFlh composition contributes to the high incidence of strain injury in this muscle. Further, we found that 38-58% of the variance in knee flexor maximum torque at isometric and at a range of concentric and eccentric velocities was attributable to differences in hamstrings muscle volume, while BFlh MHC distribution was not related to any measure of maximal or explosive strength.

The present study is the first to directly examine the BFlh muscle composition *in vivo* and our results showed that, on average, the BFlh muscle had a balanced distribution of slow and fast MHC isoforms (47.1 ± 9.1% MHC-I and 52.9 ± 9.1% total MHC-II) in young healthy men. Hamstrings muscle composition has been linked to the high injury rate seen in this muscle (Garret et al., 1984). In a much cited study, Garret et al. reported a BFlh muscle composition within a small cohort of elderly cadavers to be similar to our data (54.5 ± 2.8% type II fibres and 45.5 ± 2.8% type I of total number of sampled fibres), yet based on small differences compared to other muscles (quadriceps, 51.9%; adductor magnus, 44.8% type II fibres) they argued that the 'high proportion' of fast fibres in the hamstrings compared to other leg muscles may contribute to their susceptibility to injury. However, the VL muscle, an antagonist to BFlh muscle function, has been reported to contain a greater proportion of MHC-II isoform (66.1% total MHC-II in 95 physically active young men; Staron et al., 2000) compared to the BFlh in our cohort. Consequently, the composition of the BFlh does not seem to explain the high incidence of strain injuries within this muscle compared to other muscles. Therefore, other aspects of hamstrings structure (e.g. aponeurosis size, Evangelidis et al., 2014) or function (eccentric actions at long lengths, Schache et al., 2012) are likely to explain the high incidence of strain injuries in this muscle. On an individual basis however, the proportion of MHC-II isoforms could still be a risk factor for hamstrings strain injury. Type II fibres are selectively affected by eccentric exercise-induced muscle damage in both animals and humans (Lieber and Friden, 1988; Friden et al., 1983), even after a single eccentric contraction (Lovering and Deyne, 2004). Structural differences between fibre types (e.g. thinner Z-disks in type II fibres; Luther, 2009) may contribute to the selective damage of type II fibres (Friden and Lieber, 1992). Even though eccentric exercise-induced muscle damage is not synonymous to strain injury, it does cause disruption of the muscle structure at the microscopic level and it is possible that accumulation of such damage may eventually lead to a macroscopic injury (Brocket et al., 2004, 2001). Considering all the changes in the muscle apparatus as a result of eccentric exercise-induced damage (reduction of force-generating capacity, shift of optimum fibre length and impairment of the excitation-contraction coupling, Morgan and Allen, 1999), it would be logical to hypothesize that individuals with a high percentage of type II fibres in their hamstrings may be at an increased risk for strain injury when exposed to high-risk conditions. Within our cohort, total MHC-II isoform content ranged from 29.0-67.4% and it is possible that individuals with a high proportion of type II fibres could be at higher risk of injury. Future retrospective and prospective studies are needed to elucidate the relationship between muscle composition and the incidence of individual strain injuries.

Whilst MHC composition is a major determinant of function in single fibres (Bottinelli et al., 1996), in this study no correlation was found between BFlh muscle composition and knee flexors maximal or explosive strength *in vivo*. The lack of relevant previous data on the hamstrings prevents any direct comparison with our findings; however similar studies on knee extensors reported mixed results for the relationship of strength with muscle composition. Some studies examined this relationship within athletes with diverse training and competition backgrounds e.g. untrained, endurance, and strength and power athletes (Gür et al., 2003; Viitasalo et al., 1981; Viitasalo & Komi, 1978). Whilst this approach produces a wide range of muscle composition values, numerous other neuromuscular characteristics also likely vary between these groups (e.g. muscle size, architecture, neural drive) and these could confound any relationship of strength and muscle composition. Similar limitations confound the results of studies that reported a significant influence of muscle composition on explosive isometric strength in highly diverse cohorts (elite high jumpers vs. recreationally active individuals; Viitasalo et al., l981; elite athletes of various sports, Viitasalo and Komi, 1978). *In vivo* studies of muscle composition are typically limited to a single biopsy and it may not fully reflect the composition in other regions of the muscle (Elder et al., 1982), however fibre type distribution appears to be similar for biopsy samples taken from proximal and distal (Garret et al, 1984) as well as superficial and deep sites (Edgerton et al., 1975). In addition, BFlh muscle composition may not represent that of the other hamstrings muscles. Nevertheless, in the present study within a group of non-athletic young men, muscle composition did not explain their differences in maximal or explosive strength despite the large inter-individual variability in these measures (CV; maximal strength: 16.9-22.3%; explosive strength: 15.7-44.1%).

Our results revealed that hamstrings volume explained a significant portion of the variance in isometric (38%), concentric (50-55%) and eccentric (48-58%) knee flexor strength. These values are within the range of previous findings for the hamstrings (Akagi et al., 2012; Masuda et al., 2003; Kanehisa et al., 1994). Two small studies (n< 16) that examined the combined influence of muscle size and composition found that knee extensor strength was related to quadriceps ACSA (Johansson et al., 1987; Maughan and Nimmo, 1984), but only Johansson et al. (1987) reported a significant relationship of muscle composition with maximal concentric strength at 180° s^{-1} after accounting for muscle size. Despite the significant correlations found in this study, the variability in knee flexors torque-velocity relationship can be attributed only in part (38-58%) to differences in hamstrings muscle size. This may be partly due to the fact that other muscles (i.e. gastrocnemius, gracilis, sartorius and popliteus) also contribute to knee flexor torque but their volume was not measured. Whilst we found that MHC composition does not seem to be a determining factor, other variables likely to explain some of the remaining variance in knee flexors maximal strength include moment arm (Baxter and Piazza, 2014), muscle architecture (Aagaard et al., 2001), level of agonist activation (Westing et al., 1990) and antagonist co-activation (Kellis and Baltzopoulos, 1998).

In contrast to maximal strength, explosive strength was not influenced by muscle size apart from at high levels of absolute torque (time from rest to 90 Nm; r= -0.53, $P< 0.01$). Whilst no similar data exist on hamstrings, elbow flexors isometric explosive strength has been related to muscle volume, but only during the later stages of contraction (150 ms, r= 0.69, $P< 0.001$) at relatively high levels of force (~80% MVF; Erskine et al., 2014).

In conclusion, the balanced MHC distribution found in BFlh muscle which appears similar to other thigh muscles, and therefore seems unlikely to contribute to the high susceptibility of the BFlh to strain injury. Hamstrings muscle volume explained 38-58% of the inter-individual differences in knee flexors torque at a range of velocities while BFlh muscle composition was not associated with maximal or explosive strength.

Chapter 7

Biceps femoris aponeurosis size: A potential risk factor for strain injury?

7 CHAPTER 7 – BICEPS FEMORIS APONEUROSIS SIZE: A POTENTIAL RISK FACTOR FOR STRAIN INJURY?

7.1 INTRODUCTION

The susceptibility of the hamstrings to strain injuries is well documented with the majority of these injuries located near the proximal myotendinous junction (MTJ) of the biceps femoris long head muscle (BFlh) (Woodley and Mercer, 2004). While some risk factors for hamstrings strain injuries have been identified (e.g. previous strain injury, strength imbalances and muscle fatigue) (van Beijsterveldt et al., 2013; Opar et al., 2012) whether the anatomical structure of the BFlh muscle-tendon unit (MTU), including the aponeurosis, might influence injury risk has received very little attention. Only recently has a disproportionately small BFlh proximal aponeurosis been suggested as a potential risk factor for hamstrings strain injury, following two studies that calculated higher localised tissue strains for individuals with a narrow proximal aponeurosis using computational modelling and dynamic MR imaging (Fiorentino et al., 2012; Rehorn and Blemker, 2010).

In a pennate muscle the force from the muscle fibres is transmitted to the tendon primarily via the aponeurosis. It seems reasonable to assume that a bigger and stronger muscle would have a larger and stronger aponeurosis and tendon in order to effectively and safely transfer the contractile force to the bone. Therefore, a degree of scaling between the size of the force generator and force transmitters seems intuitive. Within the quadriceps the vastus lateralis (VL) aponeurosis area has been found to be in proportion to total quadriceps volume (Abe et al., 2012). However, whether this is the case for the hamstrings remains largely unknown. A preliminary report suggested that the width of the BFlh proximal aponeurosis was highly variable between individuals and unrelated to the size of the BFlh muscle (Handsfield et al., 2010) suggesting that the force transmitter may not be proportional to the force generator. If this is the case, a disproportionately small BFlh proximal aponeurosis may concentrate mechanical strain on the surrounding muscle tissue (Fiorentino et al., 2014; Fiorentino et al., 2012; Rehorn and Blemker, 2010) and be a risk factor for hamstrings strain injury. However, in this preliminary report aponeurosis width was measured at a single arbitrary point along the muscle, which may be a poor reflection of the size of the aponeurosis. In contrast, measuring the whole contact interface between the muscle and aponeurosis may better reflect the concentration of mechanical strain at this interface.

From a functional perspective, aponeurosis size might be expected to be most strongly related to the maximum force transmitted through these tissues, and thus muscle strength. Whilst, experimental measurement of the *in vivo* force generating capacity of the BFlh may not be possible, knee flexor torque, which is primarily due to hamstrings activation, can be assessed. Maximal isometric torque is a convenient measurement of muscle function, although higher torques can often be achieved eccentrically which likely contribute to the high risk of BFlh MTU strains during eccentric actions (Heiderscheit et al., 2005). However, the relationship between aponeurosis size and isometric or eccentric muscle strength has yet to be investigated.

The aim of this study was to examine the relationship of BFlh proximal aponeurosis area with muscle size (maximal anatomical cross-sectional area and volume) and knee flexor strength (isometric and eccentric). Based on the role of the aponeurosis as a force transmitter within the MTU, we hypothesized that BFlh proximal aponeurosis area would be positively related to muscle size and strength.

7.2 METHODS

7.2.1 Participants

Thirty healthy, recreationally active participants (age 20.7 ± 2.6 years; height 1.79 ± 0.07 m; body mass 72.2 ± 7.2 kg; mean ± SD) took part in this study. Participants had a low to moderate level of physical activity and were not involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. Their physical activity was assessed with the International Physical Activity Questionnaire short format (www.ipaq.ki.se/downloads.htm) (Craig et al., 2003) and their average energy expenditure was 1826 ± 936 metabolic equivalent-minutes/week. After completing the physical activity and health screen questionnaires, participants provided written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no history of musculo-skeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit.

7.2.2 Overview

All participants visited the laboratory on four separate occasions seven days apart at a consistent time of the day (11:00-16:00 h) for measurements on the knee flexors of their dominant leg (defined as the preferred leg when kicking a ball). The first session involved collection of the anthropometric data and familiarization with the isometric and eccentric strength measurements. The second session involved the measurement of the knee flexion isometric strength and further familiarisation with the eccentric strength measurements. In the third session, participants repeated the isometric strength testing and also performed the eccentric strength measurements. The final session involved magnetic resonance imaging (MRI) of the participants' thigh to assess the BFlh proximal aponeurosis area, hamstrings maximal anatomical cross-sectional area (ACSAmax), and BFlh/semitendinosus (ST) conjoint proximal tendon CSA.

7.2.3 Measurements and Data analysis

7.2.3.1 Isometric strength

Participants lay in a prone position on a custom-made isometric dynamometer with hip and knee joint angles of 40° and 30° respectively (0°= full extension) (Fig. 7.1). These angles were selected because of their relevance to the angles during the late swing phase in sprinting (Guex et al., 2012) when hamstrings strains are thought to occur. To minimize any extraneous movements, participants were fastened with two straps across the hips, a strap over the lower back and a strap over the distal thigh just above the knee joint. A metal ankle cuff with a lining of high density neoprene was placed ~2 cm above the medial malleolus and the distal leg was tightly secured to the cuff with straps. The distance between the knee joint space and the centre of the ankle cuff was measured and used for calculation of the knee flexion torque. Force was measured with a calibrated strain gauge (linear response up to 500 N, Force Logic UK) connected in series to the ankle cuff and positioned perpendicularly to the tibia. The force signal was amplified (x370) and sampled at 2000 Hz with an external analog-to-digital converter (Micro 1401-3, CED, Cambridge, UK). A PC recorded and displayed the data using the Spike 2 software (CED, Cambridge, UK). The force signal was filtered with a 4th order Butterworth filter with a low pass cut-off frequency of 500 Hz. The frequencies <500 Hz were used as a reference envelope for detecting the force onset during the explosive contractions (see Appendix B). A lower frequency filter (e.g. 15-20 Hz) would transform the signal into a gradually rising asymptotic curve, and therefore the sudden transition from rest to force production would be removed resulting in subjective and unreliable recognition of the force onset (Tillin et al., 2013).

Figure 7.1. Isometric measurements of the knee flexors were made with this custom-made isometric dynamometer in the joint configuration shown. Isovelocity torque measurements were made with a Con-Trex isokinetic dynamometer.

After a standardised warm-up of progressively harder contractions, participants performed 3 maximal voluntary contractions (MVCs), in which they were instructed to flex the knee as 'hard' as possible for 3-5 s with 30 s of rest between the contractions. A computer screen provided real time visual feedback by displaying the torque response. After the first MVC a target cursor was positioned at peak torque achieved so far and participants were encouraged to exceed this during subsequent attempts. Standardized verbal encouragement was given throughout the MVCs. All isometric torque values were gravity corrected by subtracting resting torque from peak torque. Isometric strength was defined as the highest instantaneous torque during any of the MVCs within that session. Data presented is an average of the two sessions. The repeatability of isometric strength measurements between the two testing sessions was high (coefficient of variation, CV= 3.9%).

7.2.3.2 Eccentric strength

The participants were seated on the dynamometer chair (Con-Trex MJ, CMV AG, Dübendorf, Switzerland) with a hip angle of 60° (0°= full extension). This was the most reclined position that could be obtained without participants sliding forwards during contractions. However, this hip angle is similar to that during late swing phase in sprinting (Guex et al., 2012). Two 3-point belts secured the torso and additional straps tightly secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint with the dynamometer rotational axis was performed during isometric contractions of >50% of isometric strength at a knee joint angle of ~65°. The dynamometer's shin brace was placed posterior to the shank ~2 cm above the medial malleolus before the shank was tightly secured to the dynamometer lever arm. The range of motion was established and anatomical zero was set at full extension of the knee joint. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque.

Participants performed a standardized warm-up protocol with five submaximal contractions of progressively higher intensity. Following the warm-up, the knee flexors were tested at two different velocities using a protocol of concentric-eccentric contractions at 50° s^{-1} and 350° s^{-1} in this order. Participants performed 3 sets of 2, and 3 sets of 3, concentric-eccentric contractions at 50° s^{-1} and 350° s^{-1} respectively, over ~100° of range of motion. There was ≥1 min rest between each set and ≥2 min rest between velocities. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen.

The torque, crank angle and crank velocity signals were sampled at 2000 Hz with a PC using Spike 2 software (CED, Cambridge, UK) and the data were smoothed with a finite impulse response filter at 15 Hz. The acceleration and deceleration phases were excluded in order to disregard torque overshoot during these phases (Schwartz et al., 2010) and the constant isovelocity period (within ±5% of the prescribed crank angular velocity) was identified. Finally, eccentric strength was defined as the highest instantaneous torque recorded within the isovelocity range of any eccentric contraction.

7.2.3.3 Magnetic resonance imaging (MRI)

A 1.5 T MRI scanner (Signa HDxt, GE) was used to scan the dominant leg in the supine position with the hip and knee joints extended. T1-weighted axial plane images were acquired from the anterior superior iliac spine to the knee joint space in two blocks and oil filled capsules were placed on the lateral side of the participants' thigh to help with the alignment of the blocks during analysis. The following imaging parameters were used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm. MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland).

BFlh aponeurosis area was defined as the contact interface distance between the BFlh muscle and the proximal aponeurosis outlined in each image where the aponeurosis was identifiable, multiplied by the slice thickness (Fig. 7.2A). The contact interface distance in each slice included both the internal and external aponeurosis. The BFlh aponeurosis:muscle area ratio was calculated by dividing the BFlh proximal aponeurosis area by the BFlh muscle ACSAmax (see below). In order to produce average muscle-aponeurosis contact interface distance data for the cohort, individual values were normalised to muscle length. This involved interpolation of individual muscle-aponeurosis contact interface data every 5% of muscle length. BFlh aponeurosis length was calculated as the sum of the slices where the aponeurosis was identifiable, multiplied by the slice thickness. For comparison with previously published data BFlh aponeurosis width was measured according to the methods of Handsfield et al. (2010) i.e. width of the aponeurosis in the most distal image at which the proximal aponeurosis was external of the BFlh.

The BFlh, biceps femoris short head, semitendinosus and semimembranosus muscles were manually outlined in every third image starting from the most proximal image in which the muscle appeared. The largest anatomical cross-sectional area of each muscle was defined as ACSAmax and muscle volume was calculated using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). To validate the use of every third image for the volume calculations, all images from six randomly selected participants were analysed and the two methods (all images vs. every third image) were compared. The average difference between methods was 1.52% (0.22 cm^2) for BFlh ACSAmax, 1.60% (0.20 cm^2) for total hamstrings ACSAmax, 0.30% (0.66 cm^3) for BFlh volume and 0.18% (1.52 cm^3) for total hamstrings muscle volume. BFlh muscle length was calculated as the sum of all images where the muscle appeared multiplied by the slice thickness. BFlh/ST proximal tendon CSA was measured in the image immediately before the first image in which the ST muscle appeared (Fig 7.2B). All manual segmentation measurements were completed by the same investigator. To examine reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later. The CV was on average 4.0% for the aponeurosis contact area, 0.6% for muscle volume, 1.1% for ACSAmax, and 5.5% for the BFlh/ST proximal tendon CSA.

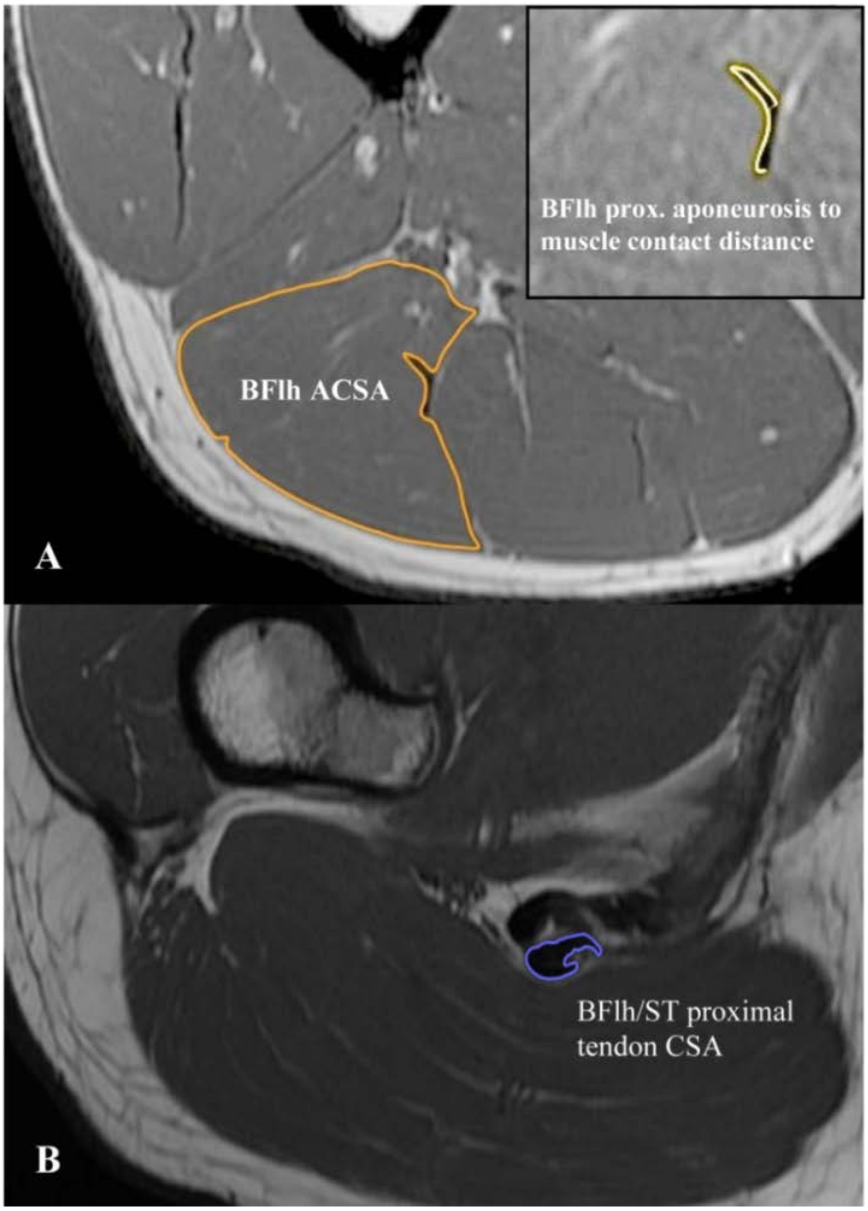

Figure 7.2. Example MR images of (A) BFlh ACSA (main) and BFlh proximal aponeurosis to muscle contact distance (inset) and (B) measurement of the BFlh/ST proximal tendon CSA.

7.2.4 Statistical analysis

One participant did not complete the eccentric strength assessment. Data are presented as mean ± SD. The bivariate relationships between the size of the different MTU components and the relationships between the size of the MTU components and the knee flexor strength measures were examined using Pearson product moment correlations between the dependent variables and the level of significance was set at $P < 0.05$. All statistical procedures were performed with IBM SPSS 20 (IBM Corporation, Armonk, NY).

7.3 RESULTS

7.3.1 Descriptive data on size of the MTU components and knee flexor strength

BFlh proximal aponeurosis area varied considerably (>4-fold) between participants ranging from 7.5 to 33.5 cm^2 (20.4 ± 5.4 cm^2, CV= 26.6%). This was a reflection of the fact that aponeurosis length was variable (16.7 ± 2.8 cm, range= 10.5-22.0 cm or 43–75% of muscle length) and muscle-aponeurosis contact interface distance was also variable along the aponeurosis length (Fig. 7.3).

Individual aponeurosis width measurements using a previously published method (Handsfield et al., 2010) appeared to occur at an arbitrary point along the aponeurosis (Fig 7.3A, i.e. not at peak aponeurosis width or a consistent point along the aponeurosis). Aponeurosis width measured in this way was 0.43 ± 0.24 cm (range= 0.19–1.22 cm, CV= 56.4%).

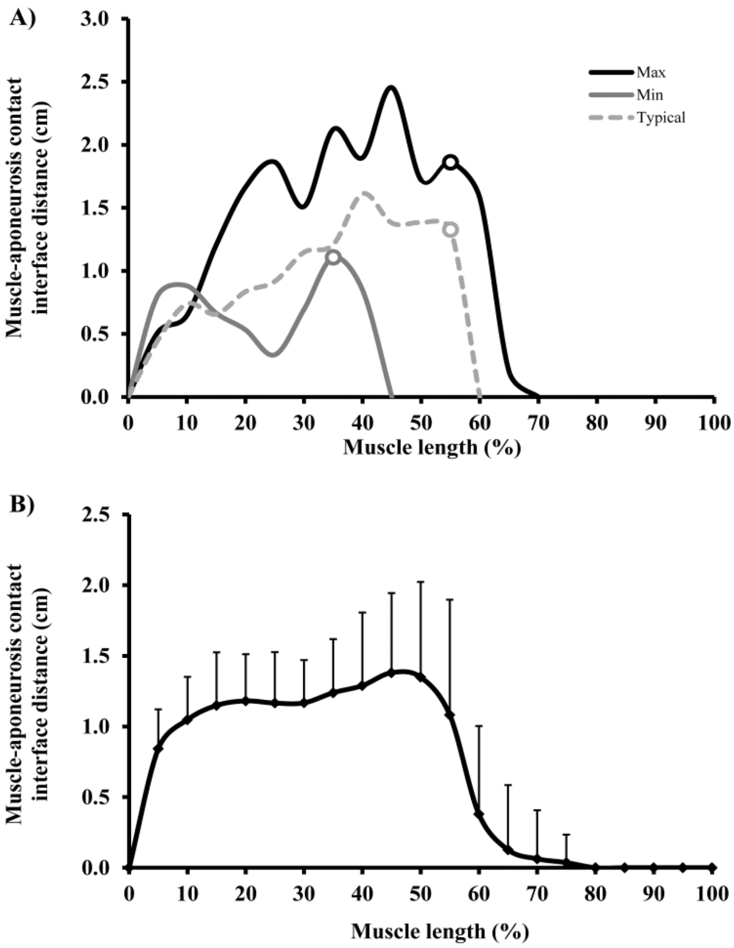

Figure 7.3. Muscle-aponeurosis contact interface distance along the length of the BFlh muscle (interpolated data every 5% of muscle length). (A) Three individual participants

(Maximum, minimum and typical (mid-range) aponeurosis area). The circles indicate the relative muscle length where the aponeurosis width measurement was performed on each individual. (B) Group mean + SD.

Participants had a mean BFlh ACSAmax of 13.6 ± 2.2 cm^2 (CV= 16.2%) while their BFlh muscle volume was 214.7 ± 37.2 cm^3 (CV= 17.3%). BFlh muscle length was 29.3 ± 2.6 cm. Large inter-individual variability was also found in proximal BFlh/ST tendon CSA (0.43 ± 0.14 cm^2, range= 0.25-0.91 cm^2, CV= 32.3%). In respect of the strength measurements, knee flexor isometric strength was 131.0 ± 19.9 Nm while eccentric strength was 134.3 ± 24.9 Nm at 50° s^{-1} and 118.2 ± 21.6 Nm at 350° s^{-1}.

7.3.2 Relationships between the size of the different MTU components

BFlh proximal aponeurosis area was not related to BFlh ACSAmax (r= 0.04, *P*= 0.830; Fig. 7.4) or volume (r= 0.35, *P*= 0.055). Consequently, the aponeurosis:muscle area ratio also exhibited high variability (6-fold), being 83% smaller in one individual than another (range 0.53 to 3.09, CV= 32.5%). BFlh proximal aponeurosis area presented a weak correlation with proximal BFlh/ST tendon CSA (r= 0.36, *P*= 0.049).

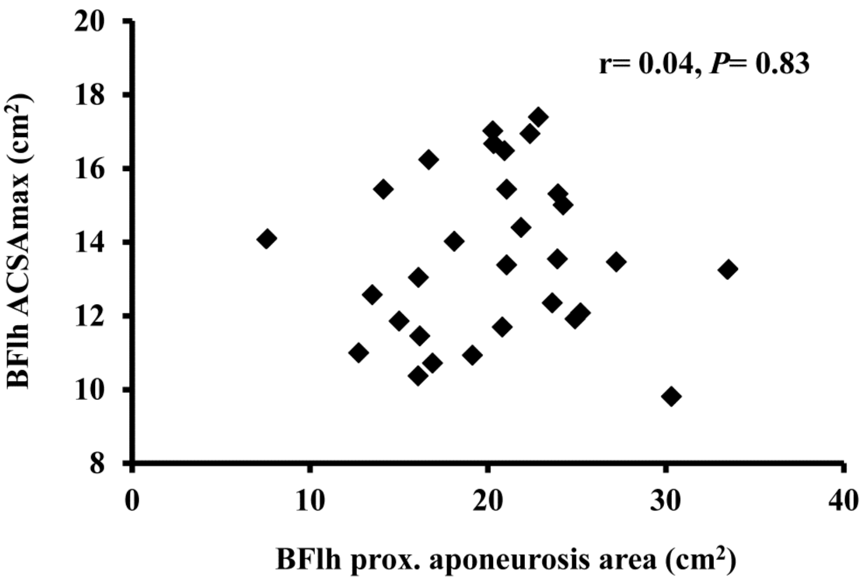

Figure 7.4. A scatter plot of BFlh proximal aponeurosis area and BFlh ACSAmax (n= 30). The individuals with the lowest and highest aponeurosis size (7.5 vs. 33.5 cm^2; >4-fold difference), had very similar sized BFlh muscles (ACSAmax, 14.1 vs. 13.3 cm^2) and thus aponeurosis:muscle size ratios of 0.53 vs. 2.52.

7.3.3 Relationships between the size of the MTU components with knee flexor strength

Whilst isometric strength was related to BFlh muscle ACSAmax and tendon CSA, there was no relationship with aponeurosis area (Fig. 7.5). Eccentric strength at both slow and fast velocities was related to BFlh muscle ACSAmax but not to aponeurosis area or tendon CSA (Table 7.1). Finally, overall hamstrings ACSAmax was related to isometric strength as well as to eccentric strength at 50° s^{-1} and 350° s^{-1} (Table 7.1).

Table 7.1. Bivariate correlations (r-values) between the size of the hamstrings muscle group and different components of the biceps femoris long head muscle-tendon unit with knee flexor isometric (n= 30) and eccentric (n= 29) strength. ACSAmax, maximal anatomical cross-sectional area; CSA, cross-sectional area. * *P*< 0.05, ** *P*< 0.01.

		Hamstrings	Biceps femoris long head		
		Muscle ACSAmax	**Muscle ACSAmax**	**Aponeurosis area**	**Tendon CSA**
Isometric strength		0.45*	0.42*	0.28	0.44*
Eccentric strength	**50**° s^{-1}	0.63**	0.44*	0.24	0.29
	350° s^{-1}	0.56**	0.50**	0.24	0.13

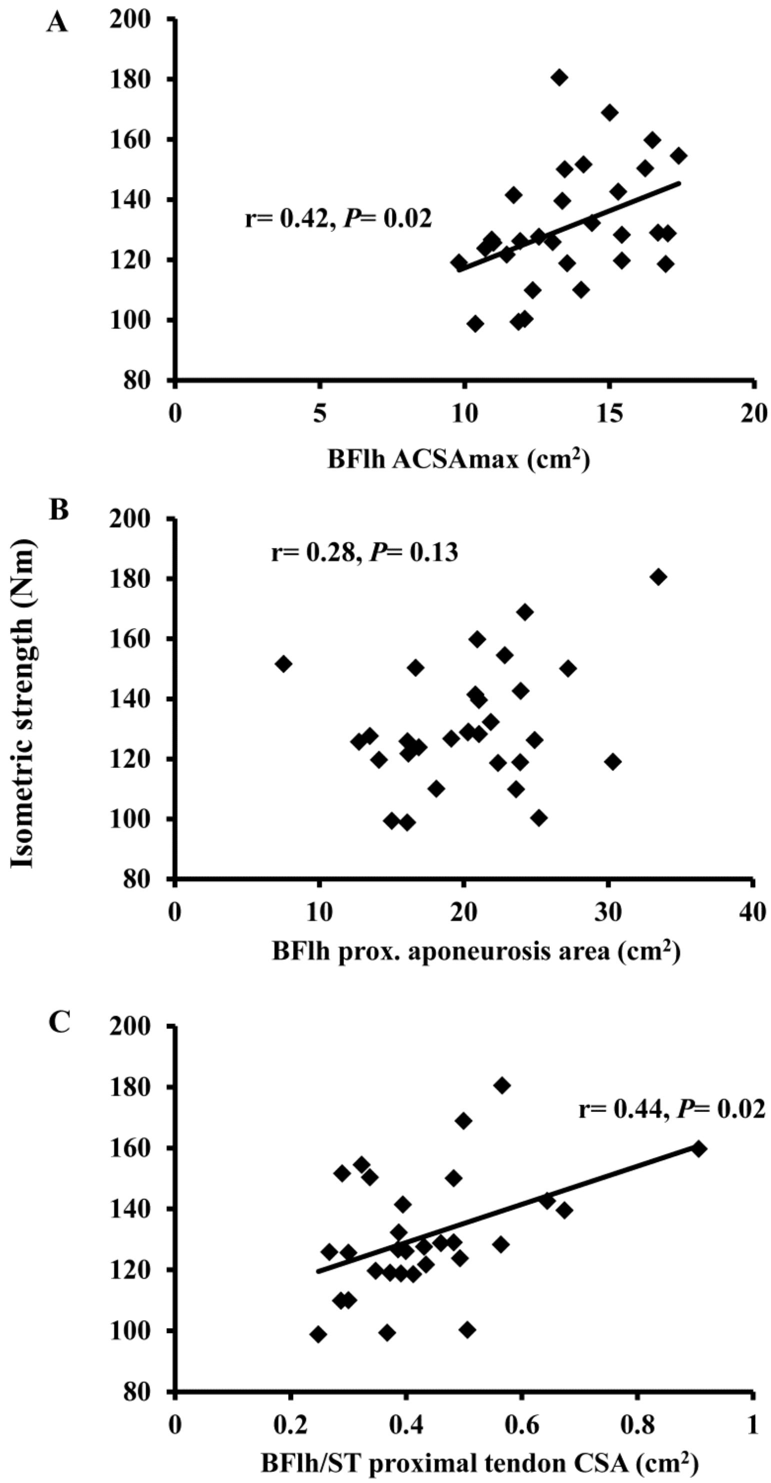

Figure 7.5. Scatter plots between knee flexors isometric strength and (A) BFlh ACSAmax, (B) BFlh proximal aponeurosis area and C) BFlh/ST proximal tendon CSA (n= 30).

7.4 DISCUSSION

The present study examined the variability of the BFlh proximal aponeurosis size in healthy, recreationally active population and the relationships of the aponeurosis size with BFlh muscle size and knee flexor function. The main finding was that the proximal aponeurosis size was highly variable between individuals and, in contrast to our hypothesis, it was not related to muscle size or knee flexor maximal isometric or eccentric strength. The disproportion between aponeurosis size and muscle size/strength suggests that individuals with a relatively small aponeurosis will be subject to greater mechanical strain in the muscle tissue surrounding the aponeurosis which may predispose them to hamstrings strain injuries.

Despite the homogenous nature of the recruited cohort there was a large 4.5-fold variability in BFlh proximal aponeurosis area between participants that was substantially greater than the variability in BFlh muscle ACSAmax (1.8-fold). Moreover, contrary to our hypothesis these two variables were unrelated, and consequently the aponeurosis:muscle area ratio in this study ranged from 0.53 to 3.09 exhibiting a 6-fold range, and being 83% smaller in one individual than another even within this relatively homogenous cohort. Interestingly, the individuals with the lowest and highest aponeurosis area in this study (7.5 and 33.5 cm^2 respectively) had similar BFlh ACSAmax (14.1 and 13.3 cm^2; Fig. 7.4) and consequently their aponeurosis:muscle area ratios were 0.53 and 2.52. Similarly, from a functional perspective, our results showed that aponeurosis size was unrelated to knee flexor strength, whilst the size of the other components of the MTU (muscle and tendon area) was associated with muscle strength (Fig. 7.5).

The lack of relationship between aponeurosis size and muscle size may have important implications for the mechanical strain within the muscle tissue surrounding the aponeurosis. Based on modelling and *in vivo* measurements of mechanical strain, individuals with a relatively small aponeurosis:muscle size ratio would be expected to experience greater mechanical strain in the muscle tissue adjacent to the aponeurosis with a greater potential for injurious muscle strains (Fiorentino et al., 2014; Fiorentino et al., 2012; Rehorn and Blemker, 2010). Therefore, our results in combination with the fact that hamstrings strain injuries typically occur near the BFlh proximal MTJ (Koulouris and Connell, 2003) suggest that individuals with a low aponeurosis:muscle size ratio may be at an increased risk of hamstrings strain injury.

This study is the first to directly examine the relationship between BFlh muscle and aponeurosis size. The only similar data we are aware of examined a different muscle and found VL aponeurosis area in a cohort of elite weightlifters and recreationally active males to be strongly related to total quadriceps muscle volume (R^2= 0.85) (Abe et al., 2012). Whilst the adaptation of tendon in response to resistance training has been shown with increases in size and stiffness (Kongsgaard et al., 2007; Seynnes et al., 2009), it remains unknown whether the aponeurosis shows similar plasticity. The greater VL aponeurosis area exhibited by the elite weightlifters compared to the recreationally active students in the study of Abe et al (2012) suggests that this difference may be an adaptive response to resistance training. If this were the case BFlh aponeurosis size may be modifiable through training and this could reduce the risk of strain injury. Interestingly, Wakahara et al. (2015) found a small increase in vastus lateralis aponeurosis width (1.9%, P= 0.050) after 12 weeks of resistance training. However, these results should be treated with caution due to methodological limitations in their study (small training cohort, n= 11, no correction for multiple tests). Nevertheless, the notion that strength training may increase the aponeurosis area has significant implications as individuals with a small aponeurosis relative to their BFlh muscle size may be able to increase their aponeurosis size and reduce the mechanical strains therein. This could be a powerful injury prevention tool that could be put in place prior to strain injury occurrence in at-risk individuals. Future studies should further examine the effect of strength training on aponeurosis size.

For comparative reasons, we measured aponeurosis width replicating the methods of a preliminary report (Handsfield et al., 2010) and the range of values obtained here (0.19–1.22 cm) were similar with their results. However, this method involved the measurement of aponeurosis width at an arbitrary point which corresponded to different relative positions along both the aponeurosis and muscle for each individual (Fig. 7.3A), nor was it the point of peak muscle-aponeurosis contact interface distance. These limitations in the width measurement do not allow for any valid comparison with aponeurosis area or examination of the differences between individuals. Furthermore, the individuals exhibiting the lowest and highest aponeurosis area (>4-fold difference) both had mid-range aponeurosis widths (0.32 vs. 0.63 cm; <2-fold difference). Therefore the aponeurosis width measurement appeared to be a limited reflection of aponeurosis size and inter-individual variability in this study.

Both aponeurosis and free tendon are considered to have a high safety factor (i.e. the ratio of failure stress to peak operating stress) such that the aponeurosis and tendon are capable of accommodating a range of loads well beyond the normal functional range with no risk of injury to these structures (Azizi et al., 2009; Biewener et al., 2005). This may partly explain the lack of relationship between muscle strength and aponeurosis size. Nevertheless, strain injuries typically occur within the muscle tissue adjacent to the aponeurosis rather than within the aponeurosis. Therefore, whilst a small aponeurosis may have a sufficient safety factor to preclude aponeurosis injury it could make the adjacent muscle tissue vulnerable to injury.

An interesting observation made during the analysis of the MR images was that the BFlh aponeurosis extends not only longitudinally along the side of the muscle belly but also transversely into the muscle (Fig. 7.2A), in agreement with a previous report (Fiorentino et al., 2012). Anecdotally, the proportion of the internal aponeurosis to the total aponeurosis area between our participants appeared highly variable. However, it is currently unknown how this aponeurosis morphology affects force transmission and stress distribution and further study is needed to elucidate its relationship with muscle size and strength.

Despite the large number of studies examining possible risk factors for strain injuries, it is still unclear how to identify individuals at high risk of strain injury, especially those with no history of injury. The emerging evidence that aponeurosis size may be a risk factor for such injuries has significant implications. Establishment of such an anatomical feature as a risk factor would greatly help to distinguish at-risk individuals before an injury occurs. For that reason, a prospective study investigating aponeurosis area relative to muscle size and strength and recording which athletes go on to suffer a strain injury would provide valuable information. Also, the possible interaction of aponeurosis area with other established risk factors (e.g. previous strain injury and strength imbalances) should be considered.

Some limitations of this study have to be considered. First, the knee joint axis of rotation was assumed to be passing through the knee joint space which was identified using superficial anatomy. It was also assumed that knee flexors strength measurement *in vivo* reflected the force generating capacity of BFlh muscle and the forces transmitted by the proximal aponeurosis.

In conclusion, the present study showed that the BFlh proximal aponeurosis size exhibits high variability within a relatively homogenous cohort of healthy young men and it was not related to muscle size or knee flexor strength. Therefore, individuals with a relatively small aponeurosis may be at increased risk of hamstrings strain injury.

Chapter 8

General Discussion

8 CHAPTER 8 – GENERAL DISCUSSION

8.1 INTRODUCTION

The main aim of this thesis was to examine the hamstrings anatomy and its influence on knee flexor muscle function *in vivo* within young healthy men. A secondary aim was to better understand the implications of hamstrings anatomy and function, and their variability, in relation to the risk of strain injury. To address these aims, a series of studies were conducted and the main findings are as follows:

1. The functional, conventional and knee joint angle-specific isometric H:Q ratios exhibited good test-retest reliability examined with a short protocol that included muscle function measurements up to high angular velocities and joint positions that closely replicated the conditions of high injury risk (Chapter 3).
2. Football players did not exhibit any difference in angle-specific or peak torque H:Q ratios (isometric, functional or conventional) compared to recreationally active controls. In addition, knee extensor and flexor strength, relative to body mass, of footballers and controls was similar for all velocities, except concentric knee flexor strength at 400° s^{-1} (footballers +40%; $P < 0.01$) (Chapter 4).
3. Muscle volume explained 30-71% and 38-58% of the differences between individuals in knee extensors and flexors torque respectively across a range of velocities. A moderate correlation was also found between the volume of these antagonistic muscle groups (R^2= 0.41). Finally, the relative volume of the knee extensors and flexors explained a significant proportion of the variance in both the isometric (~20%) and high velocity functional (~31%) H:Q ratio (Chapter 5).
4. On average, BFlh exhibited a balanced MHC isoform distribution (47.1 ± 9.1% MHC-I and 52.9 ± 9.1% total MHC-II) in young healthy men, while BFlh MHC distribution was not related to any measure of knee flexor maximal or explosive strength (Chapter 6).
5. BFlh proximal aponeurosis area varied considerably between participants (>4-fold) and was not related to BFlh ACSAmax (r= 0.04, P= 0.83). Consequently, the aponeurosis:muscle area ratio (defined as BFlh proximal aponeurosis area divided by BFlh ACSAmax) exhibited 6-fold variability (range, 0.53 to 3.09; CV= 32.5%), being 83% smaller in one individual than another. Moreover, aponeurosis size was not related

to isometric (r= 0.28, P= 0.13) or eccentric knee flexion strength (r= 0.24, $P \geq 0.20$) (Chapter 7).

8.2 HAMSTRINGS MUSCLE FUNCTION: REPLICATING THE BIOMECHANICS OF THE LATE SWING PHASE OF SPRINTING

The first step towards a better understanding of the role of knee flexors muscle function on hamstrings injuries is to assess muscle function in conditions that replicate the time when the injury occurs. Therefore, it is important to examine the knee flexors muscle function in biomechanical conditions that closely replicate those during the late swing phase of sprinting. However, most studies in the literature on hamstrings function have not accounted for these conditions. For this reason, an initial step was to develop and assess the reliability of a more ecologically valid protocol for the assessment of knee flexor function under relevant biomechanical conditions (Chapter 3). Isokinetic dynamometry, within its limitations, allows for the examination of muscle function across the torque-velocity relationship, and more importantly during eccentric contractions at controlled velocities, while the investigator can also control the hip and knee joint positions. The adoption of a seated position with a reclined back rest at 120° was selected as the most representative of the hip joint angle during the late swing phase (Fig. 2.5A), without the participants sliding forwards during contractions.

Current isokinetic dynamometers cannot reach angular velocities higher than 500° s^{-1}, which is less than half of that attained by the knee joint in sprinting (>1200° s^{-1}, Higashihara et al., 2010). However, valid isokinetic dynamometer data can be obtained only within the isovelocity phase, after the exclusion of the acceleration and deceleration phases (Baltzopoulos et al., 2012). Further, any increase of the target velocity will also increase the acceleration and deceleration phases, resulting in a progressively narrower isovelocity window. The isovelocity window for the highest angular velocity used in this thesis (400° s^{-1}) was ~20°. The application of Gaussian fitting to the raw torque data allowed mild extrapolation (5° on each side) resulting in a final isovelocity window of 30° for that velocity. The results presented in Chapter 3 suggest that the assessment of hamstrings and quadriceps torque-velocity relationships exhibited acceptable test-retest reliability when examined using a testing position that resembled the hip and knee joint angles during the late swing phase and up to high angular velocities. Therefore this protocol was applied to a subsequent study (Chapter 4).

However, due to the specific aims of the later studies (Chapters 5-7) the isokinetic protocol used in these experiments was further amended as follows:

1. Two familiarisation sessions were introduced before the isovelocity measurements.
2. The highest angular velocity was reduced to 350° s^{-1} in order to provide a sufficient isovelocity range for the examination of muscle function.

It has been shown that the discrepancy between crank angle and knee joint angle during isometric and isovelocity contractions can be up to 20° for knee extension (Tsaopoulos et al., 2011; Arampatzis et al., 2004; Kaufman et al., 1995), while similar differences might be expected for knee flexion. These discrepancies would invalidate an angle-specific approach to the examination of reciprocal muscle function and specifically H:Q strength ratios which was the main focus of Chapter 4. Therefore, the actual knee joint angles were calculated from video analysis in Chapters 3 and 4.

Any discrepancy between the knee joint and crank angle may also influence the torque measurements as the torque recorded by the dynamometer is different from that exerted by the muscle (or muscle group) under investigation (Herzog, 1988). The dynamometer torque is equal to the muscle force applied on the crank arm multiplied by the perpendicular distance from the axis of rotation of the crank to the line of force application (dynamometer moment arm). However, the torque exerted by the muscle is equal to the muscle force multiplied by the perpendicular distance from the knee joint axis of rotation to the line of force application (leg moment arm). A difference between the knee joint and the crank axis of rotation would influence the torque recorded by the dynamometer. The accurate identification of the knee joint axis can be achieved only with advanced imaging techniques not easily accessible (e.g. real-time X-ray video recordings, Tsaopoulos et al., 2011). For this reason, superficial anatomy was used and skin markers were drawn to assist the digitisation process. Although skin movement during the contractions may introduce additional error in the alignment of the knee joint with the crank, this technique is generally acceptable. While the torque values presented in this thesis correspond to the dynamometer recording, great care was taken to minimise the error due to misalignment. Specifically, the assumed knee joint centre was carefully aligned with the crank rotational axis during isometric contraction (>50% MVF). Furthermore, this was done separately for each muscle group, a procedure that to our knowledge is novel during isokinetic measurements of the antagonistic knee joint muscles for the calculation of H:Q ratio. In addition, the participants' torso, pelvis, thigh and lower leg were tightly secured to the dynamometer.

Other factors that can influence the dynamometer torque measurements are the gravity effect and the moment of inertia of the crank and leg. The gravity effect has the most significant impact on torque measurements, and also influences knee extensor and flexor torque recording differently. For knee-joint angles between 90° and 180° (180°= full extension), gravity opposes the direction of force application during knee extensions and failing to correct for this effect, the knee extensor torque would be underestimated. The opposite is true for the knee flexor torque. To account for the effect of gravity, all torque measurements in this thesis were gravity corrected. Due to the exclusive use of the torque data within the isovelocity region (excluding the acceleration and deceleration phases), the effect of the moment of inertia of both the dynamometer crank and the leg were considered to be negligible (Herzog, 1988). It must be acknowledged however that a small discrepancy between the crank velocity and the leg velocity may have been present (Herzog, 1988).

For the examination of the knee flexors explosive strength (Chapter 6), a low-compliance custom-made dynamometer was used. Similar to isokinetic dynamometer measurements, the hip and knee joint angles (140° and 150° respectively) were selected based on their relevance to the late swing phase.

8.3 STRENGTH BALANCE AROUND THE KNEE JOINT AND IMPLICATIONS FOR HAMSTRINGS STRAIN INJURIES

Chapters 4 and 5 aimed to improve the understanding on the reciprocal strength balance around the knee joint and the factors that may influence this balance. Brocket et al. (2004) reported that athletes with a history of hamstrings strain injuries exhibited an angle of peak torque at more flexed knee joint angles compared to uninjured athletes, whilst there was no difference in peak torque H:Q strength ratio. While it is unclear whether the reduced angle of peak torque pre-existed or resulted from the injuries, this finding implies that a potentially harmful imbalance may be angle-specific and more pronounced at the extended knee joint angles. The angle-specific examination of the H:Q ratio of a high-risk cohort of university-level footballers did not reveal any intrinsic strength imbalance compared to a recreationally active control group (Chapter 4). These results imply that football practice and play does not lead to potentially hazardous strength imbalances as other studies have suggested (Iga et al., 2009; Tourny-Chollet and Leroy, 2002). In support to our results, professional football players also present higher functional H:Q ratio than lower level players (Fousekis et al., 2010; Cometti et al., 2001). Nevertheless, hamstrings strains remain one of the most prevalent injuries in football. The fact that most hamstrings strains in football occur during running or sprinting (Woods et al., 2004) precludes the notion that football-specific activities (e.g. kicking, tackling) may explain the high injury rates. It seems that the sprint-specific biomechanical conditions can lead to injury. However, exposure alone to these conditions is unlikely to be singularly responsible for strain injuries, and more likely it is the summation of a number of risk factors combined with the high strains and eccentric forces in sprinting, that could lead to an injury.

The angle-specific functional H:Q ratio in both football players and normal individuals was ≥1.0 throughout the range of motion at the intermediate and high velocities (Chapter 4). A ratio of 1.0, described as point of equality (Coombs et al., 2002), suggests that the knee flexors strength is sufficient to counterbalance knee extensor strength. However, there are some limitations of the H:Q ratio measurements that should be highlighted. First, functional relevance of the angle-specific H:Q ratio would appear to rely on the assumption that the hamstrings and quadriceps muscles are active simultaneously at the specified angles, and not sequentially active in different phases of the gait cycle. Simulation and EMG studies have shown that simultaneous activation of the hamstrings and quadriceps occurs only at the more extended knee joint angles, prior to the ground contact (Chumanov et al., 2007; Thelen et al., 2005; Kyrolainen et al., 1999). Therefore, at these knee joint angles calculation of an angle-specific H:Q strength ratio is relevant and informative of the reciprocal strength balance. In contrast, at the more flexed joint angles where no simultaneous activation of hamstrings and quadriceps occurs, the angle-specific ratio may not be functionally relevant. Another limitation of the H:Q ratio is that it assumes that the hamstrings function to counter knee extension generated by the quadriceps. However, during the late swing phase the knee extension occurs mainly due to the transfer of the angular momentum of the thigh to the shank (Yeow, 2013), and only at the more extended knee joint angles do the quadriceps actively contribute to the knee extension moment. Essentially, at the beginning of the late swing phase the hamstrings are counteracting the preceding hip flexor action (primarily due to rectus femoris and iliopsoas activation). Therefore a more complete examination of the strength balance, within the context of hamstrings strain injuries, should also account for the influence of the hip flexors. The above limitations of the H:Q ratio may explain the mixed results in the literature concerning its use as a risk factor for strain injuries. Prospective studies that include some measure of hip flexor strength may enhance our understanding of the association between strength imbalances and hamstrings strain injuries.

Despite the extensive use of the H:Q ratio, there has been very little attention on the factors that determine this ratio. In contrast to previous investigations (Akagi et al., 2014, 2012), the present results showed that the muscle size ratio of the quadriceps and hamstrings contributed significantly to their strength ratio (isometric, r= 0.45, *P*= 0.024; functional 350° s^{-1}, r= 0.56, *P*= 0.003, Chapter 5). In addition, examination of the range of H:Q volume ratio values (0.34-0.51) reveals that some individuals had 50% smaller hamstrings relative to quadriceps than other individuals. Together these results suggest that within normal, previously uninjured individuals some exhibit underlying size and strength imbalances that may predispose them to strain injury, and corrective strength training might be expected to mitigate this risk.

8.4 INFLUENCE OF HAMSTRINGS ANATOMY ON MUSCLE FUNCTION, AND POTENTIAL INFLUENCE ON INJURY PREDISPOSITION

The findings in Chapters 5, 6 and 7 highlight the importance of hamstrings muscle size as the main anatomical factor that influences knee flexors function *in vivo*, while muscle composition and aponeurosis size do not seem to have a significant influence.

An interesting finding in Chapter 5 was the differential influence of muscle size on hamstrings and quadriceps torque during different types of contractions and especially eccentric strength. Hamstrings muscle size explained ~50% (48-58%) of the interindividual differences in knee flexor eccentric strength, but quadriceps size did not influence knee extensors eccentric strength. This finding implies a much smaller influence of morphological factors on eccentric quadriceps strength and may suggest a greater influence of neural factors, compared to knee flexors. In this case neural inhibition could limit the eccentric activation of the quadriceps and effective utilisation of the available muscle mass. This suggestion is in contrast to previous studies where no difference in eccentric torque relative to isometric was found between the knee extensors and flexors (Pain et al., 2013). However, the smaller sample size examined in that study (n= 15), and the fact that their data for knee flexor were 'noticeably noisier' compared to the extensors may have influenced their results. Direct examination of the hypothesis for a differential influence of neural inhibition on knee extensors and flexors eccentric strength would provide valuable information on muscle function of the main muscle groups around the knee joint. However, it is methodologically challenging as direct stimulation of hamstrings through the sciatic nerve is prevented by the overlying gluteus maximus muscle, while transcutaneous stimulation induces high discomfort at relatively low levels of stimulation (Pain et al., 2013).

Chapter 6 provided some novel data on hamstrings MHC composition and revealed that in a relatively large, young population the BFlh muscle composition does not seem to be a likely explanation for the high rate of strain injuries in this muscle. In the literature, muscle composition has been speculated to be an explanation for the common incidence of hamstrings strain injury (Noonan and Garret, 1999; Garret et al., 1990, 1984), but there is no direct evidence to support these speculations. These speculations were largely based on an early examination of the hamstrings muscle composition in a small number of elderly cadavers (n= 10, Garret et al., 1984). In that study, the total hamstrings (44.8% type I and 55.2% type II fibres) and BFlh muscle composition (45.5% type I and 54.5% type II fibres) reported were very similar to the current study (47.1 ± 9.1% MHC-I and 52.9 ± 9.1% total MHC-II). However, based on small differences compared to other muscles (quadriceps, 51.9%; adductor magnus, 44.8% type II fibres) Garrett et al. (1984) argued that the 'high proportion' of fast fibres in the hamstrings compared to these other leg muscles may contribute to their susceptibility to injury. Yet, their claim is not supported by the existing data on *in vivo* muscle composition of the thigh muscles. Specifically, within a large cohort of physically active young men (n= 95) the VL muscle was found to contain a greater proportion of MHC-II isoform (66.1% total MHC-II, Staron et al., 2000) compared to the BFlh in our cohort. Yet, the vastus lateralis does not exhibit high strain injury rates. Consequently, the composition of the BFlh does not seem to explain the high incidence of strain injuries within this muscle compared to other muscles. Nevertheless, it must be acknowledged that on an individual basis, a high proportion of MCH-II isoform could still be a risk factor for hamstrings strain injuries. In the current study, some individuals exhibited >65% of total type II fibres and they may be more susceptible to strain injuries. Future investigations are needed to elucidate any direct relationship between muscle composition and risk for strain injury.

Chapter 6 also showed that BFlh muscle composition does not influence knee flexor maximal or explosive strength. Whilst no other data on *in vivo* hamstrings MHC or fibre type composition exist, these findings are in contrast to a number of studies reporting a significant influence for quadriceps (e.g. Gür et al., 2003; Aagaard & Andersen, 1998). This discrepancy can be largely explained by the examination of diverse athletic and training populations (Gür et al., 2003) or small cohorts (Aagaard & Andersen, 1998) in these previous investigations. The selection of individuals that were not involved in structured, systematic training in the current study is likely to have reduced the variability in other neuromuscular variables such as muscle size, architecture and neural drive that may confound the relationship between strength and muscle composition.

An expected relationship between the size of the force generator (muscle) and the force transmitters (tendon and aponeurosis) was not confirmed for the BFlh MTU, and some important implications arise from these findings. Chapter 7 showed that the BFlh proximal aponeurosis size is highly variable between healthy individuals (up to 4-fold in the studied cohort), and some individuals have a disproportionally small aponeurosis relative to muscle size, manifested by a low aponeurosis:muscle area ratio. These individuals would be expected to experience greater mechanical strains along the aponeurosis and potentially a greater risk of strain injury. Previous modelling and dynamic imaging studies support this suggestion as they have calculated increased strains in individuals with small proximal aponeurosis size (Fiorentino et al., 2012; Rehorn and Blemker, 2010). However, these studies based their calculations on a crude measurement of aponeurosis width (using a single MR image at an arbitrary point) that did not fully reflect the extent of the aponeurosis size variability. In contrast, the methods applied in the current study involved the delineation of the proximal aponeurosis-muscle contact interface distance in all images that the aponeurosis could be identified. In addition, this measurement included a previously observed (Fiorentino et al., 2012) but not quantified portion of the aponeurosis that extends into the muscle belly and forms a significant part of the total aponeurosis area in some individuals. It must be noted that the proximal aponeurosis presents a complex morphology and in some individuals it was difficult to accurately distinguish the aponeurosis from other structures (e.g. epimysium). Therefore, higher resolution MR images (e.g. 3T) are recommended for future work to minimise these limitations.

The results of a previous study on quadriceps imply that aponeurosis size may adapt in response to training load (Abe et al., 2012). Indeed, Wakahara et al. (2015) reported a small increase in vastus lateralis aponeurosis width (1.9%, P= 0.050) after 12 weeks of resistance training. However, the small training cohort (n= 11), the applied statistical methods (paired *t*-tests with no correction for multiple tests) and the borderline *P*-value suggest that their findings should be treated with caution. Nevertheless, the notion that strength training may increase the aponeurosis area has significant implications as individuals with a small aponeurosis relative to their BFlh muscle size may be able to increase their aponeurosis size and reduce the mechanical strains therein. This could be a powerful injury prevention tool that could be put in place prior to strain injury occurrence in at-risk individuals. Clearly, future studies should further examine the effect of strength training on aponeurosis size.

8.5 FUTURE RESEARCH

Following the findings in the present thesis, promising areas for further research on muscle function and hamstrings strain injury prevention have been revealed. Future research could:

1. Examine prospectively the changes in angle-specific and peak torque H:Q ratio in response to football practice.
2. Assess the hip and knee joint function and strength balance, and potential combinations to better understand the function of these muscles.
3. A prospective study of muscle strength and balance as risk factors for hamstrings strain injury that includes the hip flexors, in addition to the knee extensors and flexors, would greatly enhance our understanding of the role of strength imbalances in hamstrings strains.
4. Explore further the neuromuscular factors that contribute to the different relative eccentric strength of the knee extensors and flexors. A possible muscle-specific neural inhibition warrants further investigation. Development of an appropriate methodology for hamstrings electrical stimulation may be required.
5. Examine the relationship between BFlh muscle composition and the incidence of strain injuries prospectively in high-risk athletic populations.
6. Investigate prospectively the aponeurosis area relative to muscle size and strength and record which athletes go on to suffer a strain injury. Also, further investigations could explore the effect of strength training on BFlh proximal aponeurosis size.

Appendices

9 APPENDICES

9.1 APPENDIX A – ISOKINETIC DYNAMOMETRY

9.1.1 Position and stabilisation

The participants were seated on the dynamometer chair with a hip angle of 120° (180°= full extension). This hip angle was selected because of its relevance to high injury risk situations i.e. similar to the hip angle during late swing phase in sprinting (Guex et al., 2012) when hamstrings strains are thought to occur. While this hip joint angle is more ecologically valid compared to the hip angle typically used in isokinetic dynamometer testing of the knee joint muscles (95°-105°, Fig. 9.1), the more extended hip joint resulted in an increased difficulty to stabilise the hip joint during contractions. To minimise any excessive hip joint movement, a strap was placed across the pelvis, on the anterior superior iliac spine, that was in addition to the two 3-point built-in straps across the torso and pelvis. Care was taken not to cause any posterior pelvis tilt with the additional pelvis strap.

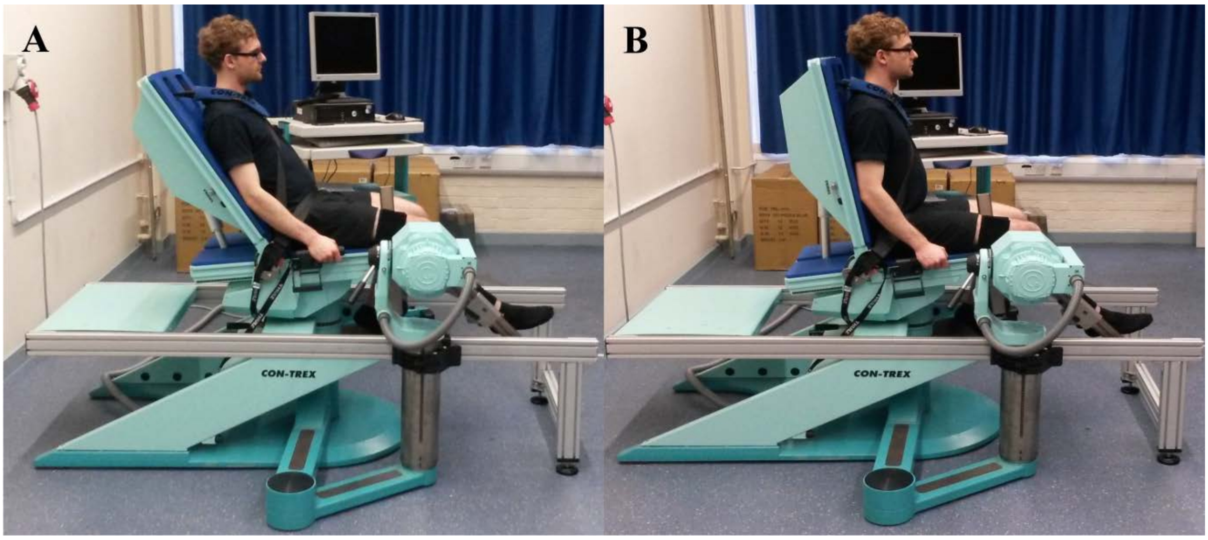

Figure 9.1. Hip joint angle of A) 120° (180°= full extension) replicating the late swing phase of sprinting and adopted in this thesis) and B) 95° which is typically used in knee joint isokinetic dynamometry testing.

To ensure maximal stabilisation of the participants, the distal thigh was also secured with a 10-cm wide velcro strap while a brace was placed in front of the non-involved leg. Finally, participants were instructed to grasp the handles next to the seat during maximal contractions.

The dynamometer's shin brace was placed ~2 cm above the medial malleolus, anterior to the shank for knee extension contractions and posterior for knee flexion contractions, prior to the shank being tightly secured to the dynamometer lever arm. During the knee extension contractions, an additional moulded rigid plastic shin pad, lined with 2 mm of high density foam, was tightly secured to the tibia to avoid any discomfort to the shin during maximum contractions.

9.1.2 Knee joint alignment and gravity correction

The alignment of the knee joint with the dynamometer rotational axis during active muscle contractions was done separately for knee extension and flexion contractions. Specifically, in each case the alignment was done during isometric contractions of >50% MVF at a knee joint angle of ~115°.

The range of motion was established and anatomical zero was set at the most (passively) extended position where participants felt comfortable and without excessive stretch of their hamstrings. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque by the dynamometer software. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen.

9.1.3 Ankle-joint position during knee flexion contractions

Gastrocnemius, due to its action at the knee joint as knee flexor, may influence the recorded torque during the isovelocity contractions. Therefore, the ankle-joint position during knee flexion contractions was consistent between participants. During pilot testing, it was observed that most participants preferred to maintain a dorsiflexed ankle joint during knee flexion contractions. Therefore, all individuals were instructed to maintain a dorsiflexed ankle joint during maximal knee flexion contractions (Fig 9.2).

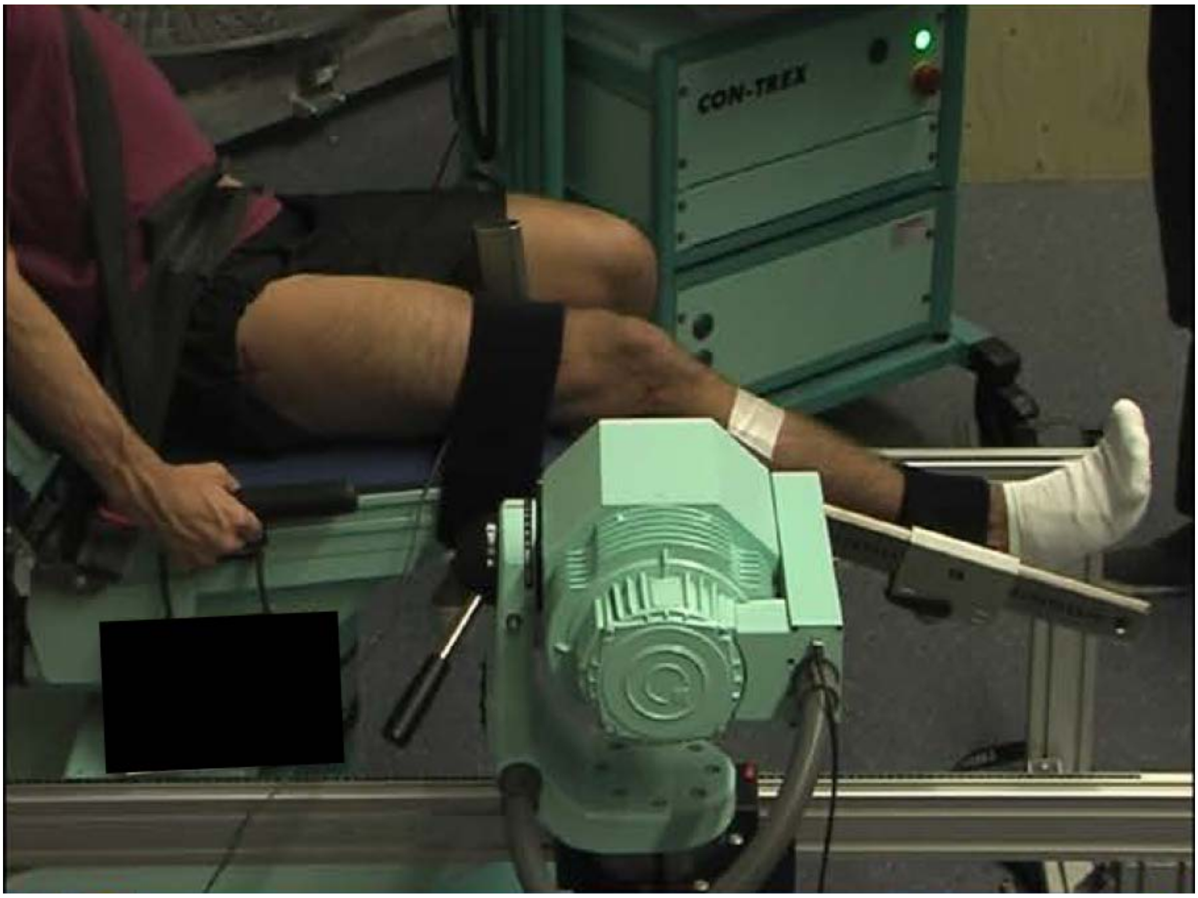

Figure 9.2. Example of the ankle-joint position during maximal knee flexion contraction. Participants were instructed to maintain a dorsiflexed ankle joint in order to control for the contribution of gastrocnemius to the knee flexor torque.

9.1.4 Isovelocity range identification

The acceleration and deceleration phases were excluded in order to disregard torque overshoot during these phases (Schwartz et al., 2010) and the constant isovelocity period (within ±10% (for Chapters 3 and 4) or ±5% (for Chapters 5-7) of the prescribed crank angular velocity) was identified (Fig.9.3-9.4).

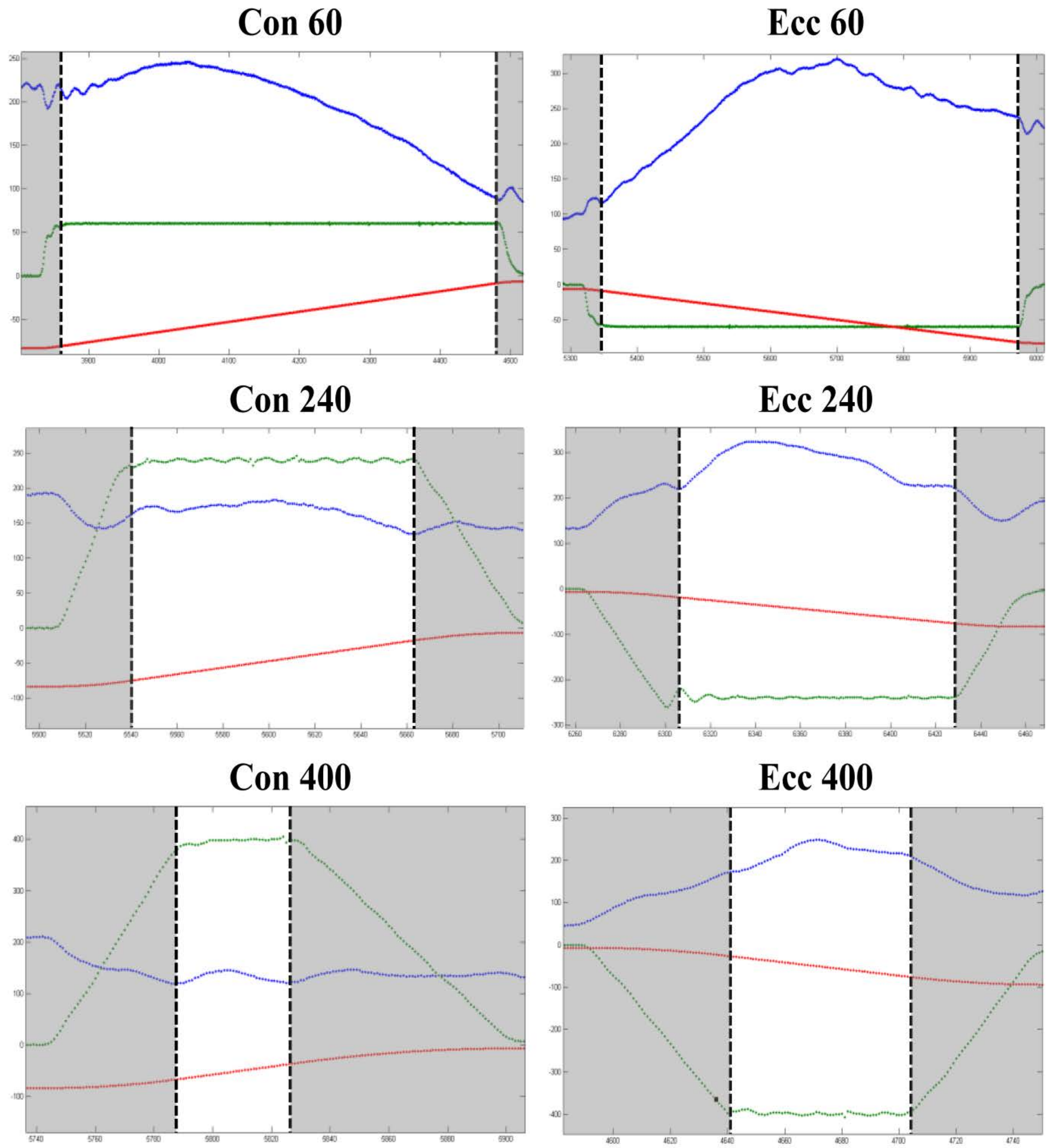

Figure 9.3. Example of the torque (blue), crank velocity (green) and crank angle (red) raw data during knee extensors concentric (Con) and eccentric (Ecc) isokinetic contractions at 60, 240 and 400° s^{-1}. The isovelocity data within ±10% of the prescribed crank velocity was identified by removing the acceleration and deceleration phases (grey areas).

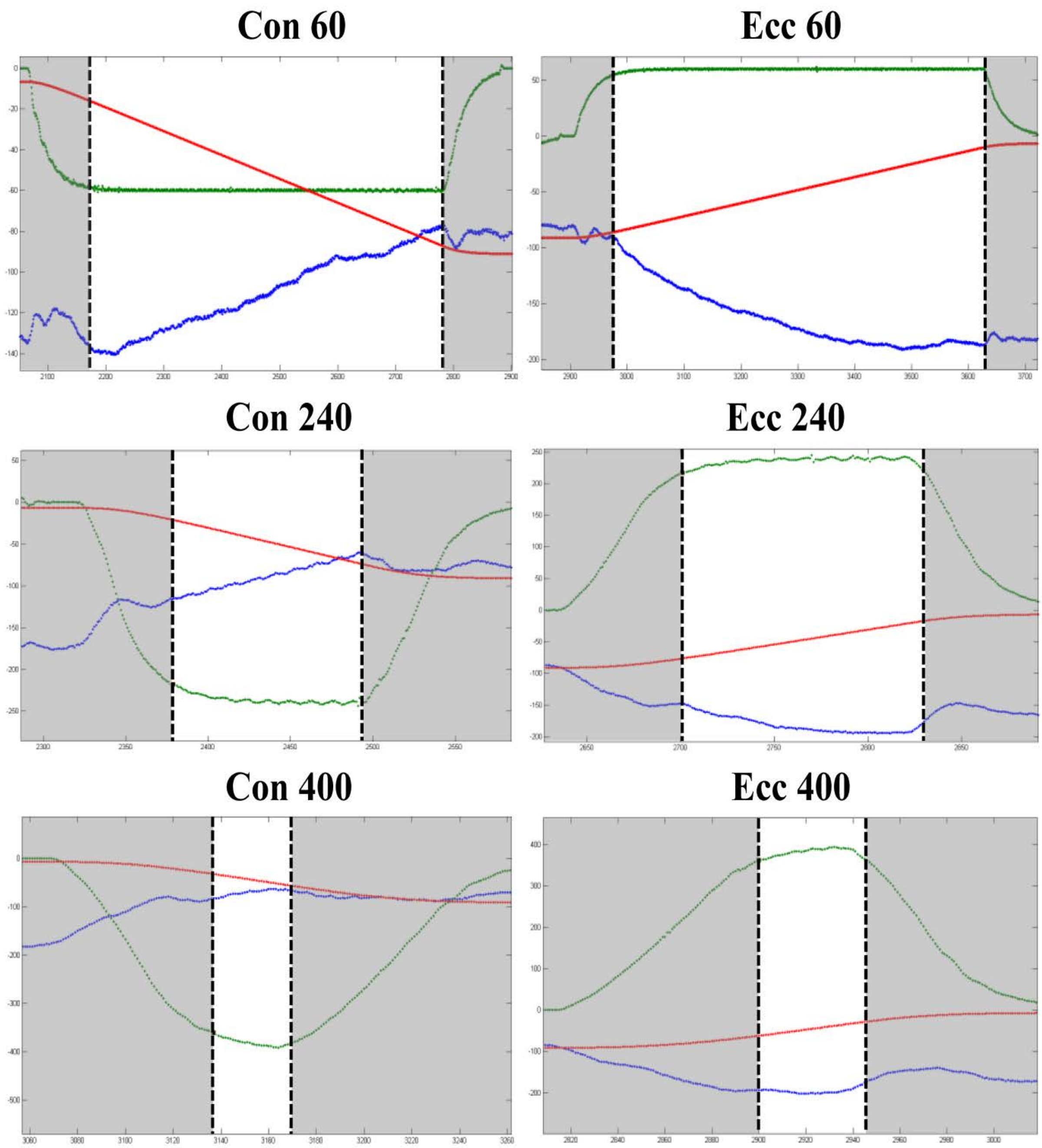

Figure 9.4. Example of the torque (blue), crank velocity (green) and crank angle (red) raw data during knee flexors concentric (Con) and eccentric (Ecc) isokinetic contractions at 60, 240 and 400° s^{-1}. The isovelocity data within ±10% of the prescribed crank velocity was identified by removing the acceleration and deceleration phases (grey areas).

9.1.5 Angle-specific torque

In Chapters 3 and 4, the angle-specific torque was calculated. For the isometric contractions (Chapter 3), this was done by smoothing torque-knee joint angle data for each muscle group by performing 2nd order polynomial fitting to the raw torque values. Then the polynomial fit was used to interpolate torque values for knee joint angles at 105, 120, 135, 150 and 165°. For the concentric and eccentric contractions (Chapter 4), the isovelocity torque-knee joint angle data at each velocity, for each muscle group was smoothed by performing Gaussian fitting (Forrester et al., 2011) using a root mean square method to minimise the error to the raw torque values (Fig. 9.5-9.6, Matlab, The Mathworks, Inc., Natick, MA, USA). Then the Gaussian fit was used to interpolate torque values for knee joint angles every 5° over the relevant isovelocity range for each angular velocity: 100-160° for 60° s^{-1}; 105-160° for 240° s^{-1}; and 115-145° for 400° s^{-1}. Data from contractions in which participants failed to maximally activate the examined muscle group throughout the range of motion were discarded.

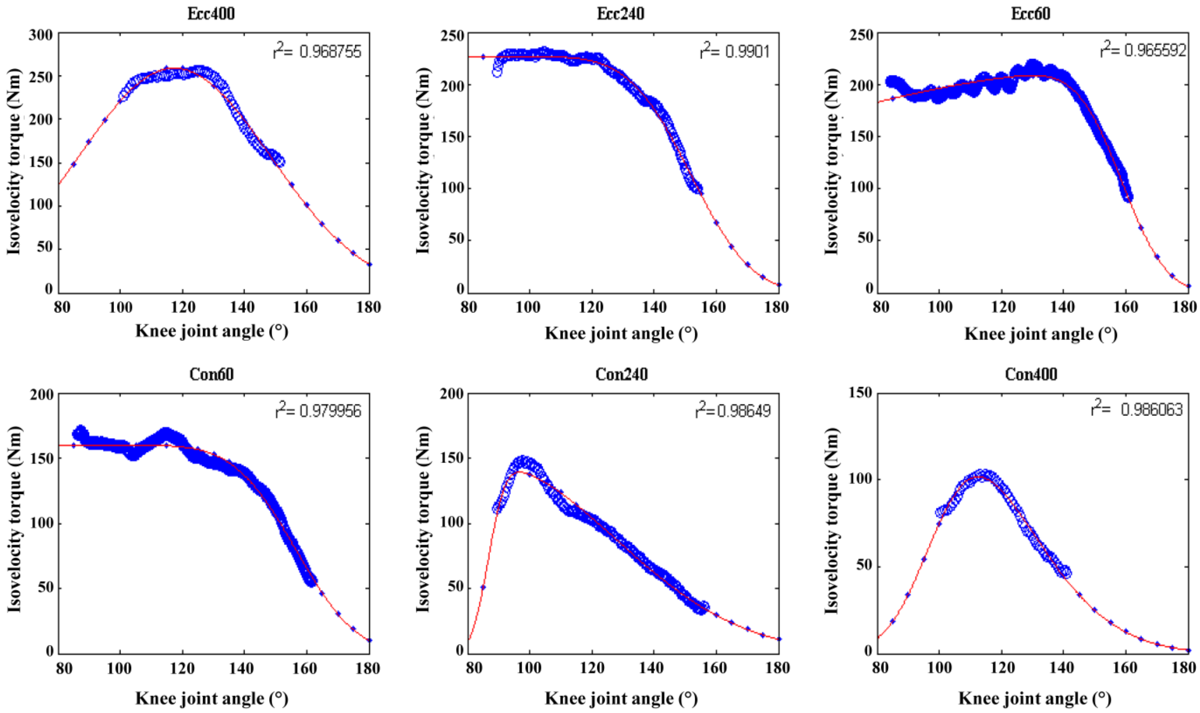

Figure 9.5. Example of Gaussian fitting on raw knee extensor concentric (Con) and eccentric (Ecc) isovelocity strength data at 60, 240 and 400° s^{-1}.

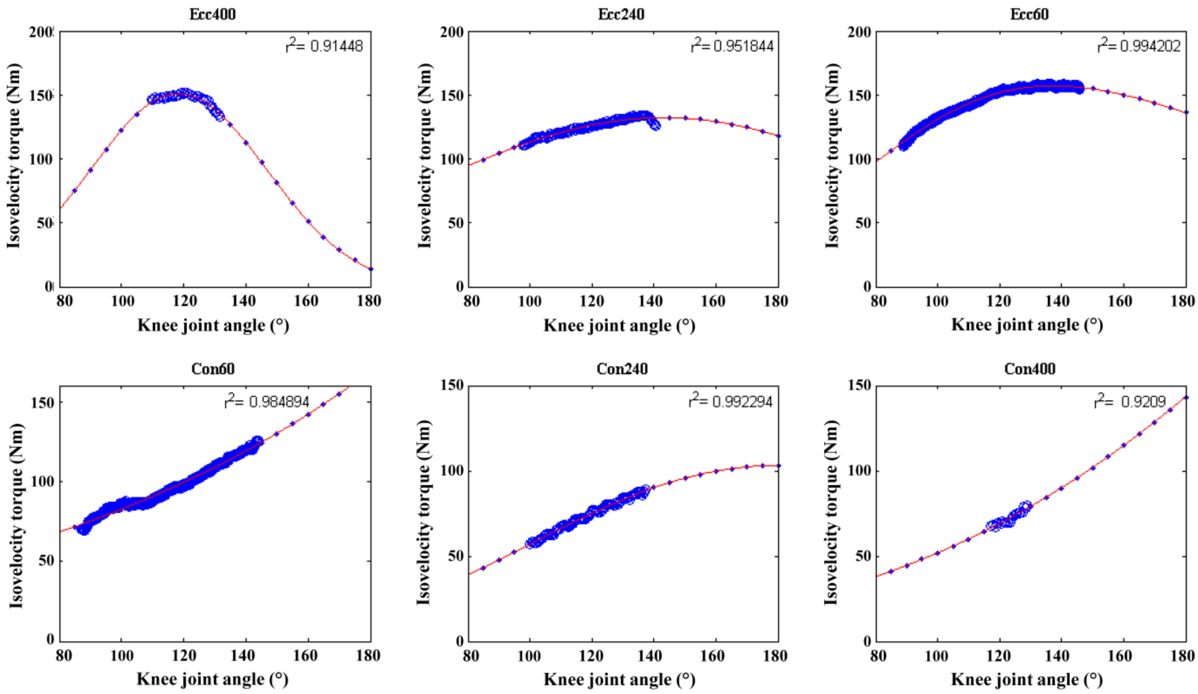

Figure 9.6. Example of Gaussian fitting on raw knee flexor concentric (Con) and eccentric (Ecc) isovelocity strength data at 60, 240 and 400° s^{-1}.

9.2 APPENDIX B – FORCE SIGNAL FILTERING

9.2.1 Explosive isometric contractions

The force signal was filtered with a 4th order Butterworth filter with a low pass cut-off frequency of 500 Hz. The frequencies <500 Hz were used as a reference envelope for detecting the force onset during the explosive contractions (Fig 9.7). A lower frequency filter would transform the signal into a gradually rising asymptotic curve, and therefore the sudden transition from rest to force production would be removed resulting in subjective and unreliable recognition of the force onset (Tillin et al., 2013).

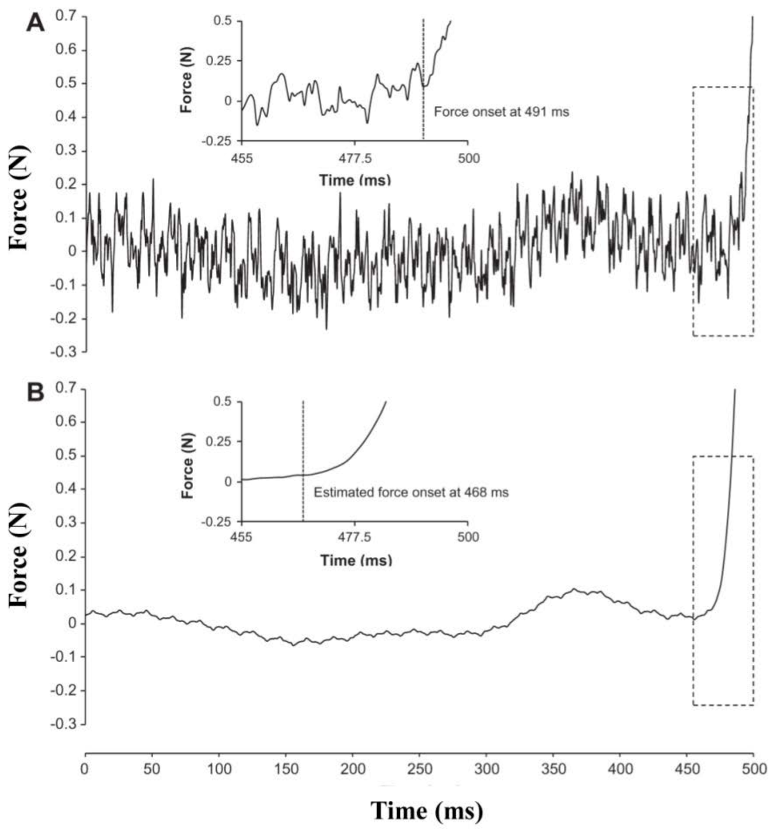

Figure 9.7. The force signal during knee flexors explosive isometric contractions was filtered with a 4th order Butterworth filter with a low pass cut-off filter of 500 Hz (A). The inclusion of frequencies up to 500 Hz provided a reference envelope that facilitated the accurate and reliable identification of the force onset. In contrast, application of a low frequency smoothing filter (e.g. 100-point moving average, B) would result in an asymptotic curve, making the identification subjective and unreliable (adapted from Tillin et al., 2013).

9.3 APPENDIX C – MAGNETIC RESONANCE IMAGING

9.3.1 Scanning parameters

A 1.5 T MRI scanner (Signa HDxt, GE) was used to scan the dominant leg in the supine position with the hip and knee joints extended. T1-weighted axial plane images were acquired from the anterior superior iliac spine to the knee joint space in two blocks and oil filled capsules were placed on the lateral side of the participants' thigh to help with the alignment of the blocks during analysis. The following imaging parameters were used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm. MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland).

9.3.2 MR images analysis

9.3.2.1 Muscle anatomical cross-sectional area and volume

MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland). The hamstrings (biceps femoris long head, biceps femoris short head, semitendinosus, semimembranosus) and quadriceps (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) muscles were manually outlined in every third image starting from the most proximal image in which the muscle appeared (Fig. 9.8). The largest anatomical cross-sectional area of each muscle was defined as ACSAmax and muscle volume was calculated using cubic spline interpolation to interpolate the CSA between the analysed images (Fig. 9.9, GraphPad Prism 6, GraphPad Software, Inc.). Two investigators conducted the image analysis and all manual segmentation measurements of each muscle were completed by the same investigator. To examine the reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later and the coefficient of variation (CV) was calculated. The CVs for measurements of muscle volume and ACSAmax were 0.5% and 1.2% (quadriceps), and 0.5% and 1.1% (hamstrings).

To validate the use of every third image for the volume calculations, all images from six randomly selected participants were analysed and the two methods (all images vs. every third image) were compared. The average difference between methods was 1.52% (0.22 cm^2) for BFlh ACSAmax, 1.60% (0.20 cm^2) for total hamstrings ACSAmax, 0.30% (0.66 cm^3) for BFlh volume and 0.18% (1.52 cm^3) for total hamstrings muscle volume.

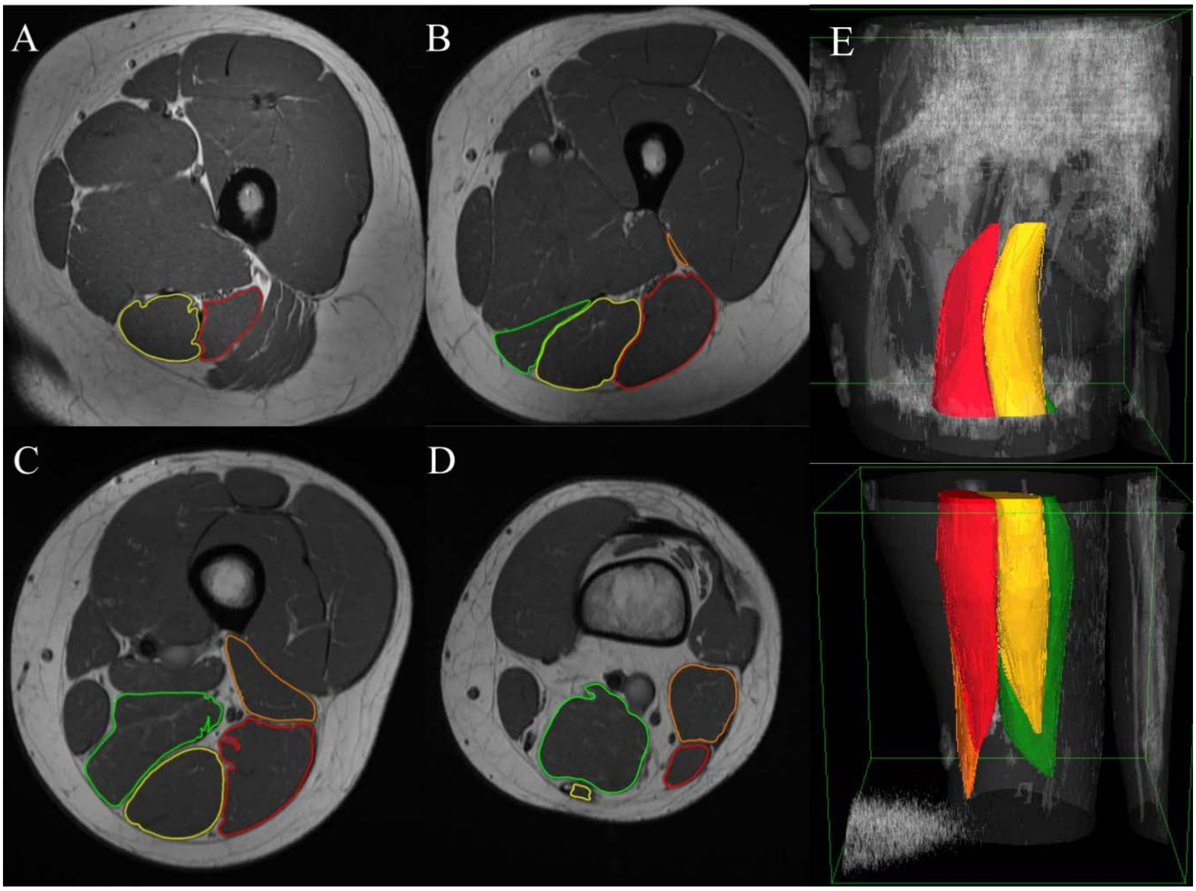

Figure 9.8. A-D) Example of (left) hamstrings muscles (biceps femoris long head (red), biceps femoris short head (orange), semitendinosus (yellow), semimembranosus (green)) segmentation in magnetic resonance images at 20, 40, 60 and 80% of hamstrings length (defined as the distance from the most proximal to the most distal image in which hamstrings were identified). E) Three-dimensional reconstruction of the hamstrings muscles (posterior view of the left leg).

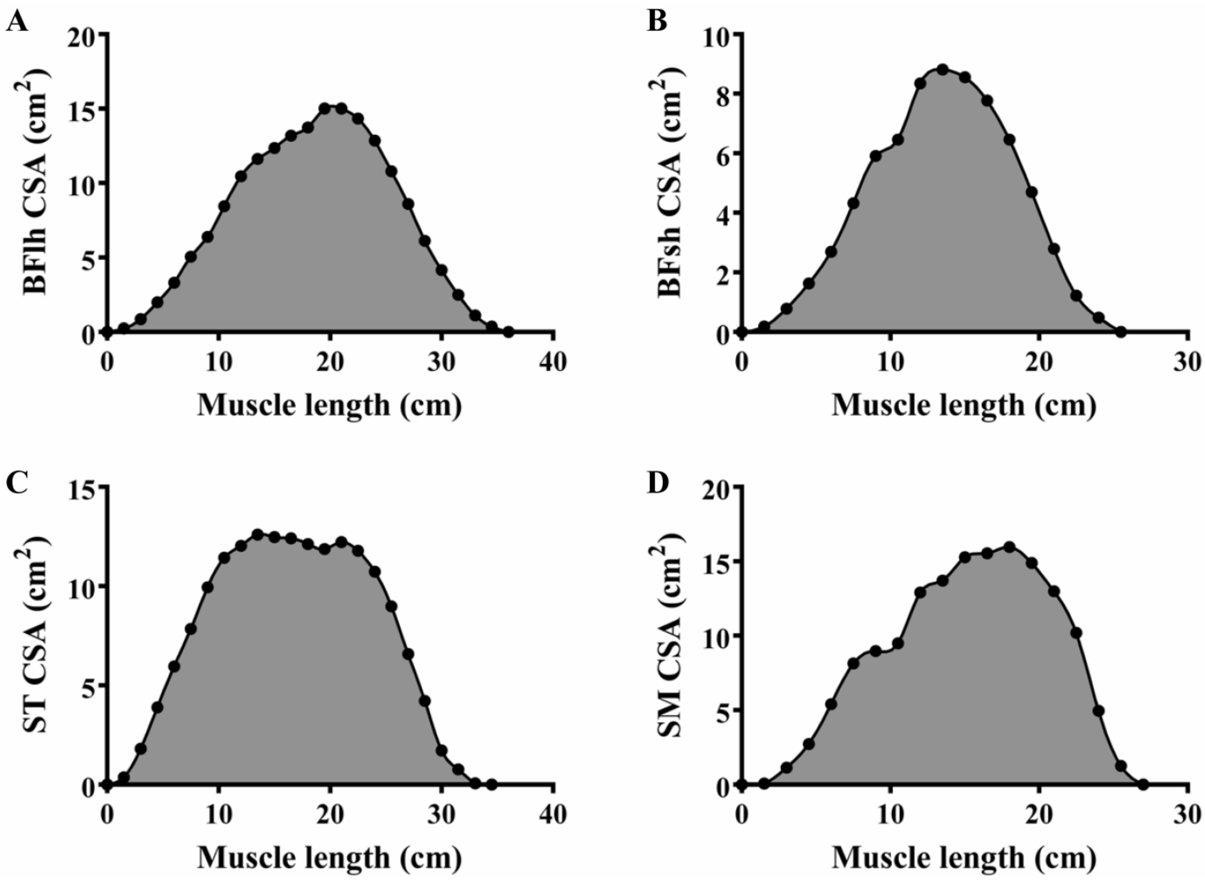

Figure 9.9. Example of hamstrings muscle volume calculation. Cubic spine interpolation was used to interpolate the muscle cross-sectional area (CSA) between the analysed magnetic resonance images and volume was calculated as the area under curve (grey area). BFlh: biceps femoris long head, BFsh: biceps femoris short head, ST: semitendinosus, SM: semimembranosus.

9.3.2.2 BFlh proximal aponeurosis area and BFlh/ST proximal tendon CSA

BFlh aponeurosis area was defined as the contact interface distance between the BFlh muscle and the proximal aponeurosis outlined in each image where the aponeurosis was identifiable, multiplied by the slice thickness (Fig. 9.10). The contact interface distance in each slice included both the internal and external aponeurosis. The BFlh aponeurosis:muscle area ratio was calculated by dividing the BFlh proximal aponeurosis area by the BFlh muscle ACSAmax (see above).

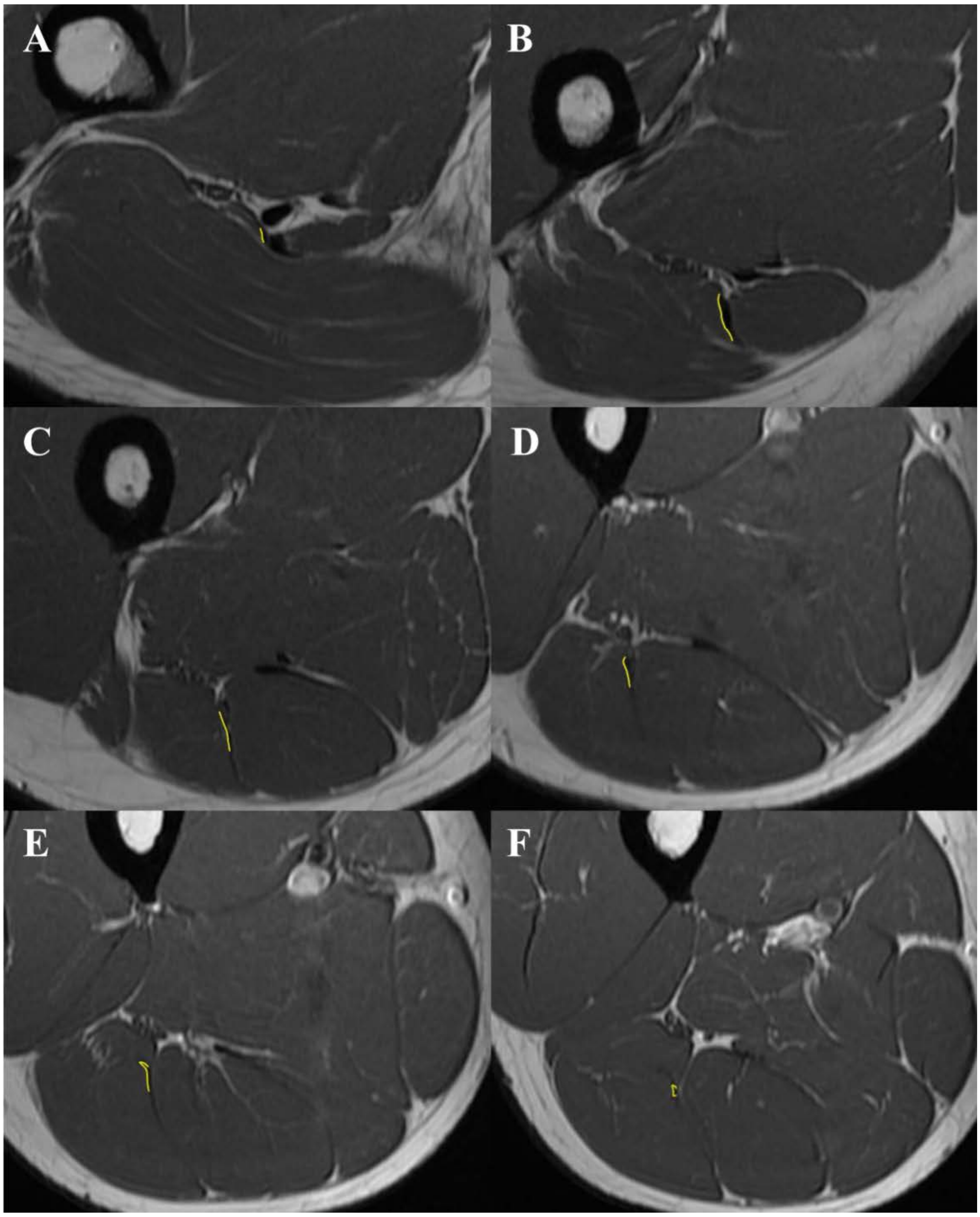

Figure 9.10. Example of biceps femoris long head (right) proximal muscle-aponeurosis contact interface distance delineation at 20, 40, 60 and 80% of proximal aponeurosis length.

In order to produce average muscle-aponeurosis contact interface distance data for the cohort, individual values were normalised to muscle length (Fig 9.11). This involved interpolation of individual muscle-aponeurosis contact interface data every 5% of muscle length. BFlh aponeurosis length was calculated as the sum of the slices where the aponeurosis was identifiable, multiplied by the slice thickness. BFlh muscle length was calculated as the sum of all images where the muscle appeared multiplied by the slice thickness.

BFlh/ST proximal tendon CSA was measured in the image immediately before the first image in which the ST muscle appeared (Fig 9.12).

All manual segmentation measurements were completed by the same investigator. To examine reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later. The CV was on average 4.0% for the aponeurosis contact area and 5.5% for the BFlh/ST proximal tendon CSA.

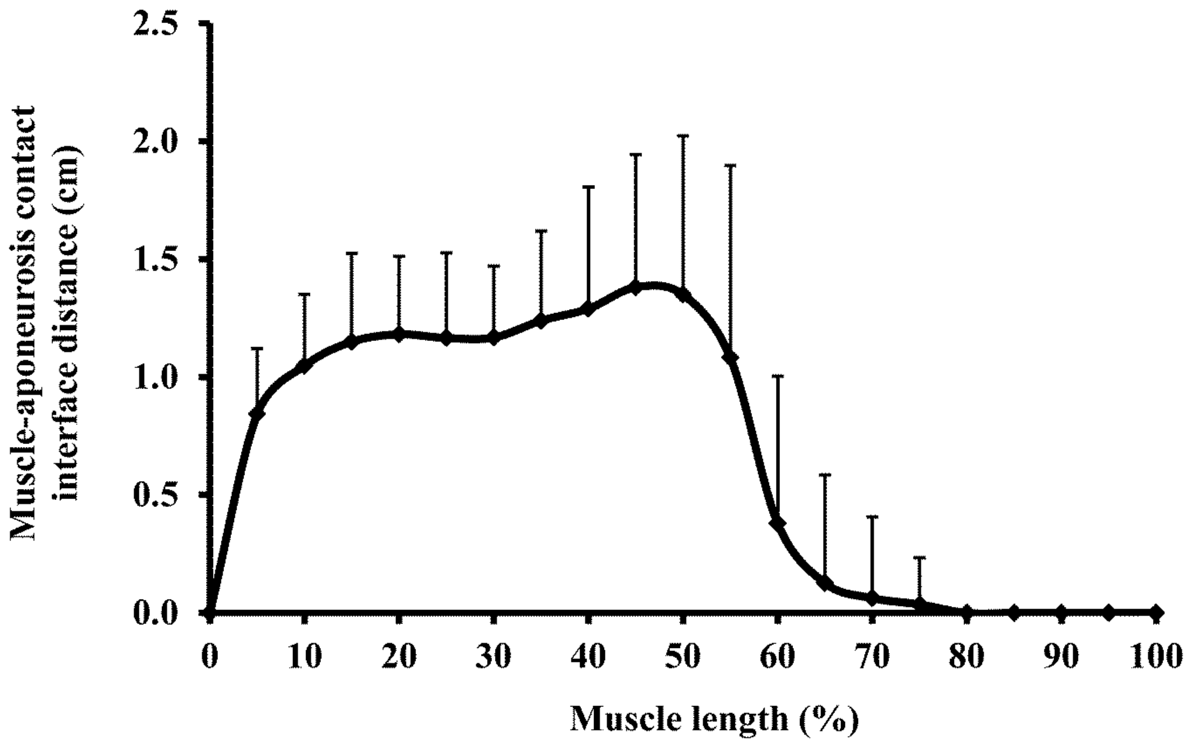

Figure 9.11. Muscle-aponeurosis contact interface distance along the length of the BFlh muscle (interpolated data every 5% of muscle length). Data presented as group mean + SD.

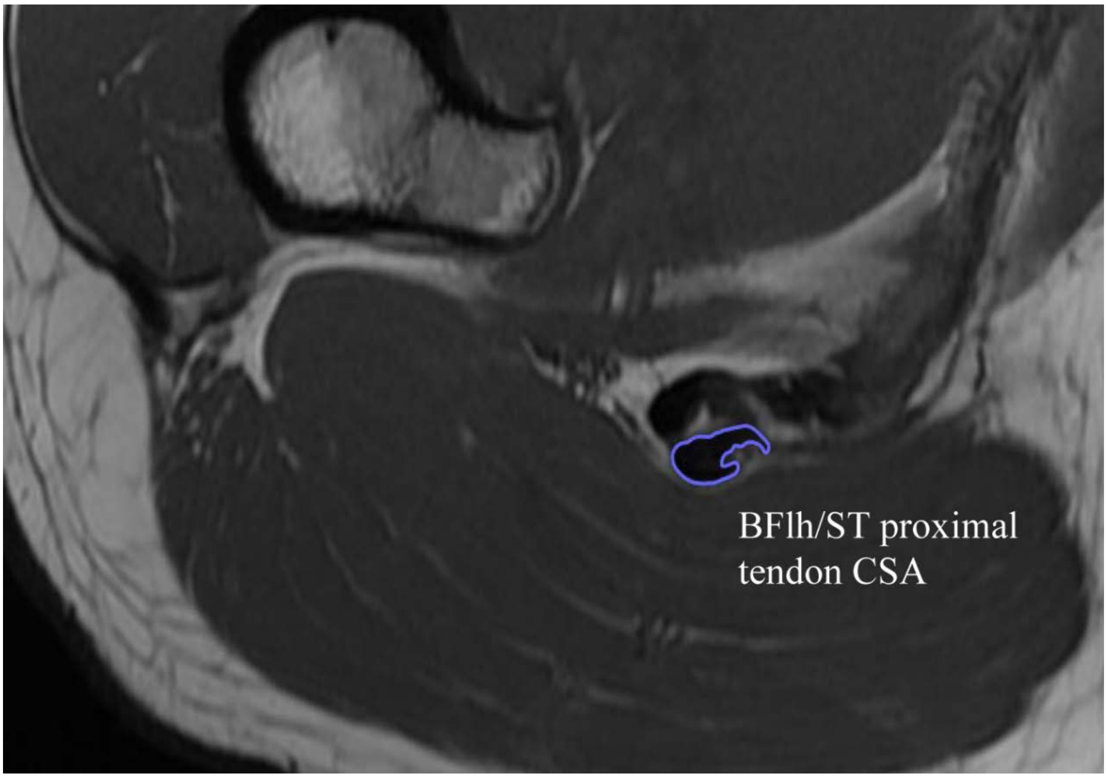

Figure 9.12. Example MR image of the BFlh/ST proximal tendon CSA.

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