Title:

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

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ABSTRACT

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

What are the new findings:

- Possessing short BFlh fascicles (a newly identified risk factor) increased the risk of a future hamstring strain injury
- Low levels of eccentric knee flexor strength increased the risk of a hamstring strain injury occurring in the subsequent season
- The increased risk associated with increasing age and a history of hamstring strain injury can be mitigated with greater levels of eccentric knee flexor strength and longer BFlh fascicle lengths.

INTRODUCTION

Paragraph 1

Hamstring strain injuries (HSI) are the most prevalent cause of lost playing and training time in elite soccer and account for approximately 37% of all muscle strain injuries[1-3]. Of these HSIs the majority occur in the biceps femoris long head (BF_{lh}) [1-3]. Despite a concerted scientific effort over the past decade, the incidence of HSIs has not declined in elite soccer[4]. What is known is that a number of non-modifiable risk factors, including increasing age and previous injury history, have been shown to increase the risk of a future HSI in elite soccer[5-7]. More recently greater attention has been directed to modifiable risk factors that can be altered via a range of interventions[8-10]. These risk factors include isokinetically derived eccentric knee flexor strength [10] and muscle imbalances (between-limb and hamstring:quadriceps ratios) [10 11].

In addition a recent prospective cohort study in elite Australian Rules Football identified eccentric weakness during the Nordic hamstring exercise as risk factor for a future HSI [9]. This study showed that there was a decreased risk of sustaining a future HSI in older athletes and those with a prior HSI if coupled with high levels of eccentric knee flexor strength [9]. Despite this evidence, there is still no consensus regarding the role that eccentric knee flexor strength plays in the aetiology of a HSI in soccer and this warrants further attention[12].

Paragraph 2

Despite a lack of direct evidence, it has been proposed that hamstring muscle fascicle length may alter the risk for a future HSI[13-15]. One retrospective study has shown BF_{lh} fascicles are shorter in previously injured muscles than in the contralateral uninjured muscles [16], but due to the retrospective nature of the available evidence [16], it is not possible to determine if these differences in fascicle length increased the risk of a HSI occurring or were the result of the initial insult.

Paragraph 3

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

METHODS

Participants and study design

Paragraph 4

This prospective cohort study was completed during pre-season (June 2014 to July 2014) and in-season period (October 2014 to May 2015) of the 2014/2015 elite, professional Australian Football (soccer) competition. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee (approval number: 2014 26V). Eight of the ten invited teams elected to take part in the study. Recent staffing changes resulted in two teams deciding not to participate. All outfield members of the playing squad (approximately 18-22 athletes per team) were approached and provided written, informed consent. In total, 152 elite male football (soccer) players participated in this study. Club medical staff completed a retrospective injury questionnaire that detailed each athlete's history of hamstring, quadriceps, groin and calf strain injuries and chronic groin pain in the past 12 months, as well as the history of anterior cruciate ligament (ACL) injury at any stage throughout the athlete's career. Playing positions were defined as: defender (n=52), midfielder (n=59) and attacker (n=41) as per previous research[17]. The athletes had their maximal voluntary isometric contraction strength (n=141) (MVIC), BF$_{lh}$ architecture (with relaxed hamstrings (n=152) and while performing isometric knee flexion at 25% of MVIC (n=141)) and eccentric knee flexor strength (n=131) assessed at the beginning of pre-season. Some athletes did not complete the maximal eccentric and isometric strength assessments at the advice of their team's medical department.

BF$_{lh}$ architecture assessment

Paragraph 5

Muscle thickness, pennation angle and fascicle length of the BF$_{lh}$ was determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-*i*, Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BF$_{lh}$. All architectural assessments were performed with participants in a prone position and the hip neutral following at least 5 minutes of inactivity. Assessments at rest were always performed first followed by an isometric contraction protocol. During all assessments of the BF$_{lh}$ architectural characteristics (passive and 25% of MVIC), the knee joint was fully extended. Assessment of the MVIC of the knee flexors was undertaken in the same position and was performed in a custom made device[18] [19]. Participants were instructed to contract their knee flexors maximally over a five second period. The peak force value during this effort was used to determine their MVIC strength. The assessment of the BF_{lh} architectural characteristics during a 25% isometric contraction then occurred in the same position and device, with the participants shown the real-time visual feedback of the force produced to ensure that target contraction intensities were met. To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures[20]. Finally, the orientation of the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel. Ultrasound image analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazevich and colleagues [21]. Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BF_{lh}. A fascicle of interest, which was the clearest and could be seen across the entire field of view, was outlined and marked on the image. The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line across the captured image[21 22]. Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe it was estimated via the following equation from Blazevich and colleagues[21 22]:

$$FL=\sin(AA+90°) \times MT/\sin(180°-(AA+180°-PA))$$

where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle. Fascicle length was reported in absolute terms (cm) and relative to BFlh length. The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers during the analysis. Reliability of the assessor (RGT) and processes used for the determination of the BF_{lh} architectural characteristics have been reported [16].

Eccentric hamstring strength

Paragraph 6

The assessment of eccentric knee flexor strength using the Nordic hamstring device has been reported previously [9 16 18 19]. Participants were positioned in a kneeling position over a padded board, with the ankles secured superior to the lateral malleolus by individual ankle braces that were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK). The ankle braces and load cells were secured to a pivot that ensured that force was always measured through the long axis of the load cells. Following a warm up set of three submaximal efforts with a subsequent 1 minute rest period, participants were asked to perform one set of three, maximal bilateral repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean forward at the slowest possible speed while maximally resisting this movement with both lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands held across the chest. Verbal encouragement was given throughout the range of motion to ensure maximal effort.

Prospective hamstring strain injury reporting

Paragraph 7

A HSI was defined as any acute posterior thigh pain that resulted in the immediate cessation of exercise and was later diagnosed by the club medical staff. The injury diagnosis also included the presence of pain during an isometric contraction and during any knee flexor muscle length test (stretch). Injury reports were not completed for injuries that did not fulfil the criteria (e.g. acute posterior thigh pain, however completed the exercise). A recurrent injury was a HSI that occurred on the same side of the body that had already suffered an injury in the current season. For all recurrent and new HSIs that fit the above criteria, the club medical staff completed a standard injury report form that detailed which limb was injured (dominant/non dominant, left/right), the muscle injured (BF_{lh}/biceps femoris short head/semimembranosus/semitendinosus), location of injury (proximal/distal, muscle belly/muscle-tendon junction), activity type performed at time of injury (e.g running, kicking etc), grade of injury (I, II or III) [23 24] and the number of days taken to return to full participation in training/competition. These reports were forwarded to the investigators throughout the season.

Injury specifics and rates

Paragraph 8

The determination of playing time missed as a result of a HSI was measured as missed matches per club per season [25]. Recurrence rate was defined as the number of recurrent injuries in the same season as a percentage of new injuries [25]. Additionally time lost as a result of the injury was defined as the amount of days from when the injury occurred to the resumption of full training participation.

Data analysis

Paragraph 9

Whilst positioned in the custom made device, shank length (m) was determined as the distance from the lateral tibial condyle to the mid-point of the brace that was placed around the ankle. This measure of shank length was used to convert the force measurements (collected in N) to torque (Nm). Knee flexor eccentric and MVIC strength force data were transferred to a personal computer at 100Hz through a wireless USB base station (Mantracourt, Devon, UK). The peak force value during the MVIC and the three Nordic hamstring exercise repetitions for each of the limbs (left and right) was analysed using custom made software. Eccentric knee flexor strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the average of the peak forces from the 3 repetitions for each limb, resulting in a left and right limb measure [18]. Knee flexor MVIC strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the peak force produced during a 5 second maximal effort for each limb.

Paragraph 10

Between limb imbalance of BF_{lh} fascicle length, muscle thickness, eccentric and MVIC knee flexor strength was calculated as a left:right limb ratio for the uninjured players and as an uninjured:injured limb ratio in the injured players. As recommended, between limbs imbalance was converted to a percentage difference using log transformed raw data followed by back transformation[26]. Negative percentage imbalances indicate that the variable of the left limb was greater than the right limb in the uninjured players, or that the injured limb was variable was greater than the uninjured limb in the injured players. For athletes who did not suffer a HSI, as the limbs did not differ for any variables ($p>0.05$) the left and right limb were averaged to give a single control ‘score’.

Statistical analyses

Paragraph 11

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

Paragraph 12

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

- those with or without prior
    - hamstring (past 12 months)
    - calf (past 12 months)
    - quadriceps (past 12 months)
    - ACL (at any stage in their career)
    - chronic groin injury (past 12 months)
- those with passive fascicle lengths above or below
    - 10.56cm
        - This threshold was determined utilising receiver operator characteristic (ROC) curves based on the fascicle threshold that maximised the difference between sensitivity and 1- specificity.
- those with 25% MVIC fascicle lengths above or below
    - 9.61cm

    - Threshold determined as above
- those with passive muscle thickness threshold above or below
  - 2.35cm
    - Threshold determined as above
- those with 25% MVIC muscle thickness threshold above or below
  - 2.61cm
    - Threshold determined as above
- those with average eccentric knee flexor strength threshold above or below
  - 337N
    - Threshold determined as above
- those with MVIC knee flexor strength threshold above or below
  - 400N
    - Threshold determined as above
- those with limbs above or below arbitrarily selected cut offs of 10%, 15% and 20% between limb imbalance for
  - passive fascicle length
  - 25% MVIC fascicle length
  - average eccentric knee flexor strength
  - MVIC knee flexor strength
- athletes above these age cut offs (which represent the 10th, 25th, 50th, 75th and 90th percentiles for this sample)
  - 18.0 years
  - 20.4 years

  - 23.7 years
  - 28.8 years
  - 32.6 years
- athletes above and below the height (182.3cm) and weight (77.9kg) means as defined previously by Hagglund and colleagues [27]

Paragraph 13

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

Paragraph 14

As per a previous investigation in elite Australian Football [9], to improve the understanding of the risk from the univariate analysis and remove the possible confounding effects, multivariate logistic regression models were built using risk factors from previously published evidence [1-3 5 9]. The first model included passive fascicle length (average of both limbs) and history of HSI and their interaction. The second model included fascicle length (average of both limbs) and age and their interaction. The third model included mean eccentric strength (average of both limbs) and history of HSI and their interaction. The fourth model included mean eccentric strength (average of both limbs) and age and their interaction. The final model included both fascicle length (average of both limbs) and mean eccentric strength (average of both limbs) and their interaction.

Additionally for this final model the Nagelkerke R^2 coefficient was used to display the strength of the association between the two continuous independent variables (eccentric strength and fascicle length) with a prospective HSI occurrence [28]. Significance was set at a $p<0.05$ and where possible Cohen's *d* [29] was reported for the effect size of the comparisons, with the levels of effect being deemed small (*d* = 0.20), medium (*d* = 0.50) or large (*d* = 0.80) as recommended by Cohen (1988).

RESULTS

Power calculations

Paragraph 15

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

Participant and injury details

Paragraph 16

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

Paragraph 17

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

Univariate analysis

Paragraph 18

Eccentric and isometric knee flexor strength, BF_{lh} architectural characteristics and between limb asymmetries of the injured and uninjured limbs from the injured players and the average of both limbs from the uninjured players can be found in Table 1.

BF_{lh} architectural characteristics

Paragraph 19

The subsequently injured limbs had shorter BF_{lh} fascicle lengths than the two-limb-average of uninjured players when assessed at rest (mean difference: 1.37cm; 95% CI=0.8 to 1.8; p=<0.001; d=1.08; Table 1) and during 25% MVIC (mean difference: 1.02cm; 95% CI=0.5 to 1.5; p=<0.001; d=0.92; Table 1). In comparison to the contralateral uninjured limb, the BF_{lh} fascicle length of the subsequently injured limbs was significantly shorter when assessed at rest (mean difference: 1.05cm; 95% CI=0.6 to 1.5; p=<0.001; d=0.91; Table 1) and during 25% MVIC (mean difference: 0.65cm; 95% CI=0.3 to 1.0; p=<0.001; d=0.57; Table 1). Whereas, the BF_{lh} architectural characteristics of the left and right limbs in the uninjured players were not significantly different when assessed in when relaxed or during 25% MVICs ($p>0.05$).

Using univariate logistic regression, BF_{lh} fascicle length (OR = 0.261; 95% CI = 0.10 to 0.57; p=0.002) had a significant inverse relationship with the incidence of prospectively occurring HSIs. For every 0.5cm increase in BF_{lh} fascicle length, the risk of HSI was reduced by 73.9%. Muscle thickness measures of the BF_{lh} (at rest and during 25% MVIC) from the subsequently injured limbs were no different from either the contralateral uninjured limbs or the two-limb-average of the uninjured players ($p<0.05$, d range=0.13 to 0.23; Table 1).

Paragraph 20

The measures of between limb asymmetry in BF_{lh} fascicle length and muscle thickness, assessed at rest and at 25% MVIC, did not differ significantly between the injured and uninjured players ($p<0.05$, d range=0.03 to 0.48; Table 1).

Eccentric and isometric knee flexor strength

Paragraph 21

Between-limb differences in absolute eccentric knee flexor forces between the left and right limbs of uninjured players and between the subsequently injured and contralateral uninjured limbs of injured players, were not significant ($p>0.05$, *d* range=0.02 to 0.21; Table 1). However, between group comparisons of absolute eccentric knee flexor force showed that subsequently injured limbs were weaker (260.6N±82.9) than the two-limb-average of uninjured players (309.5N±73.4) (mean difference: 48.9N; 15.8%; 95% CI=16.2 to 81.5N; p=0.004; *d*=0.62; Table 1). Additionally, the uninjured limbs of the injured players were also significantly weaker (262.6N±63.2) than the uninjured players' two-limb-average (mean difference: 46.9N; 15.1%; 95% CI=15.9 to 77.9N; p=0.003; *d*=0.68; Table 1).

Eccentric strength represented as knee flexor torque showed similar differences, with the subsequently injured limbs (115.2Nm±37.1) being weaker than the two-limb-average (135.5Nm±33.7) of uninjured players (mean difference: 20.3Nm; 14.9%; 95% CI=5.3 to 35.1Nm; p=0.008; *d*=0.57; Table 1). Similarly, the uninjured limbs (116.2Nm±28.7) from the injured players were weaker than the two-limb-average of the uninjured players (135.5Nm±33.7; mean difference: 19.3Nm; 14.2%; 95% CI=4.9 to 33.4Nm; p=0.008; *d*=0.62; Table 1).

Using univariate logistic regression, eccentric knee flexor strength (OR = 0.910; 95% CI = 0.85 to 0.97; p=0.004) had a significant inverse relationship with the incidence of prospectively occurring HSIs. For every 10N increase in eccentric knee flexor strength, the risk of HSI was reduced by 8.9%. Comparisons of between-limb imbalance in eccentric knee flexor strength did not differ between the subsequently injured and uninjured players (mean difference: 9.6%; 95% CI=-3.6 to 22.7; p=0.147; *d*=0.40; Table 1).

Paragraph 22

There were no significant differences in knee flexor MVIC strength between either the subsequently injured limbs or the contralateral uninjured limbs of the injured players and the two-limb-averages of uninjured players ($p>0.05$; *d* range=0.07 to 0.22; Table 1)

Relative Risk

Paragraph 23

The univariate relative risks of a future HSI associated with all variables examined can be found in Table 2. Athletes with a relaxed BF_{lh} fascicle length shorter than that of the ROC-curve-determined threshold of 10.56cm (area under the curve = 0.71; sensitivity = 0.70; 1-specificity = 0.29) were 4.1 times more likely to suffer a subsequent HSI than those with longer fascicles (RR = 4.1; 95% CI=1.9 to 8.7; $p<0.001$). Similar RR values were seen for BF_{lh} fascicle length assessed during 25% MVIC (Table 2). Furthermore, athletes with average eccentric knee flexor forces below the ROC-curve determined threshold of 337N (area under the curve = 0.65; sensitivity = 0.96; 1-specificity = 0.68) had 4.4 times greater risk of a subsequent HSI than stronger players (RR = 4.4; 95% CI=1.1 to 17.6; $p=0.013$). Similar RR values were seen for the other measures of knee flexor strength (torque, force/kg body mass and torque/kg body mass) (Table 2). No measure of MVIC strength or between-limb imbalance in this measure led to a statistically significant increase in RR (Table 2).

Multivariate logistic regression

Paragraph 24

Details of all of the logistic regression models can be found in Table 3 and Figures 1 to 5. All of the models were significant (model 1: prior HSI and BF_{lh} fascicle length, $p<0.001$; model 2: age and BF_{lh} fascicle length; $p<0.001$; model 3: prior HSI and eccentric strength, $p=0.009$; model 4:

age and eccentric strength, p=0.007; model 5: eccentric strength and BF_{lh} fascicle length; p<0.001), however none of the interactions reached significance (Table 3). For all models in which fascicle length was included, it made the most significant contribution to the model. A Nagelkerke R^2 coefficient of 0.31 was found, when using a binary logistic regression, to determine the strength of the association between the two continuous independent variables (eccentric strength and fascicle length) with the dependant variable of a prospective HSI occurrence (yes/no).

DISCUSSION

Main findings

Paragraph 25

This is the first study that has examined the role that BF_{lh} fascicle length plays in the aetiology of HSI. The main findings were that 1) athletes that suffered a HSI contained shorter BF_{lh} fascicle lengths than those that remained uninjured; 2) athletes that suffered a HSI were weaker during eccentric contractions than those who remained uninjured; 3) eccentric between-limb imbalances were not different between the injured or uninjured groups and between-limb imbalances did not infer any increased HSI risk; 4) the probability of future HSI associated with non-modifiable factors (increasing age and a history of HSI) appears to be influenced by BFlh fascicle length and eccentric knee flexor strength and 5) measures of MVIC knee flexor strength were not different between the injured and uninjured groups and did not infer any increased HSI risk.

BFlh fascicle length and the risk of a future HSI

Paragraph 26

In the current study short BF_{lh} fascicle lengths were associated with an increased risk of future HSI in elite soccer players. One previous retrospective investigation reported that individuals with a unilateral HSI history have shorter BF_{lh} fascicles in the previously injured limb than the contralateral uninjured limb [16]. It was previously hypothesized that shorter fascicles, with fewer in-series sarcomeres, may be more susceptible to being over-stretched and having damage caused by powerful eccentric contractions, like those performed during the terminal swing phase of high speed running [13 30]. Given that more than two thirds of the HSIs noted in the current study occurred during high speed running, the shorter BF_{lh} fascicle lengths in the subsequently injured limbs may have increased the susceptibility of the muscle to damage and altered their HSI risk.

Knee flexor strength and HSI risk

Paragraph 27

Low levels of eccentric knee flexor strength during the Nordic hamstring exercise increased the risk of a future HSI in elite soccer players. This has also been recently observed in elite Australian footballers [9]. As the hamstrings are required to contract eccentrically during the terminal swing phase of the gait cycle[31], low levels of eccentric strength may reduce the hamstrings ability to do this and as a result potentially lead to an acute injury. Interestingly, low levels of isometric knee flexor strength were not associated with future HSI rates and this suggests that the contraction mode of strength tests is a critical factor in determining their predictive value. This is of particular relevance given that isometric assessments of the knee-flexors have been developed and advocated as clinically convenient [32] and minimally ‘intrusive’ in athlete training programs [33] given the low levels of muscle damage and soreness involved. Without discounting the value and convenience of such isometric tests as measures of strength and indicators of fatigue [33], the present results suggest that eccentric hamstring tests are of greater value in determining injury risk.

Between-limb imbalance and HSI risk

Paragraph 28

The current study also found that a larger between-limb strength imbalance during the Nordic hamstring exercise did not increase the risk of future HSI and this is consistent with a similar recent study in AFL players [9] but contrary to previous findings in elite soccer, which indicated that isokinetically derived between-limb eccentric strength imbalances are associated with an increased risk of HSI [11]. Bourne and colleagues have also recently observed, in a prospective study, that between-limb imbalances in the Nordic strength test (as employed in this study) are associated with elevated HSI rates in rugby union players while absolute strength levels are not [34]. The diverse findings in these studies are hard to explain. The different physical demands of these three football codes are readily apparent [34]and the mode of testing may also influence the results of these prospective studies.

Multivariate comparisons

Paragraph 29

Multivariate exploration into combinations of variables including BF_{lh} fascicle length, eccentric strength, age and a HSI history provides novel insights regarding HSI risk. Advanced age and a history of HSI have both been previously reported to increase the risk of a future HSI in elite soccer[5-7]. The data in the current study indicates that the risk of a future HSI is lower in older athletes or those who have a previous history of HSI when coupled with longer BFlh fascicle lengths and/or high levels of eccentric knee flexor strength. Most notably, older athletes with shorter BF_{lh} fascicles and lower levels of eccentric strength were at an increased risk when compared to younger athletes.

As an example, the results of the current study allow us to estimate that a 33 year old athlete with BF_{lh} fascicle length of 10cm has a 65% probability of HSI occurring, while a 22 year old has a 17% probability of injury. Similarly, 33 year old athletes with two-limb-average eccentric strength level of 200N have an estimated probability of HSI injury of 46% while a 22 year old player has an injury probability of 27%. Despite these results, the Nagelkerke R^2 coefficient indicated that eccentric strength and BF_{lh} fascicle length accounted for approximately 30% of the risk associated with a prospective HSI occurrence. Therefore future research is still needed to identify the other 70% of the risk associated with a prospective HSI that is not accounted for.

Limitations

Paragraph 30

The authors acknowledge that there are limitations in the current study. Firstly, there is a lack of athlete exposure data and this does not allow for the determination of injury incidence relative to exposure to training and match play. Future work should focus on determining the interaction between high speed running demands and the risk of a future HSI. Secondly, the study was undertaken in elite soccer players and as such generalizing the results to athletes of different sports may be done with caution. For example, in Australian footballers (AFL) the ROC-curve determined threshold for an elevated risk of future HSI was 256N at the start of pre-season[9]. This differs when compared to the 337N found in the current study. One explanation for this variance is the different sporting populations utilised in the two studies that highlights the population specificity of the results. It also indicates that the need for future research in other sporting cohorts is warranted. It should be noted that the thresholds for elevated risk of future HSI determined using the ROC-curve approach should not be compared across studies as an indicator of which cohort possess greater strength. Thirdly, the measures of eccentric knee flexor strength were not made relative to an anterior muscle group such as the knee extensors or the hip flexors. Doing so may have enabled the determination of a hamstring-to-quadriceps ratio, or something of similar nature. Despite the lack of this relative comparison, the eccentric knee flexor strength measures in this study provided valuable information regarding HSI risk, which suggests such ratios may not be crucial.

The assessment of muscle fascicle length was only performed on the BF_{lh}. Considering the high rates of BF_{lh} strain injury in the current study, the authors believe it was justified to focus on this muscle. Future research could aim to assess the risk associated with short fascicle lengths in the other hamstring muscles.

Conclusion

Paragraph 31

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

CONTRIBUTORS

RT was the principle investigator and was involved with study design, recruitment, analysis and manuscript write up. MB was involved with recruitment, analysis and the manuscript preparation. AS, MW, CL and DO were involved with the study design, analysis and manuscript preparation. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY DECLARATION

The lead author* (RT) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. * = The manuscript's guarantor.

COPYRIGHT DECLARATION

DATA SHARING

Consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

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COMPETING INTERESTS

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) A Faculty of Health Research Grant from the Australian Catholic University partially funded the research; (2) AS and DO are listed as co-inventors on a patent filed for a field test of eccentric hamstring strength (PCT/AU2012/001041.2012) as well as being shareholders in a company responsible for commercialising the device; (3) RT, MB, MW and CL have no relationships with companies that might have an interest in the submitted work in the previous 3 years; (4) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (5) RT, MB, AS, MW, CL and DO have no financial interests that may be relevant to the submitted work beyond what is already declared.

ETHICAL APPROVAL

This study was approved by the Human Research Ethics Committee of the Australian Catholic University (approval number: 2014 26V) and informed consent was provided to all participants.

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Figure 1. The interaction between BF_{lh} fascicle length, history of HSI and the probability of a future HSI (error bars indicate 95% CI).

Figure 2. The interaction between BF_{lh} fascicle length, age and the probability of a future HSI. The ages are representative of the 10th, 25th, 50th, 75th and 90th percentile of the cohort. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 9, 10, 11, 12, 13 and 14cm for all groups (error bars indicate 95% CI).

Figure 3. The interaction between eccentric knee flexor strength, history of HSI and the probability of a future HSI (error bars indicate 95% CI).

Figure 4. The interaction between eccentric knee flexor strength, age and the probability of a future HSI. The ages are representative of the 10th, 25th, 50th, 75th and 90th percentile of the cohort. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 100, 200, 300, 400 and 500N for all groups (error bars indicate 95% CI).

Figure 5. The interaction between eccentric knee flexor strength, BF_{lh} fascicle length and the probability of a future HSI. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 100, 200, 300, 400 and 500N for all groups (error bars indicate 95% CI).

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

	Uninjured group		Injured group			Compared to uninjured group average		
BFlh architecture	Two-limb average	Between-limb imbalance (%)	Between-limb imbalance (%)	Uninjured limb	Injured limb	Uninjured group vs injured limb (95% CI)	*p*	Effect size (*d*)
Passive FL (cm)	11.20 (±1.2) (n=125)	11.2 (±8.2)	13.8(±1.3)	10.90 (±0.9) (n=27)	9.85 (±1.3)$^{\#\#}$ (n=27)	1.35 (0.8 to 1.8)	0.001**	1.08
25% MVIC FL (cm)	9.53 (±1.2) (n=116)	11.7 (±9.2)	11.3 (±1.0)	9.16 (±1.2) (n=25)	8.51 (±1.0) $^{\#\#}$ (n=25)	1.02 (0.5 to 1.5)	0.001**	0.92
Passive MT (cm)	2.54 (±0.3) (n=125)	8.0 (±6.1)	7.8 (±6.2)	2.58 (±0.2) (n=27)	2.47 (±0.3) (n=27)	0.07 (-0.1 to 0.2)	0.357	0.23
25% MVIC MT (cm)	2.66 (±0.3) (n=116)	9.8 (±8.0)	6.4 (±5.8)	2.63 (0.3) (n=25)	2.62 (±0.3) (n=25)	0.04 (-0.1 to 0.2)	0.671	0.13
	Uninjured group		Injured group			Compared to uninjured group average		
Knee flexor strength measures	Two-limb average	Between-limb imbalance (%)	Between-limb imbalance (%)	Uninjured limb	Injured limb	Uninjured group vs injured limb (95% CI)	*P*	Effect size (*d*)
Eccentric force (N)	309.5 (±73.4) (n=105)	10.1 (±8.8)	19.7 (±32.4)	262.6 (±63.2) (n=26)	260.6 (±82.9) (n=26)	48.9 (16.2 to 81.5)	0.004*	0.62
Eccentric torque (Nm)	135.5 (±33.7) (n=105)			116.2 (±28.7) (n=26)	115.2 (±37.1) (n=26)	20.3 (5.3 to 35.1)	0.008*	0.57
Relative eccentric force (N/Kg)	4.11 (±0.9) (n=105)			3.47 (±0.9) (n=26)	3.46 (±1.2) (n=26)	0.65 (0.2 to 1.1)	0.004*	0.61
Relative eccentric torque (Nm/Kg)	1.79 (±0.4) (n=105)			1.54 (±0.4) (n=26)	1.53 (±0.5) (n=26)	0.26 (0.1 to 0.5)	0.007*	0.57
Isometric force (N)	373.7 (±75.6) (n=116)	10.4 (±7.56)	12.1 (±13.7)	365.2 (±73.9) (n=25)	367.9 (±72.7) (n=25)	5.8 (-26.1 to 39.4)	0.690	0.08
Isometric torque (Nm)	163.2 (±34.2) (n=116)			160.2 (±30.1) (n=25)	161.4 (±34.2) (n=25)	1.8 (-13.1 to 16.7)	0.811	0.05
Relative isometric force (N/Kg)	4.99 (±1.0) (n=116)			4.81 (±1.1) (n=25)	4.81 (±1.1) (n=25)	0.18 (-0.3 to 0.6)	0.428	0.17
Relative isometric torque (Nm/Kg)	2.18 (±0.4) (n=116)			2.10 (±0.4) (n=25)	2.11 (±0.4) (n=25)	0.07 (-0.1 to 0.3)	0.495	0.18

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

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

Risk Factor	*n*	Percentage from each group that sustained a HSI	RR (95% CI)	*p*
Passive fascicle length	152			
<10.56 cm	56	33.9	4.1 (1.9-8.7)	0.001**
≥10.56 cm	96	8.3		
Passive fascicle length relative to BFlh length	152			
<0.254	38	39.4	3.7 (1.9-7.3)	0.001**
≥0.254	114	10.5		
25% MVIC fascicle length	141			
<9.61 cm	79	25.3	3.2 (1.2-7.9)	0.008*
≥9.61 cm	62	8.0		
Passive fascicle length imbalance	152			
<10% imbalance	80	16.3	1.2 (0.6-2.4)	0.673
≥10% imbalance	72	19.4		
<15% imbalance	99	15.1	1.5 (0.7-3.0)	0.271
≥15% imbalance	53	22.6		
<20% imbalance	125	16.8	1.3 (0.6-3.0)	0.579
≥20% imbalance	27	22.2		
25% MVIC fascicle length imbalance	141			
<10% imbalance	78	20.0	1.3 (0.6-2.6)	0.512
≥10% imbalance	63	20.6		
<15% imbalance	100	22.7	0.7 (0.3-1.7)	0.630
≥15% imbalance	41	14.6		
<20% imbalance	118	22.0	0.4 (0.1-1.7)	0.249
≥20% imbalance	23	8.7		
Passive muscle thickness	152			
<2.35 cm	36	11.1	0.56 (0.2-1.5)	0.320
≥2.35 cm	116	19.8		
25% MVIC muscle thickness	141			
<2.61 cm	58	20.6	1.3 (0.6-2.7)	0.504
≥2.61 cm	83	15.6		
Eccentric strength	131			
<337 N	96	25.0	4.4 (1.1-17.5)	0.013*
≥337 N	35	5.7		
<145 Nm	89	25.8	3.6 (1.2-11.4)	0.017*
≥145 Nm	42	7.1		
<4.35 N/kg	82	25.6	2.5 (1.1-6.2)	0.041*
≥4.35 N/kg	49	10.0		
<1.86 Nm/kg	78	26.9	2.9 (1.1-7.1)	0.011*
≥1.86 Nm/kg	53	9.4		
Eccentric strength imbalance	131			
<10% imbalance	76	19.7	1.0 (0.5-2.0)	1.000
≥10% imbalance	55	20.0		
<15% imbalance	98	18.3	1.3 (0.6-2.7)	0.459
≥15% imbalance	33	24.2		
<20% imbalance	117	18.8	1.5 (0.6-3.8)	0.476
≥20% imbalance	14	28.5		
MVIC strength	141			
<400 N	93	21.5	2.0 (0.8-5.2)	0.161
≥400 N	48	10.4		
<172 Nm	88	20.4	1.5 (0.7-3.5)	0.364
≥172 Nm	53	13.2		
<4.60 N/kg	52	23.1	1.5 (0.7-3.2)	0.254
≥4.60 N/kg	89	14.6		

<2.07 Nm/kg	62	22.6	1.6 (0.8-3.3)	0.192
≥2.07 Nm/kg	79	13.9		
MVIC strength imbalance	141			
<10% imbalance	81	19.7	0.8 (0.4-1.8)	0.826
≥10% imbalance	60	16.6		
<15% imbalance	110	16.3	1.3 (0.6-2.9)	0.434
≥15% imbalance	31	22.6		
<20% imbalance	126	17.4	1.1 (0.4-3.3)	0.732
≥20% imbalance	15	20.0		
Prior injury	152			
HSI	30	30.0	2.0 (1.0-4.0)	0.063
No HSI	122	14.7		
ACL	16	31.1	1.9 (0.8-4.4)	0.164
No ACL	136	16.1		
Calf strain	13	23.1	1.3 (0.4-3.8)	0.713
No calf strain	139	17.3		
Quadriceps strain	21	28.6	1.8 (0.8-3.9)	0.215
No Quadriceps strain	131	16.0		
Chronic groin pain	13	23.1	1.3 (0.5-3.8)	0.703
No chronic groin pain	139	17.3		
Age (yr)	152			
≤18.0	8	12.5	1.5 (0.2-9.7)	1.000
>18.0	144	18.7		
≤20.4	37	2.7	8.4 (1.1-59.5)	0.005*
>20.4	115	22.6		
≤23.7	74	6.7	4.2 (1.6-10.4)	0.001*
>23.7	78	28.2		
≤28.8	116	13.8	2.2 (1.1-4.3)	0.043*
>28.8	36	30.5		
≤32.6	136	12.5	0.7 (0.2-2.6)	0.739
>32.6	16	18.4		
Height (cm)				
≤182.3	111	15.3	1.6 (0.8-3.4)	0.206
>182.3	41	24.4		
Weight (kg)				
≤77.9	102	15.7	1.4 (0.7-2.8)	0.370
>77.9	50	22.0		

RR=relative risk, HSI=hamstring strain injury, BFlh=biceps femoris long head, MVIC=maximal voluntary isometric contraction, 95%CI=95% confidence intervals, cm=centimetres, N=newtons, Nm=newton metres, N/Kg = newtons per kilogram of body weight, Nm/kg = newton metres per kilogram of body weight, ACL=anterior cruciate ligament, yr=years, kg=kilogram,*=$p<0.05$, **=$p<0.001$ when comparing the RR of future HSI between groups

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

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

[a] Determined as the average of both left and right limb. AUC, area under the curve. *$p<0.05$