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Prediction of hamstring injuries in Australian Football using biceps femoris architectural risk factors derived from soccer

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Title:

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ABSTRACT

Purpose: To assess the generalisability of previously established hamstring strain injury (HSI) risk factors, including demographic data, injury history and biceps femoris long head (BFlh) architecture; to predict HSI in a cohort of elite Australian footballers.

Methods: Demographic, injury history and BFlh architectural data were collected from elite Australian soccer (n=152) and Australian football players (n=169) at the beginning of preseason for their respective competitions. Any prospectively occurring HSIs were reported to the research team. Optimal cut points for continuous variables used to determine association with HSI risk were established from the previously published data in soccer and subsequently applied to the Australian football cohort in order derive the relative risk (RR) for these variables. Logistic regression models were built using data from the soccer cohort and used to estimate the probability of injury in the Australian football cohort. Area under the curve (AUC) and Brier scores were the primary outcome measures to assess the performance of the logistic regression models.

Results: Twenty-seven and 30 prospective HSIs occurred in the soccer and Australian football cohorts, respectively. When using cut points derived from the soccer cohort and applying these to the Australian football cohort, only older athletes (≥ 25.4 years, RR = 2.7, 95% confidence intervals [95% CIs] = 1.4 to 5.2) and those with a prior HSI (RR = 2.5, 95% CIs = 1.3 to 4.8) were at an increased risk of HSI. Using the same approach stature, mass, BFlh fascicle length, muscle thickness, pennation angle and relative fascicle length were not significantly associated with an increased risk of HSI in Australian footballers. The logistic regression model constructed using age and prior HSI performed the best (AUC = 0.67, Brier score = 0.14), with the worst performing model being that which was constructed using pennation angle (AUC = 0.53, Brier score = 0.18).

Conclusions: Using HSI risk factor cut points derived from previously published data in soccer on a dataset from Australian football identified older age and prior HSI, but none of the modifiable factors, to be associated with injury. The transference of HSI risk factor data between different cohorts appears limited and suggests that cohort specific cut points must be established.

Key Words: risk, injury prediction, hamstring injury, muscle injury, fascicle length

INTRODUCTION

Hamstring strain injuries (HSIs) are the most common injury in team sports such as soccer [5, 6, 10] and Australian football [15], lead to a reduced performance following return to play [27] and pose a significant financial burden for athletes and their sporting organisations [11]. As such, identifying factors that increase the risk of HSI have been the focus of ongoing research. Two of the most consistently identified risk factors are older age and a prior HSI, both of which are non-modifiable [8]. Recent work has focused on identifying modifiable factors that could be targeted via intervention to, potentially, mitigate the risk of future HSI. Amongst this work, a study conducted in elite soccer players reported that athletes with biceps femoris long head (BFlh) fascicles shorter than 10.56 cm were approximately 4 times more likely to sustain a prospective HSI than their 'longer' counterparts [24]. However, this cut point was determined retrospectively from the data it was ultimately applied to. Whilst such an approach is commonly used to establish associations between factors and the risk of injury, their use to identify the risk of injury at an individual level requires further validation.

In order to determine the predictive ability of injury risk factors, Bahr [1] proposes a three-step process. Firstly, a risk factor and its associated cut point must be established in a specific cohort. Subsequently, the generalisability of risk factors and their cut points must be validated in separate cohorts (whose data were not used to determine the cut point). The final step is to implement randomised control trials to test the effectiveness of a combination of risk factor screening (based on data generated from the first two steps) and interventions targeted at those deemed "at-risk". It should be noted that the framework outlined by Bahr [1] specifically relates to the application of dichotomised risk factor data (a cut point used to assign a high and low risk group) and whilst this is an appropriate series of steps to determine the utility and generalisability of risk factors it does not directly assess the predictive performance of continuous variables. Bahr[1] further states that the eventual goal of injury prediction is the successful development of a screening tool. As an extension of the Bahr framework [1], techniques such as logistic regression, which establish univariate and multivariate models to estimate the probability of future (hamstring strain) injury, can be used to assess the performance of factors associated with future HSI to predict injury occurrence at the individual level. Whilst logistic regression is a commonly employed statistical approach, there is a dearth of work in sports injury which has developed logistic regression models in one cohort and then applied these models to a separate cohort in a different sport. The addition of a separate cohort is necessary for the theoretical screening tool that is suggested by Bahr[1], who further suggests that such tools need to be validated in all cohorts that could use the tool. Such an approach would allow for a more thorough understand of the generalisability of factors, potentially associated with future HSI, across cohorts[23].

Despite architectural characteristics of the BFlh being associated with future risk of HSI in elite soccer players, no research has investigated the generalisability of these risk factor cut points when applied to a separate cohort nor has the predictive ability of these data in another cohort of athletes, from a different sport, been determined. Accordingly, this study aimed to assess the ability of BFlh architecture, in conjunction with age and prior injury data, that has had previously established cut-points determined in elite soccer players, to identify the risk of HSI in elite Australian footballers, at both a group and an individual level. We hypothesise that previously established risk factor cut points for HSI derived from BFlh architecture, age and prior HSI data in soccer players will be associated with HSI in Australian footballers.

METHODS

Study design

Data for this prospective cohort study were collected during the 2014-15 A-League season and the 2018 Australian Football League (AFL) season. The A-League and the AFL are the premier competitions in Australia for soccer and Australian football, respectively. For both cohorts, demographic (age, height, weight), injury history and BFlh architecture data were collected at the beginning of the pre-season periods (soccer cohort: June 2014; Australian football cohort: November 2017). Any prospectively occurring HSIs throughout the pre- and in-season periods (excluding finals) of both leagues (soccer cohort: June 2014 – May 2015; Australian football cohort: November 2017 – August 2018) were reported to the research team, this includes injuries incurred in training or in match play. Data collected during the 2014-15 A-League season has been previously published [24]. Ethical approval for the collection of both datasets was granted by the University Human Research Ethics Committee (approval numbers: 2014-26V [soccer dataset] and 2017-208H [Australian football dataset]).

Participants

A total of 321 athletes (152 soccer players from nine teams; 169 Australian footballers from four teams) provided informed consent to participate prior to data collection. The age (years), stature (cm) and mass (kg) of each athlete was provided at the beginning of the pre-season period for both cohorts. Additionally, team medical staff completed a retrospective injury questionnaire that reported in a binary manner (Y/N) each athlete's history of hamstring in the past 12 months, as well as the history of ACL injury at any stage throughout the athlete's career.

Biceps femoris long head architectural assessment

Collection of the BFlh architectural characteristics of both cohorts was undertaken as previously reported [12, 16-18, 24]. Muscle thickness, pennation angle and fascicle length of the

BFlh were determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12 MHz; depth, 8 cm; field of view, 14×47 mm) (GE Healthcare Vivid-i, Wauwatosa, USA). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the BFlh. All architectural assessments were performed with participants in a relaxed, prone position with the hips in neutral and knees fully extended. 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 with the hips in normal, unrestrained rotation. Care was taken to ensure minimal pressure was placed on the skin by the probe and the operator. Finally, the orientation of the probe was manipulated slightly by the operator in order to optimise fascicle identification. Ultrasound image analysis was undertaken off-line (MicroDicom, V.0.7.8, Bulgaria). For each image, six points were identified as described by Kellis et al. [13]. Muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BFlh. 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 defined as the pennation angle. The angle of both the superficial and intermediate aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line input across the captured image. Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the probe's field of view it was estimated via the following validated equation [25]:

$$\mathrm{FL} = \sin\left(\mathrm{AA} + 90^\circ\right) \times \mathrm{MT}/\sin\left(180^\circ - \left(\mathrm{AA} + 180^\circ - \mathrm{PA}\right)\right)$$

where FL, fascicle length; AA, aponeurosis angle; MT, muscle thickness; PA, pennation angle. Fascicle length was reported in absolute terms (cm) and relative to muscle thickness (the quotient of FL and MT). All BFlh architecture assessments and analyses were completed by the same operator (RGT) with published reliability (Intraclass correlation coefficient range = 0.95 – 0.99; % typical error range = 2.1 to 3.4%) [25]. The extrapolation technique and equation have been validated against cadaveric tissues [13].

Prospective hamstring strain injury data

A prospective HSI was defined as acute pain in the posterior thigh that resulted in the cessation of activity. Each injury was confirmed by clinical examination conducted by the medical officials (i.e. physiotherapist, doctor) of each club. Club medical officials subsequently provided the research team with a medical report detailing the injured limb, the location and the mechanism of the injury, as well as number of days taken to return to full match availability.

Statistical analysis

Differences between the two cohorts were assessed using independent t-tests. Following this, using the soccer cohort dataset, receiver operator characteristic (ROC) curves were used to determine optimal cut points for continuous variables. These cut points were established as the value that maximised the difference between sensitivity and 1-specificity, as described in the original work [24]. All cut points derived from the soccer cohort were then applied to the Australian football cohort to determine relative risks (RRs), the associated 95% confidence intervals (95% CIs) as well as sensitivity and specificity. As prior HSI is a dichotomous variable, the cut point used to determine RR of future HSI in the Australian football cohort was determined by comparing those with and without a history of HSI. All variables included in the RR analyses can be found in Table 1. A RR was deemed to be significant when the 95% CIs did not cross 1.0. Following the determination of RR, univariable and multivariable logistic regression models were built using the soccer dataset and then subsequently applied to the

Australian football data to assess the generalisability and predictive performance of these models. The variables included in these models and the process by which they are built can be found in Table 1 and Figure 1, respectively.

Table 1. The variables used to determine relative risks (RRs) and to build univariable and multivariable logistic regression models. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head. No interaction terms were included in any of the models.

RR was calculated for	Univariable logistic regression models	Multivariable logistic regression models
Age (years)	Age	Age and prior HSI
Prior HSI	Prior HSI	Age, prior HSI and fascicle length
Stature (cm)	Fascicle length	Age, prior HSI and pennation angle
Mass (kg)	Pennation angle	Age, prior HSI and relative fascicle length^
Fascicle length (cm)	Relative fascicle length^	All variables#
Muscle thickness (cm)		
Pennation angle (degrees)		
Relative fascicle length^		

^Relative fascicle length refers to fascicle length relative to muscle thickness. #All variables (age, prior HSI, stature, mass, fascicle length, muscle thickness, pennation angle and relative fascicle length) were included in a stepwise regression model. The final model was built using the subset of variables that minimised the model's Akaike information criterion

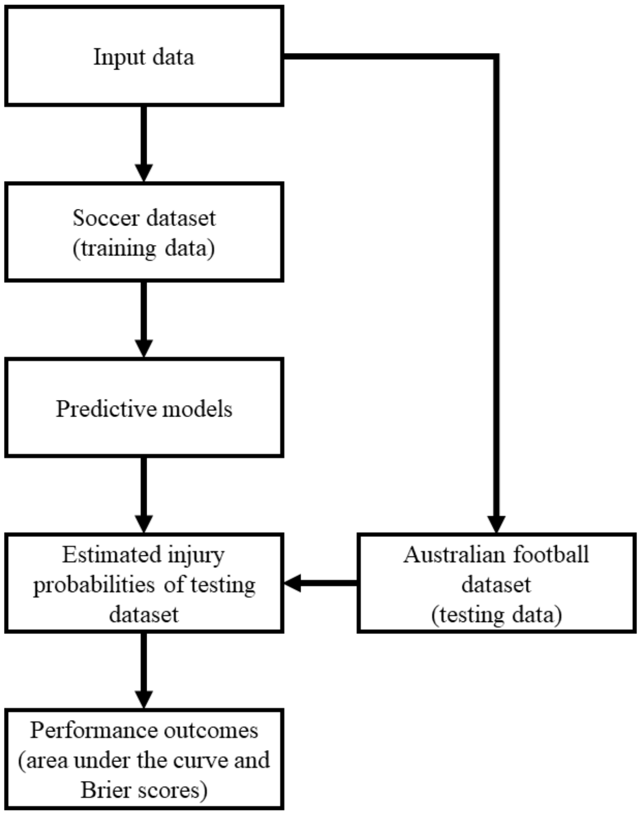

Figure 1. The logistic regression modelling approach implemented in this study.

To assess the performance of each logistic regression model to predict future HSI in the Australian football cohort, the area under the curve (AUC) and the Brier score was determined. Area under the curve, determined from a ROC curve, measures the ability of a model to distinguish between prospectively injured and uninjured observations. An AUC of 1.0 indicates that the predicted injury probabilities for the prospectively injured athletes are all greater than the predicted injury probabilities for the uninjured athletes. An AUC of 0.5 indicates classification no better than random chance. The AUC could also be considered as analogous to a percentage, where an AUC of 0.5 equates to successful prediction 50% of the time and an AUC of 1.0 is a successful prediction 100% of the time. Brier scores, measured on a scale of 0 to 1, are a measure of the precision of probabilistic predictions, with a Brier score closer to 0 indicating better precision. Calibration plots for all logistic regression models were constructed to provide a visual representation of how well a model can estimate the probability of an event (i.e. prospective HSI) across the spectrum of predicted probabilities.

All statistical analyses were performed using the R statistical programming language [19] and the following packages: caTools, dplyr, ggplot2, DescTools, scoring, OptimalCutpoints and ggpubr.

RESULTS

Participant characteristics

Complete prospective follow up was obtained for all participants. A total of 152 soccer players (age, 24.7 ± 5.0 years; stature, 179 ± 6 cm; mass, 75.6 ± 6.6 kg) and 169 Australian footballers (age, 23.6 ± 3.5 years; stature, 188 ± 8 cm; mass, 86.4 ± 8.7 kg) were included in the analyses. All descriptive data and differences between the two cohorts can be observed in Supplementary Material 1. Of the athletes that were included in this study, 27 soccer players and 30 Australian footballers sustained a prospective HSI during their respective seasons. For both cohorts more HSIs were sustained in matches (soccer, 20; Australian football, 17) compared to training (soccer, 6; Australian football, 13), although not all injuries had this information available (soccer, 1; Australian football, 0). For soccer players, the number of HSIs sustained per position: midfielder, 11; forwards, 9; and defenders, 7. For Australian football: midfielder, 6; forwards, 11; backs, 11; and rucks, 2. Descriptive statistics for both the prospectively injured and uninjured athletes of both cohorts can be found in Table 2.

Table 2. Descriptive statistics for athletes that sustained a prospective hamstring strain injury (HSI) and uninjured athletes from the soccer and Australian football cohorts. Data are presented as mean ± standard deviation for continuous variables or as total number for dichotomous variables. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head.

	Australian football cohort			Soccer cohort		
	Injured (n = 30)	Uninjured (n = 139)	p	Injured (n = 27)	Uninjured (n = 125)	p
Age (years)	24.9 ± 3.5	23.3 ± 3.5	0.029	27.0 ± 3.8	24.2 ± 5.1	0.002
Prior HSI	10	18	<0.001	9	21	0.063
Stature (cm)	186 ± 7	188 ± 8	0.850	180 ± 7	179 ± 6	0.395
Mass (kg)	84.8 ± 8.5	86.8 ± 8.7	0.253	76.4 ± 6.7	75.4 ± 6.6	0.463
Fascicle length (cm)	10.10 ± 0.89	10.20 ± 0.60	0.581	10.30 ± 1.48	11.10 ± 1.49	0.018
Muscle thickness (cm)	2.60 ± 0.22	2.61 ± 0.26	0.862	2.52 ± 0.31	2.51 ± 0.32	0.918
Pennation angle (degrees)	15.6 ± 1.0	15.4 ± 1.2	0.561	14.2 ± 1.4	13.2 ± 1.5	0.002
Relative fascicle length^	3.88 ± 0.31	3.94 ± 0.26	0.217	4.11 ± 0.45	4.44 ± 0.50	0.001

^Relative fascicle length refers to fascicle length relative to muscle thickness
NA; comparisons for binary data are not applicable

Relative risk, sensitivity and specificity

The RR of Australian footballers sustaining a prospective HSI, as well as sensitivity and specificity values, based on the cut points derived from the soccer cohort, can be found in Figure 2. Older athletes (≥ 25.4 years, RR = 2.7, 95% CIs = 1.4 to 5.2) and those with a prior HSI (RR = 2.5, 95% CIs = 1.3 to 4.8) were at an increased risk of HSI. Stature, mass, BFlh fascicle length, muscle thickness, pennation angle and relative fascicle length were not associated with an increased risk of HSI in Australian footballers when using cut points derived from the soccer cohort (Figure 2). All RR, sensitivity and specificity data can be found in Supplementary Material 2.

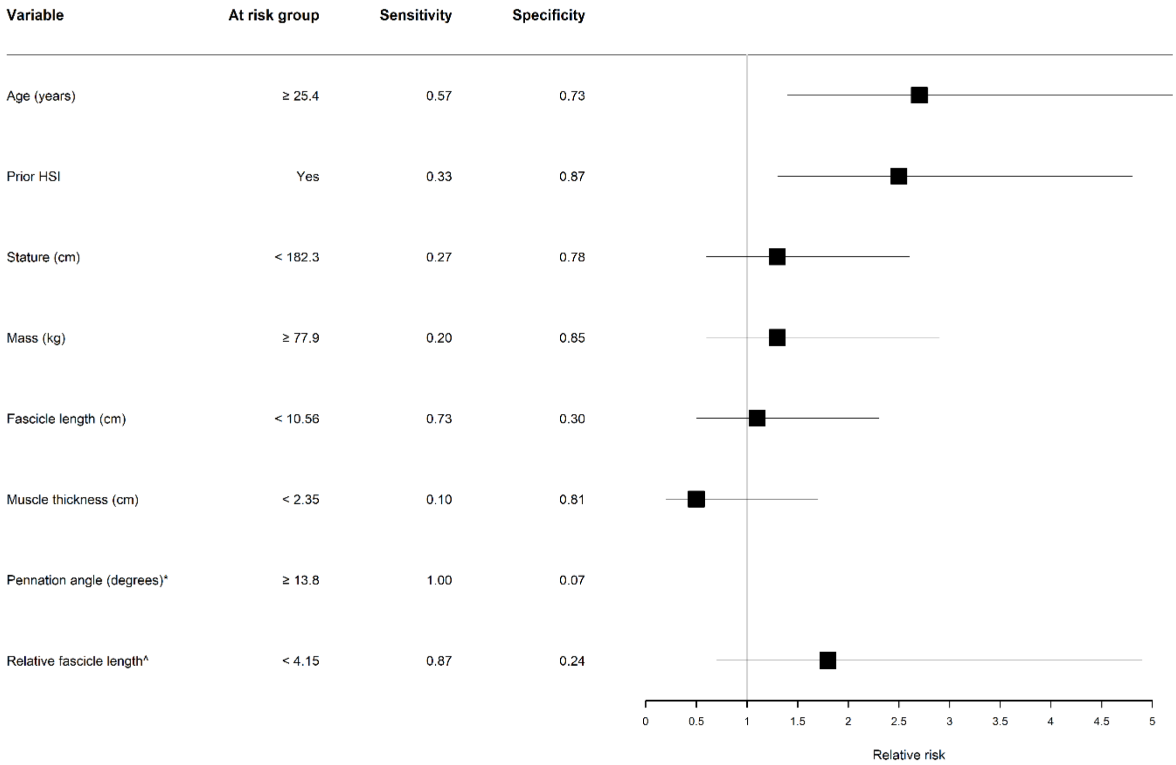

Figure 2. The relative risk (RR) of the Australian football athletes sustaining a prospective hamstring strain injury (HSI), as well as sensitivity and specificity values, based on the cut points derived from a previously collected dataset in soccer. If the 95% confidence intervals (represented by the black horizontal lines) cross the grey vertical line (RR = 1.0), this indicates

a non-significant RR. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head (BFlh). *RR and 95% confidence intervals for pennation angle could not be calculated due to a sensitivity value of 1.00, which indicates that there were no HSIs in the low-risk group. ^Relative fascicle length refers to BFlh fascicle length relative to muscle thickness.

Logistic regression models

The AUC and Brier score of each logistic regression model that was built using the soccer dataset and subsequently applied to the Australian football dataset can be found in Table 3 (model coefficients are provided in Supplementary Material 3 and variable importance for each individual model is provided in Supplementary Material 4). The model constructed using age and prior HSI performed best (AUC = 0.67, Brier score = 0.14) with the worst performing model a univariable model containing pennation angle (AUC = 0.53, Brier score = 0.18). The calibration of each univariable and multivariable model is illustrated in Figure 3 and Figure 4 respectively.

Table 3. The area under the curve (AUC) and Brier scoreof each logistic regression model. Models were built using data from the soccer cohort and used to estimate the probability of prospective hamstring strain injury (HSI) in the Australian football cohort. Estimated injury probabilities were compared to the actual outcomes to determine the predictive performance of each model. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head.

	Model Composition	AUC	Brier score
	Age	0.64	0.14
	Prior HSI	0.60	0.14
Univariable	Fascicle length	0.54	0.15
	Pennation angle	0.53	0.18
	Relative fascicle length^	0.56	0.16
	Age and prior HSI	0.67	0.14
	Age, prior HSI and fascicle length	0.65	0.14
Multivariable	Age, prior HSI and pennation angle	0.62	0.17
	Age, prior HSI and relative fascicle length^	0.65	0.15
	Stepwise regression#	0.65	0.15

^Relative fascicle length refers to fascicle length relative to muscle thickness. #All variables (age, prior HSI, stature, mass, fascicle length, muscle thickness, pennation angle and relative fascicle length) were included in the stepwise regression model. The final model was built using the subset of variables that minimised the model's Akaike information criterion. The final variables included were: age, prior HSI and relative fascicle length. Note that these are the same variables that were included in one of the a-priori determined models.

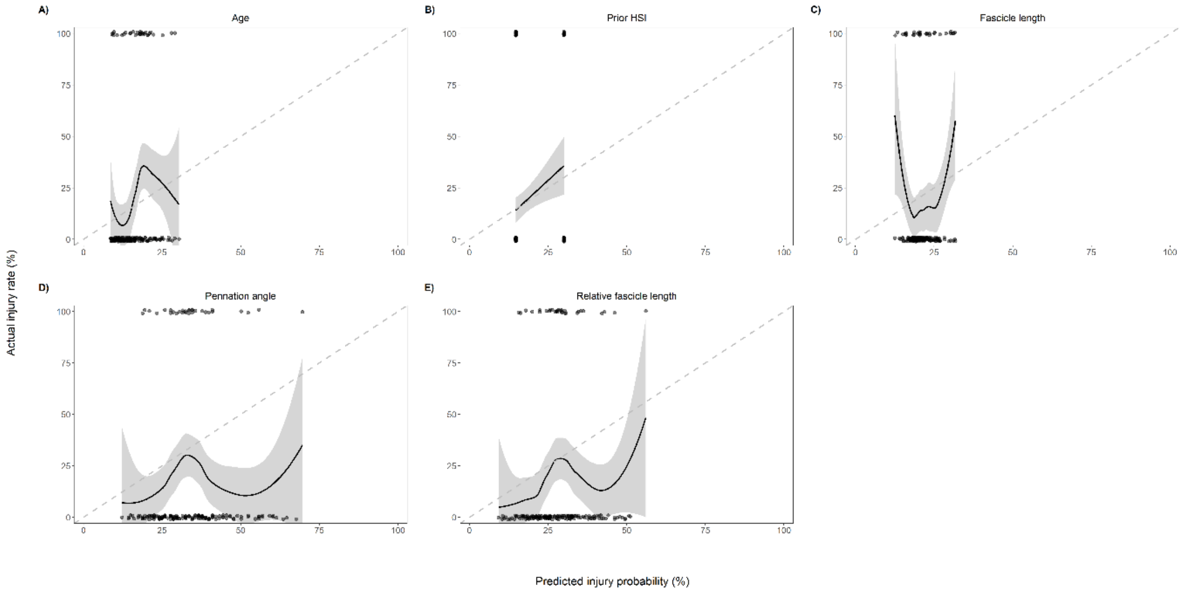

Figure 3. Calibration plots for all univariable logistic regression models with actual and predicted rates of hamstring strain injury (HSI). Calibration is a measure of how well a model can estimate the probability of an event. For example, if we were to take every observation with a predicted injury probability of 25%, a perfectly calibrated model would suggest that the actual rate of injury for these observations was equal to 25%. The 45-degree diagonal line represents perfect calibration and the grey shaded areas indicate the 95% confidence intervals. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head. Relative fascicle length refers to fascicle length relative to muscle thickness. Points at 100 on the y-axis represent predicted injury probabilities of subsequently injured athletes (with predicted probabilities shown on the x-axis) while points at 0 on the y-axis represent predicted injury probabilities of athletes who avoid subsequent injury. Excluding Plot B (prior HSI), all points are separated by height for visual clarity. A) age; B) prior HSI; C) biceps femoris long head (BFlh) pennation angle; D) BFlh fascicle length; E) BFlh fascicle length relative to muscle thickness.

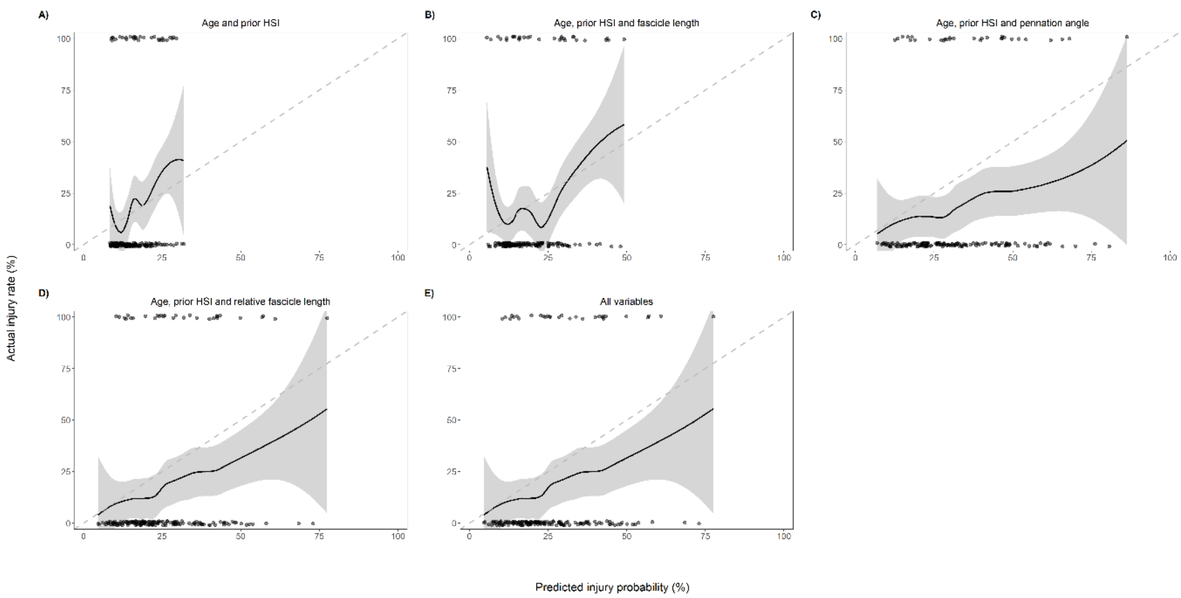

Figure 4. Calibration plots for all multivariable logistic regression models with actual and predicted rates of hamstring strain injury (HSI). Calibration is a measure of how well a model can estimate the probability of an event. For example, if we were to take every observation with a predicted injury probability of 25%, a perfectly calibrated model would suggest that the actual rate of injury for these observations was equal to 25%. The 45-degree diagonal line represents perfect calibration and the grey shaded areas indicate the 95% confidence intervals. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head. Relative fascicle length refers to fascicle length relative to muscle thickness. Points at 100 on the y-axis represent predicted injury probabilities of subsequently injured athletes (with predicted probabilities shown on the x-axis) while points at 0 on the y-axis represent predicted injury probabilities of athletes who avoid subsequent injury. A) age and prior HSI; B) age, prior HSI and biceps femoris long head (BFlh) fascicle length; C) age, prior HSI and BFlh pennation angle; D) age, prior HSI and BFlh fascicle length relative to muscle thickness; E) stepwise regression including all variables as inputs (final model included age, prior HSI and BFlh fascicle length relative to muscle thickness).

DISCUSSION

The key finding of this study was that previously reported risk factors associated with cut points derived from BFlh architectural variables in soccer players were not associated with future risk of HSI in Australian footballers. However, risk of future HSI in Australian footballers was associated with older age (≥25.4 years) and prior HSI, with the age cut point generated from a previously collected dataset in soccer. This study is the first to apply statistical cut points derived in one sporting cohort to determine the risk of HSI in another sporting cohort, which is recommended as a critical step in establishing the predictive ability of risk factor data [1, 20].

In contrast to the findings of the original investigation of the soccer cohort[24], cut points derived from BFlh architectural variables in soccer players were not associated with risk of HSI when applied to an Australian football cohort. Prior research has reported that soccer players with BFlh fascicles shorter than 10.56 cm were at a 4-fold increased risk of HSI compared to their longer counterparts [24]. In the current study, however, BFlh fascicle length was not associated the risk of HSI, with Australian football athletes possessing fascicles less than 10.56 cm being at similar level or risk (RR = 1.1) compared to the athletes with longer BFlh fascicles. This suggests that whilst BFlh architecture may play an important role in identifying elite soccer players' risk of future HSI, injury risk cut points derived from this cohort are not generalisable to Australian football. There are a number of potential reasons as to why data from soccer may not readily transfer to Australian football, not least differences in anthropometric and architectural characteristics between cohorts (Supplementary Material 1). Whilst it might be expected that risk factor cut point data from one sport, subsequently applied to another, is unlikely to have transference, in reality, practitioners from various sports rely on literature not specific to their sport to guide their HSI prevention and/or risk mitigation strategies. The present work provides evidence that an assumption of transference between sports cannot be guaranteed for modifiable risk factor cut points and highlights the importance of replication work, across different cohorts, for variables found to be associated with future HSI. However, age and prior HSI were associated with an increased risk of HSI in the soccer cohort [24] and, when these same cut points were applied to Australian footballers, an association was still present. These findings add to the existing body of evidence reporting age and prior HSI as strong, albeit non-modifiable, risk factors for future HSI [9].

When identifying the risk of HSI at an individual level, via logistic regression, the model built using age and prior HSI was superior to all other models. In prior research, models built using BFlh tended to outperform other models [24], however, in the current study, including BFlh architectural variables in the models typically reduced their predictive performance. The results of the logistic regression models are in line with the RR (association) data, suggesting that age and prior HSI offered the best predictive ability within the Australian football cohort. Despite this, the model built using age and prior HSI only had an AUC of 0.66. This value suggests that if we were to randomly select a prospectively injured athlete and an uninjured athlete, the likelihood that the best performing model would have allocated the prospectively injured athlete with a higher predicted injury probability (compared to the uninjured athlete) is only equal to 66%. Whilst there is no consensus on how to subjectively describe and/or interpret AUC data, an AUC of above 0.75 indicates that model performance was closer to perfect prediction than random chance. Given that all AUC of the logistic regression models reported in the current work were ≤ 0.67, this suggests their ability to correctly classify the prospectively injured and uninjured athletes was closer to random chance than it was to perfect predictive performance, as illustrated by the multivariable calibration curves (Figure 4), the models tended to overestimate the probability of future HSI. This is likely a function of the models being built using data from the soccer cohort, in which BFlh architecture influenced the risk of

HSI and highlights the fragility of the transference of logistic regression models between different sports.

Prior research has attempted to investigate the ability of other variables to identify the risk of HSI in elite Australian footballers. For example, an association between high-speed running distances and the risk of HSI at a group level in elite Australian footballers has been reported previously [22]. At an individual level, one study [4] has investigated the ability of internal and external training load data to predict lower limb non-contact injuries in elite Australian footballers. In this study, data from two seasons were used to predict injury occurrences in a third season. The best performing model was able to classify the athletes that sustained a prospective hamstring injury and the uninjured athletes with an AUC of 0.72. Whilst this study utilised an independent training and testing dataset (as per the current methods), it is important to note that the cohorts were not entirely independent. Whether the ability of internal and external training load to predict the occurrence of HSI is generalisable across cohorts from different sports remains to be seen. These results do, however, suggest that the addition of internal and external training load data may contribute to the improvement of multivariable HSI prediction models in Australian footballers.

Another study of a similar design to the current investigation has reported on the predictive performance of HSI risk factors in elite Australian footballers [23]. Low levels of eccentric knee flexor strength, in addition to older age and a history of HSI, have previously been associated with an increased risk of HSI in a cohort of elite Australian footballers [14]. A follow-up to the original investigation used these data to build predictive models and identify the risk of injury in another cohort of elite Australian footballers two years later [23]. Despite age, prior HSI and eccentric knee flexor strength being strongly associated with the risk of HSI in the original dataset [14], the predictive models built using these variables were only able to classify the prospectively injured and uninjured athletes in the follow-up cohort with a mean AUC of 0.52

[23]. In comparison, the worst performing multivariable model in the current study was that which was built using age, prior HSI and pennation angle (AUC = 0.62). The current findings suggest that the architectural variables included in this study, whilst not displaying a significant association with the risk of HSI, may facilitate better predictive performance than eccentric knee flexor strength. However, as aforementioned, prior research used independent training and testing datasets from the same sport [4, 23]. Accordingly, the results may be difficult to compare to current study, which is the first to use testing and training datasets from two different sporting cohorts.

Recent recommendations [3], have suggested using Brier scores as a predictive performance metric, which has been rarely, if ever, reported in the sports injury literature. Whilst the Brier scores did not offer a different interpretation of the current results in comparison to the AUC, it is important for researchers and practitioners alike to understand how to interpret Brier scores as a means to facilitate comparisons between future work. Measured on a scale of 0 to 1, Brier scores are a measure of the precision of probabilistic predictions, with a lower Brier score indicating better precision. When building predictive models, it is important to assess not only the ability of a model to distinguish between the prospectively injured and uninjured athletes (for which AUC is a metric well suited to do so), but also how precise the predicted injury probabilities are. Brier scores reflect the ability of a model to correctly predict the actual rates of injury observed. In the current study, the multivariable model with the lowest Brier score was built using age and prior HSI (Brier score = 0.14). The addition of fascicle length to this model did not negatively impact the Brier score, although it did reduce the AUC from 0.67 to 0.65. The addition of all other architectural variables, however, negatively impacted the Brier score (Table 3). The calibration curves illustrated in Figure 3 and Figure 4 provide a visual representation of each model's ability to correctly predict the actual observed injury rates. The use of calibration curves, whilst requiring a subjective interpretation, can provide a more granular understanding of model error, when considered in conjunction with AUC and Brier score data. These curves suggest that the addition of the architectural variables to the multivariable models tends to result in overestimation of injury rates (Figure 4) and this would have been indeterminant based on the objective performance measures only.

From a practical perspective, the results of this study suggest that practitioners must proceed cautiously when interpreting and translating the findings of an investigation in one sporting cohort to another sporting cohort, as it relates to HSI risk factors. It may be tempting, based on the seminal work [24], to conclude that 10.56 cm is an appropriate cut point for classifying athletes as having either short or long BFlh fascicles. However, this cut point was determined retrospectively from the data it was applied to and as a result, is closely fit to the original soccer cohort. Whilst this cut point displays some level of predictive ability in the soccer cohort [24], it was not appropriate for identifying Australian footballers at an increased risk of HSI. The best performing model in the current study achieved an AUC of 0.67. This indicates that if we were to randomly observe a prospectively injured and uninjured athlete, the likelihood that the model will have allocated the prospectively injured athlete with a higher predicted injury probably is equal to 67%. These results suggest a poor ability to correctly identify the risk of HSI at an individual level, even using previously reported risk factors. Accordingly, practitioners should be cautious when using risk factor data from a different sport to make inferences regarding their athletes' risk of future HSI.

There are limitations in this study that must be acknowledged. Firstly, the measure of BFlh fascicle length is an estimation made from the validated equation reported in the methods [2, 13]. This estimation is necessary due to the small transducer field of view utilised in this study. The methodology and equation employed for this estimation has been compared against cadaveric hamstring samples and has been reported as valid and reliable [13, 25] and has also been associated with the risk of injury [24]. However, the utilisation of other methods for determining BFlh architecture [7] may have provided different results. This notwithstanding, the validity of the current work is strengthened as the same method of BFlh architectural assessment and analysis was consistent across both cohorts. Secondly, the data used to build the predictive models in this study were only collected at the beginning of pre-season training for each study period. It is unknown whether more frequent measures of the architectural variables included in this study would have impacted predictive performance. Additionally, although prior HSI was significantly associated with the risk of injury in this study, prior research has suggested that more frequent measures of the impact of prior injury (such as measures of session availability) may provide more insight [21]. Thirdly, BFlh architectural data was used to predict all HSI While the exclusive prediction of BFlh injuries may have resulted in alternate findings, it would also negatively impacted statistical power. Finally, the current study does not report running exposure data from either cohort. Previous literature has shown that Australian footballers cover significantly higher distances during high-velocity running and sprinting as well as significantly more sprint efforts [26]Differences in running exposure between the two cohorts may have influenced our findings, however, we were unable to account for this.

In conclusion, modifiable HSI risk factors and their cut points, previously established in a cohort of elite soccer players, were not able to identify the risk of HSI in a cohort of elite Australian footballers, at both a group and an individual level. Currently, the ability of predictive models to correctly identify athletes at an increased risk of HSI is sub-optimal. Whilst the efficacy of the current methods to identify risk and predict the occurrence of HSI may warrant further investigation, practitioners should proceed with caution when interpreting and implementing the findings of previous research that is not specific to their cohort of interest.

Figure captions:

Figure 1. The logistic regression modelling approach implemented in this study.

Figure 2. The relative risk (RR) of the Australian football athletes sustaining a prospective hamstring strain injury (HSI), as well as sensitivity and specificity values, based on the cut points derived from a previously collected dataset in soccer. If the 95% confidence intervals (represented by the black horizontal lines) cross the grey vertical line (RR = 1.0), this indicates a non-significant RR. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head (BFlh). *RR and 95% confidence intervals for pennation angle could not be calculated due to a sensitivity value of 1.00, which indicates that there were no HSIs in the low-risk group. ^Relative fascicle length refers to BFlh fascicle length relative to muscle thickness.

Figure 3. Calibration plots for all univariable logistic regression models with actual and predicted rates of hamstring strain injury (HSI). Calibration is a measure of how well a model can estimate the probability of an event. For example, if we were to take every observation with a predicted injury probability of 25%, a perfectly calibrated model would suggest that the actual rate of injury for these observations was equal to 25%. The 45-degree diagonal line represents perfect calibration and the grey shaded areas indicate the 95% confidence intervals. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head. Relative fascicle length refers to fascicle length relative to muscle thickness. Points at 100 on the y-axis represent predicted injury probabilities of subsequently injured athletes (with predicted probabilities shown on the x-axis) while points at 0 on the y-axis represent predicted injury probabilities of athletes who avoid subsequent injury. Excluding Plot B (prior HSI), all points are separated by height for

visual clarity. A) age; B) prior HSI; C) biceps femoris long head (BFlh) pennation angle; D) BFlh fascicle length; E) BFlh fascicle length relative to muscle thickness.

Figure 4. Calibration plots for all multivariable logistic regression models with actual and predicted rates of hamstring strain injury (HSI). Calibration is a measure of how well a model can estimate the probability of an event. For example, if we were to take every observation with a predicted injury probability of 25%, a perfectly calibrated model would suggest that the actual rate of injury for these observations was equal to 25%. The 45-degree diagonal line represents perfect calibration and the grey shaded areas indicate the 95% confidence intervals. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) were derived from the biceps femoris long head. Relative fascicle length refers to fascicle length relative to muscle thickness. Points at 100 on the y-axis represent predicted injury probabilities of subsequently injured athletes (with predicted probabilities shown on the x-axis) while points at 0 on the y-axis represent predicted injury probabilities of athletes who avoid subsequent injury. A) age and prior HSI; B) age, prior HSI and biceps femoris long head (BFlh) fascicle length; C) age, prior HSI and BFlh pennation angle; D) age, prior HSI and BFlh fascicle length relative to muscle thickness; E) stepwise regression including all variables as inputs (final model included age, prior HSI and BFlh fascicle length relative to muscle thickness).

Supplementary materials:

Supplementary Material 1. Descriptive statistics comparing demographic and biceps femoris long head (BFlh) architectural data from Australian football and soccer cohorts. Data are presented as mean ± standard deviation for continuous variables or as total number for dichotomous variables.

Supplementary Material 2. The relative risk (RR) of Australian footballers sustaining a prospective HSI, as well as area under the curve (AUC), sensitivity and specificity values, based on the cut points derived from the soccer cohort. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head (BFlh).

Supplementary Material 3. Model coefficients results for all constructed models. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head (BFlh).

Supplementary Material 4. Variable importance plot of each variable within each model. All architectural variables (fascicle length, muscle thickness, pennation angle, relative fascicle length) are derived from the biceps femoris long head (BFlh). Variable importance was determined as the absolute value of the z-test value (the absolute coefficient divided by the standard error). For Model 10 all variables (age, prior HSI, stature, mass, fascicle length, muscle thickness, pennation angle and relative fascicle length) were included in a stepwise regression model. The stepwise regression model (Model 10 was built using the subset of variables that minimised the model's Akaike information criterion. The final variables included were: age, prior HSI and relative fascicle length. These variables were identical to those included in Model 9, hence the coefficients for both Models 9 and 10 are presented together.

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