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HAMSTRING MUSCLE ARCHITECTURE AND VISCOELASTIC PROPERTIES: RELIABILITY AND RETROSPECTIVE COMPARISON BETWEEN PREVIOUSY-INJURED AND -UNINJURED ATHLETES

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Background: The architecture of the biceps femoris (BF) and stiffness of the hamstrings have been found to be associated with injury risk. However, less is known about the architecture of the equally voluminous semitendinosus (ST), and viscoelastic properties of both muscles in individuals with a prior injury. Methods: BF and ST of 15 athletes (previously injured, n=5; control, n=10) were assessed using ultrasonography and myotonometry. Mean architecture (muscle thickness, pennation angle and fascicle length) and viscoelastic measures (stiffness, oscillation frequency and decrement) were compared between the previously-injured and contralateral uninjured limb, and between the previously-injured and control limbs (mean of both limbs of the control group). Control group participants returned for a duplicate measurement. Findings: Both muscles exhibited high reliability between sessions (ICC=0.89–0.98) for architecture. BF pennation angle was larger in the previously injured than both uninjured (+1.1°, d=0.65) and control (+1.51°, d=0.71). BF fascicles were shorter in the previously injured limb compared to the uninjured (-0.4 cm, d=0.65) and control (-0.6cm, d=0.67). BF was stiffer in the previously injured compared to uninjured (+9.2Nm^{-1}, d=1.28). ST architecture and viscoelasticity were similar across limbs. Conclusion: A prior hamstring strain injury is associated with a stiffer BF characterized by larger pennation angles and shorter fascicles.

Keywords: biceps femoris; semitendinosus; thickness; pennation angle; fascicle length; stiffness

1. Introduction

Hamstring strain injuries (HSI) are common in many activities that involve high-speed running. Football and sprinting in athletics are examples of such activities. HSIs have been reported to account for 37% of muscular injuries in football.[1] In a retrospective study documenting injuries among Australian Football League players, HSIs were found to be responsible for approximately 15% of total injuries reported in a single season.[2] The nature of HSIs results in lengthy rehabilitation periods that affect both recreational and elite players. The extended time loss and accompanying financial costs highlight the need for evidenced based strategies to reduce the incidence of HSIs in sport.

In recent years, majority of research on reducing HSI risk has investigated the efficacy of a number of prophylactic exercises. One such exercise that has received considerable attention is the Nordic hamstring lowers.[3, 4] The results of these studies provide strong evidence that exercises which strengthen the biceps femoris (BF) eccentrically may be more effective in reducing HSI risk compared to conventional training modalities through concentric strengthening.[5, 6] Since almost 80% of HSIs involve the BF,[7] it is understandable that the current literature is skewed towards investigation of the BF. However, with up to one-fifth of such injuries taking place at the similarly superficial and voluminous semitendinosus (ST),[7] a better understanding of the structural characteristics of both the BF and ST may provide a more comprehensive insight into the risk factors of HSI.

Among individuals with a history of HSI, previous studies have reported deficits in knee flexor eccentric strength,[8] rate of torque development and differences in the angle of optimal torque generation.[9, 10] The observed change in the angle of optimal torque generation at shorter muscle lengths has been postulated to be a consequence of fewer in-series sarcomeres, an accompanying structural feature of muscles with shorter fascicles.[11] A retrospective study had found shorter fascicles and larger pennation angles in the BF long head to be associated with previously injured hamstrings.[12] Although it has been suggested that shorter fascicles in the BF may increase an individual's risk of sustaining an HSI,[13] there is insufficient evidence to conclusively show that shorter fascicles are an characterize the architecture of hamstrings which have been strained, either as a consequence or intrinsic risk factor present prior to injury. Thus, before further investigations to establish the underlying mechanisms underpinning the contribution of shortened fascicles to HSIs, it is important to first characterize the differences in hamstring muscle architecture between previously injured and healthy hamstrings.

Apart from architecture, the viscoelastic properties of muscles have been suggested as a risk factor of injuries.[14] Individuals who had previously sustained a HSI were found to have poorer hamstring flexibility,[10] and poor hamstring flexibility is a likely consequence of hamstring stiffness.[15] In a recent study, elevated muscle stiffness was found to be related to injury incidence among elite athletes.[16] Therefore, measurements of viscoelastic properties which provide useful information of muscle stiffness could help to determine the risk of sustaining an HSI. To the authors' best knowledge, there are no published data on the viscoelastic properties of previously injured hamstring muscles.

In recent years, the advent and availability of high-fidelity medical imaging technology have made it easier to image muscle architecture through relatively affordable methods. Two-dimensional (2-D) ultrasonography is an imaging method commonly used to assess muscle architecture in-vivo and can provide useful information. Examples include quantified measures of muscle architecture such as muscle thickness, pennation angle and fascicle length.[17, 18] Specific to the hamstrings, good reliabilities of BF and ST parameters have been reported in healthy volunteers.[18] In recreationally active males, high reliability of BF muscle architecture was also found.[12] Regarding muscle viscoelastic properties measurements, excellent reliability has been reported for BF parameters such as stiffness, oscillation frequency, and decrement among generally healthy individuals.[19, 20] There are, however, no data on the reliability of hamstrings muscle architecture or viscoelastic properties specifically in sprint-based athletes who are more susceptible to HSI.

Therefore, the purpose of this study was to first determine the test-retest reliability of muscle architecture (muscle thickness, pennation angle and fascicle length) and viscoelastic properties. Subsequently, muscle architecture and viscoelastic properties of previously injured and -uninjured hamstrings were measured to provide a retrospective comparison. It was hypothesized that compared to the contralateral uninjured muscle and a control group, a previously injured hamstring would be characterized by features such as shorter fascicle length, larger pennation angle, and higher stiffness.

2. Methods

2.1. *Participants*

Fifteen male athletes participating in collegiate level sports involving long bouts of high-speed running were recruited for this study (Table 1). Ten participants with no history of HSIs in the 18 months prior to the start of the study were recruited to the control group. The previously injured group comprised five participants who had completely recovered from a single episode of a self-reported unilateral BF strain injury sustained in the 18 months prior to the start of the study. The criteria for complete recovery was a minimum period of three months from the onset of injury and a return to pre-injury training intensity before the start of the study. Confirmation of a HSI was defined by participant description of a sharp pain in the posterior aspect of the thigh which resulted in the immediate termination of an ongoing exercise.[21] All participants in the previously-injured group reported the location of this pain as the lateral side of the posterior thigh. The control group was both the comparison and test-retest group for reliability measures of muscle architecture and viscoelastic properties. All participants provided written informed consent prior to experimental procedures. The study was approved by the Nanyang Technological University Institutional Review Board (IRB-2015-02-005).

Table 1. Participant characteristics of male athletes from sprint-based sports.

	Control (n=10)	Previously injured (n=5)	P-value
Age [years]	23.2 (2.1)	22.8 (1.9)	0.726
Height [m]	1.75 (0.32)	1.73 (0.18)	0.900
Body mass [kg]	69.5 (3.2)	67.0 (2.9)	0.166

2.2. Procedure

Participants from the *control group* reported to the laboratory on two separate occasions, with a minimum of seven days between sessions. Previously injured *group* participants underwent a single measurement session. Participants from both groups were instructed to avoid activities involving excessive physical exertion in the 48 hours prior to the start of each measurement session.

2.3. ***Architecture assessment***

Reporting to the laboratory at the same times, participants were asked to lay prone on a standard physiotherapy bed with both feet hanging off the edge. A cushion was placed under the participant's head for support. Two-dimensional, B-mode ultrasonography (frequency = 12 MHz, depth focus = 8 cm, field of view = 14 - 47 mm; GE Healthcare Vivid-i, Wauwatosa, WI) was used to image the muscles of interest. Images were obtained over the muscle belly along its longitudinal axis, with the probe positioned on the mid-point between the ischial tuberosity and the knee joint fold, along corresponding lines for the BF and ST (Ref 12; Fig. 1). A minimum of five images with good fidelity were obtained at each site. The linear array ultrasound probe was carefully placed on the skin and maneuvered over the site of interest with hypoallergenic ultrasound gel. The probe was held perpendicular to the surface of the posterior thigh and aligned longitudinally. In order to reduce the effect of probe pressure on the accuracy of measurement results,[22] minimal pressure was used to stabilize the probe.

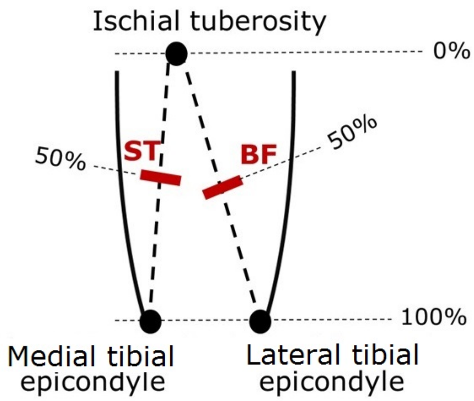
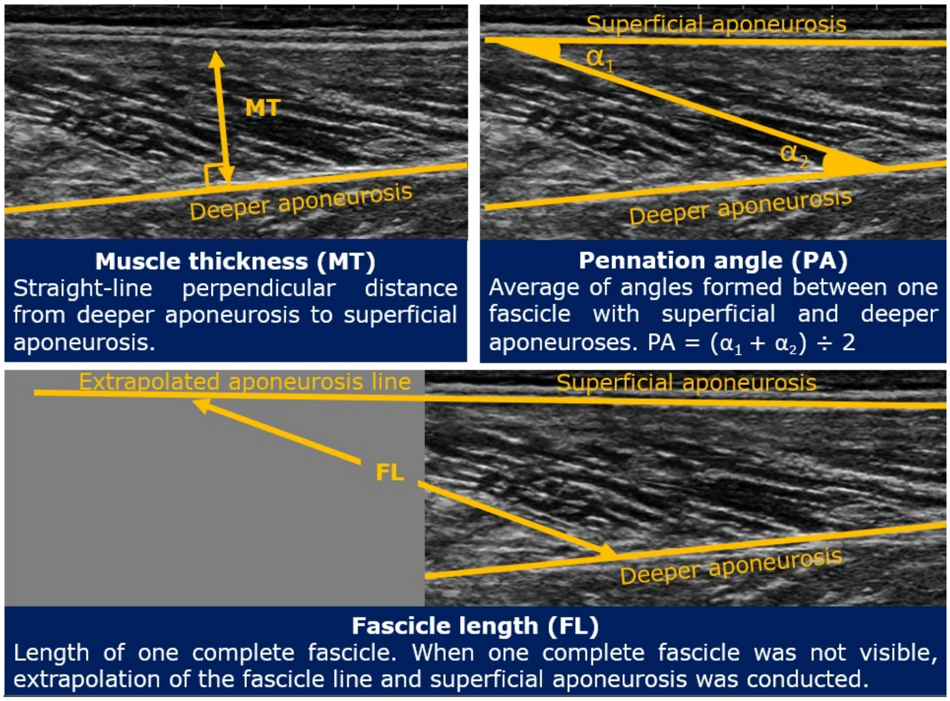


Fig. 1. Measurement sites on biceps femoris (BF) and semitendinosus (ST).

Ultrasound images were exported for offline analysis (Kinovea 0.8.15, Kinovea). Muscle architecture properties were measured according to the methods shown in Fig. 2. Muscle thickness (MT) was measured as the straight-line perpendicular distance between the deeper and superficial aponeuroses. To measure pennation angle (PA), a fascicle was traced on the image and marked out. PA was calculated as the average of angles formed between this fascicle with the lines of both the deeper and superficial aponeuroses. Fascicle length (FL) was defined as the length of a complete fascicle identified end-to-end between aponeuroses. On images where the fascicle is not fully visible, the lines of the fascicle and aponeuroses were extrapolated for analysis. For both PA and FL, five fascicles which could be most clearly identified were measured on the same image. The recorded measurement was calculated as the average of these five individual values.



Muscle thickness (MT)
Straight-line perpendicular distance from deeper aponeurosis to superficial aponeurosis.

Pennation angle (PA)
Average of angles formed between one fascicle with superficial and deeper aponeuroses. PA = (α_1 + α_2) ÷ 2

Fascicle length (FL)
Length of one complete fascicle. When one complete fascicle was not visible, extrapolation of the fascicle line and superficial aponeurosis was conducted.

Fig. 2. Muscle architecture variables measured on ultrasound images.

2.4. *Viscoelastic assessment*

Measurements of viscoelastic properties were obtained using a hand-held myotonometer (MyotonPRO, Myoton AS). The myotonometer was able to measure muscle stiffness, decrement and oscillation frequency – viscoelastic properties of interest in this study. The use of the myotonometer has been found to have high inter-day reliability (ICC=0.92) in the measurement of BF stiffness and has been suggested to be generally reliable for clinical assessments of muscle viscoelastic properties.[20] This method was also observed to demonstrate good intra-session (ICC=0.81) and inter-rater reliability (ICC=0.83).[23] Two measurements (five consecutive taps per measurement, one second interval) were taken at the same site where ultrasound scans were taken and exported for analysis. Special care was taken to ensure that contact pressure was similar between all repeated measurements.

2.5. *Statistical analysis*

Statistical analyses were performed using the statistical software SPSS Version 24.0 (IBM Corporation, Chicago, IL). Descriptive statistics of architectural and viscoelastic properties of the BF and ST of both limbs in the control group calculated and presented as means and standard deviations. For the analysis of test-retest reliability, intraclass correlation coefficient (ICC), standard error of measurement (SEM) and minimum detectable change (MDC) at 95% confidence interval were calculated. MDC was calculated according to the formula SEM × 1.96 × √2. An ICC equal to or higher than 0.90 was considered high, between 0.80 and 0.89 was moderate, and less than 0.79 was interpreted as poor.

Since there were no differences ($p > .05$) in the muscle architecture and viscoelastic measures between both limbs (left and right) in the control group, the average value was used for subsequent comparison with the previously injured athletes. Due to the small sample size in the previously injured group ($n = 5$), the use of inferential statistics will not provide a meaningful interpretation of results. Instead, differences were interpreted using effect size (Cohen's d).[24] Cohen's d was reported to represent the effect size of comparisons, with the levels of effect being classed as small ($d = 0.20$), medium ($d = 0.50$), or large ($d = 0.80$) as recommended by Cohen.[24]

3. Results

3.1. *Inter-session reliability*

In general, BF and ST muscle architecture variables in both right and left limbs of the control group demonstrated high reliability between sessions (Table 2). ICC values were higher than 0.94 for all BF muscle architecture variables in both limbs. In the ST of both limbs, ICC values were high for measurements of both pennation angle (≥0.92) and fascicle length (≥0.95). ICCs for ST muscle thickness ranged from moderate (left, 0.89) to high (right, 0.93). For muscle viscoelastic properties, reliability measures of stiffness and decrement in the BF of both limbs were high (≥0.90). However, oscillation frequency measures showed poorer reliability between sessions.

3.2. *Hamstring architecture comparisons*

When comparing pennation angle and fascicle length between limb groups, a close to large effect size was found for some differences (Table 3). In the BF, larger pennation angles (+1.54°, $d = 0.71$) and shorter fascicles (-0.58 cm, $d = 0.67$) were reported in the IL when compared to the CL. Similarly, BF in the IL had larger pennation angles (+1.06°, $d = 0.48$)

compared to the UL. Fascicles in the ST of the CL were also found to be longer (+1.03 cm) compared to the IL, with a large effect size (d = 1.65).

Table 2. Descriptive statistics (mean ± standard deviation) and test-retest reliability data from the control group (n=10) for muscle architecture and viscoelastic properties of biceps femoris and semitendinosus.

	Session 1	Session 2	ICC Mean (95% CI)	SEM	MDC
Architecture					
Biceps femoris					
Right leg					
MT [cm]	2.30 ± 0.33	2.26 ± 0.33	0.97 (0.86–0.99)	0.12	0.34
PA [°]	16.9 ± 2.8	17.1 ± 2.7	0.96 (0.86–0.99)	0.99	2.75
FL [cm]	8.90 ± 1.06	9.02 ± 0.86	0.95 (0.80–0.99)	0.41	1.15
Left leg					
MT [cm]	2.24 ± 0.25	2.25 ± 0.19	0.95 (0.78–0.99)	0.09	0.26
PA [°]	16.4 ± 2.6	16.6 ± 2.3	0.96 (0.85–0.99)	0.91	2.52
FL [cm]	8.75 ± 1.15	8.80 ± 1.10	0.98 (0.92–1.00)	0.30	0.82
Semitendinosus					
Right leg					
MT [cm]	2.31 ± 0.28	2.27 ± 0.22	0.93 (0.72–0.98)	0.13	0.36
PA [°]	14.4 ± 3.2	14.0 ± 2.2	0.96 (0.84–0.99)	1.08	2.99
FL [cm]	10.05 ± 0.48	10.11 ± 0.56	0.95 (0.78–0.99)	0.24	0.65
Left leg					
MT [cm]	2.22 ± 0.26	2.24 ± 0.20	0.89 (0.57–0.97)	0.14	0.38
PA [°]	14.1 ± 2.6	13.3 ± 1.9	0.92 (0.69–0.98)	1.37	3.81
FL [cm]	10.33 ± 0.68	10.27 ± 0.48	0.95 (0.81–0.99)	0.25	0.68
Viscoelastic property					
Biceps femoris					
Right leg					
Stiffness [Nm^{-1}]	263.7 ± 51.2	258.3 ± 49.5	0.93 (0.72–0.98)	25.11	69.61
Oscillation freq [Hz]	18.3 ± 1.9	16.1 ± 2.7	0.77 (0.09–0.94)	2.92	8.10
Decrement	1.66 ± 0.30	1.55 ± 0.23	0.94 (0.75–0.99)	0.16	0.44
Left leg					
Stiffness [Nm^{-1}]	255.6 ± 39.5	253.7 ± 28.9	0.91 (0.64–0.98)	18.84	52.22
Oscillation freq [Hz]	18.6 ± 1.2	17.4 ± 2.4	0.51 (-0.97–0.88)	2.41	6.67
Decrement	1.69 ± 0.16	1.68 ± 0.11	0.91 (0.65–0.98)	0.08	0.21
Semitendinosus					
Right leg					
Stiffness [Nm^{-1}]	231.9 ± 19.3	232.2 ± 15.2	0.77 (0.07–0.94)	14.27	39.56
Oscillation freq [Hz]	18.7 ± 1.2	17.9 ± 1.1	0.72 (-0.11–0.93)	1.28	3.54
Decrement	1.73 ± 0.08	1.74 ± 0.11	0.65 (-0.40–0.91)	0.10	0.29
Left leg					
Stiffness [Nm^{-1}]	236.6 ± 14.8	241.9 ± 20.0	0.84 (0.36–0.96)	13.49	37.38
Oscillation freq [Hz]	17.8 ± 1.9	17.3 ± 1.5	0.85 (0.40–0.96)	1.27	3.51
Decrement	1.68 ± 0.16	1.70 ± 0.12	0.91 (0.62–0.98)	0.08	0.23

Note: MT–muscle thickness; PA–pennation angle; FL–fascicle length; CI–confidence interval; ICC–intraclass correlation; SEM–standard error of measurement; MDC–minimum detectable change at 95% CI.

Table 3. Muscle architecture comparisons between control limb (CL), uninjured limb (UL) and previously injured limb (IL).

		Control (n=10)	Previously injured group (n=5)		CL versus IL		UL versus IL	
		Mean of both limbs (CL)	Uninjured limb (UL)	Previously injured limb (IL)	Mean difference (95% CI)	Effect size (d)	Mean difference (95% CI)	Effect size (d)
BF	MT [cm]	2.27 ± 0.28	2.33 ± 0.11	2.26 ± 0.13	0.01 (-0.28–0.30)	0.05	0.07 (-0.10–0.24)	0.53
	PA [°]	16.6 ± 2.3	17.1 ± 0.9	18.2 ± 2.2	-1.54 (-4.25–1.18)	0.71	-1.06 (-3.64–1.52)	0.48
	FL [cm]	8.82 ±1.08	8.66 ±0.71	8.24 ± 0.58	0.58 (-0.55–1.71)	0.67	0.41 (-0.97–1.80)	0.37
ST	MT [cm]	2.26 ± 0.27	2.26 ± 0.32	2.21 ± 0.37	0.05 (-0.30–0.41)	0.15	0.05 (-0.03–0.14)	0.78
	PA [°]	14.2 ± 2.6	14.4 ± 1.9	13.7 ± 3.0	0.48 (-2.75–3.71)	0.18	0.64 (-1.63–2.91)	0.35
	FL [cm]	10.19 ± 0.54	9.08 ± 0.63	9.16 ± 0.70	1.03 (0.32–1.73)	1.65	-0.08 (-1.22–1.05)	0.09

Note: Effect size: small (d = 0.20), medium (d = 0.50), or large (d = 0.80) (Ref. 24).

3.3 *Hamstrings viscoelastic property comparisons*

The BF in the IL was stiffer (+9.2 Nm-1, d = 1.28) compared to the UL (Table 4.). Oscillation frequency of the BF was lower in the IL when compared to the CL (-1.0 Hz, d = 0.88) and UL (-0.7 Hz, d = 1.03); and lower on the ST of the IL when compared to the UL (-0.5 Hz, d = 0.73).

Table 4. Viscoelastic property comparisons between control limb (CL), uninjured limb (UL) and previously injured limb (IL).

		Control (n=10)	Previously injured group (n=5)		CL versus IL		UL versus IL	
		Mean of both limbs (CL)	Uninjured limb (UL)	Previously injured limb (IL)	Mean difference (95% CI)	Effect size (d)	Mean difference (95% CI)	Effect size (d)
BF	Stiffness $[Nm^{-1}]$	259.7 ± 43.8	257.3 ± 3.7	266.5 ± 9.5	-6.9 (-50.4–36.7)	0.21	-9.2 (-23.1–4.7)	0.83
	Oscillation freq [Hz]	18.4 ± 1.4	18.1 ± 0.6	17.4 ± 0.8	1.0 (-0.5–2.4)	0.88	0.7 (-0.1–1.5)	1.03
	Decrement	1.89 ± 0.21	1.81 ± 0.03	1.83 ± 0.08	-1.55 (-0.37–0.06)	0.53	-0.02 (-0.08–0.05)	0.02
ST	Stiffness $[Nm^{-1}]$	234.3 ± 15.5	244.0 ± 6.0	245.9 ± 7.1	-11.6 (-27.6–4.4)	0.96	-1.9 (-6.9–3.1)	0.48
	Oscillation freq [Hz]	18.2 ± 1.1	18.5 ± 0.5	18.0 ± 0.7	0.2 (-1.0–1.4)	0.22	0.5 (-0.3–1.2)	0.73
	Decrement	1.71 ± 0.12	1.84 ± 0.06	1.79 ± 0.03	-0.09 (-0.21–0.04)	0.91	0.04 (-0.06–0.13)	0.56

Note: Effect size: small (d = 0.20), medium (d = 0.50), or large (d = 0.80) (Ref. 24).

4. *Discussion*

In the first part of this study, the inter-session reliability of hamstring muscle architecture and viscoelastic property measurements were determined through the use of 2-D ultrasonography and myotonometry, respectively. It was found that muscle architecture measures, when obtained by the same operator, were reliable with high ICCs reported. This finding further substantiates the reliability of ultrasound measures of the hamstrings that have been previously reported.[12, 25] Viscoelastic property assessments utilizing a handheld myotonometer, on the other hand, demonstrated lower inter-session reliability values for majority of measured variables. However, inter-session reliability for these variables was still good, with BF stiffness measures exhibiting similar ICCs to architectural variables measured. Overall, the main finding in this part of the study suggests that the quantifications of hamstrings architecture and viscoelastic properties through the methods used can be reliably interpreted. These same methods could be replicated in future similar studies investigating hamstrings structure, or possibly in other muscle sites.

When muscle architecture parameters between athletes without and with a history unilateral HSI were compared, it was found that the BF in the IL had larger pennation angles and shorter fascicles. In this study, all hamstring injuries were reported to have been sustained on the lateral side of the posterior thigh which corresponds to the location of the BF. This finding suggests that a previous injury to the BF is associated with more pennate and shorter muscle fascicles. This finding is similar to the results of a previous retrospective study characterizing the architecture of the BF following a hamstring injury.[12] The main implication of this finding is that the differences in BF architecture between previously-injured and uninjured limbs, particularly the presence of fewer in-series sarcomeres within the muscle which is an accompanying feature of shorter fascicles, may be a reason why some hamstrings are more susceptible to damage during powerful eccentric contractions - a mechanism of muscle contraction found in movements which involve high-speed running.

Although the hamstrings contain an almost equally voluminous ST, there is limited knowledge about its possible role in the onset of HSIs, either as the injured site or contributor to a BF injury. In this study, little difference in ST architecture was found between contralateral limbs in an athlete with a prior HSI. Based on this finding, it is likely that either ST architecture has no contributory role to the occurrence of HSIs in the BF, or injuries to the BF have no consequence on ST architectural properties. Further investigations of individuals who have sustained a previous ST strain injury would provide meaningful information to profile the association between pennation angle and fascicle length, with injury across both hamstring muscles. Currently, results from previous work and evidence from this study suggest it is likely that an association between BF architecture and injury exists. However, it is important to note that this association may either be retrospective or prospective in nature.

The effects of hamstring flexibility on HSI risk have been reported with contradicting results.[26, 27] Hamstring flexibility has been assessed with a variety of methods involving both passive,[28, 29] and active methods.[30, 31] However, the utility of these assessments is limited in isolating the outcome measure of flexibility as they involve several confounding variables such as lumbar spine flexibility and neural extensibility. Passive muscle stiffness has been found to be positively correlated to joint flexibility,[32] and could possibly provide a more accurate assessment of passive hamstring flexibility. Stiffness measures of the BF measured in this study were found to be higher on the IL compared to the UL. In this investigation, the use of a hand-held myotonometer which involved a passive assessment environment, eliminated the presence of several factors which might have had a confounding influence. Coupled with the good inter-session reliability of stiffness measures that has been previously reported and found in this study, it is possible that poor hamstring flexibility is associated with HSIs. This could be explained by higher muscular stiffness at the hamstrings which results in more tensile forces at the musculotendinous unit during lengthening as compared to a less stiff muscle,[27] increasing the stress at the muscle and reducing its ability to sustain loads.

The main limitation to this study is the relatively small number of participants recruited who had previously sustained a hamstring injury. Therefore, interpretation of results was based majorly on calculated mean differences, 95% CI and effect size (Cohen's d)[24]. This statistical interpretation was chosen to provide a more meaningful understanding of any observed differences found in this study. Additionally, although the reliability of muscle architecture and viscoelastic measurement methods was established in this study, they were not validated. However, previous literature provides strong evidence for the validity of 2D-ultrasound imaging data with cadaveric measurements;[33] and readings obtained from a hand-held myotonometer with other viscoelastic measurement methods.[34]

4.1. *Conclusion*

The results from the first part of this study suggest that 2-D ultrasonography and hand-held myotonometry can be reliably utilized for quantifying muscle architecture and viscoelastic properties of the hamstrings, respectively. In a hamstring with a prior strain injury, the BF is characterized by greater muscle stiffness, larger pennation angle and shorter fascicles (when compared to the UL and CL). To determine if these features are an intrinsic risk factor or a consequence of injury to the hamstring, further investigations need to be undertaken. Future work should progress to using a prospective study design to establish the role of both hamstring muscle architecture and viscoelastic properties in the onset of HSI.

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Disclosure

There are no potential conflicts of interest.

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