RESEARCH ARTICLE

A retrospective comparison of the biceps femoris long head muscle structure in athletes with and without hamstring strain injury history

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Abstract

Introduction

Hamstring strain injuries (HSI) and re-injuries are endemic in high-speed running sports. The biceps femoris long head (BFlh) is the most frequently injured muscle among the hamstrings. Structural parameters of the hamstring muscle are stated to be susceptible to strain injuries at this location. This retrospective study targeted comparing the BFlh's structural parameters between previously injured and uninjured athletes.

Methods

Nineteen male athletes with previous BFlh strain injury history and nineteen athletes without former lower extremity injury history were included in this study. Fascicle length, mid-muscle belly and distal musculotendinous (MTJ) passive stiffnesses of the biceps femoris long head (BFlh) were examined via b-mode panoramic ultrasound scanning and ultrasound-based shear-wave elastography. Parameter comparisons of both legs within and between athletes with and without injury history were performed.

Results

Comparison of the BFlh fascicle length between the injured leg of the injured group and the legs of the controls revealed a trend to shorter fascicle lengths in the injured leg ($p = 0.067$, $d = -0.62$). However, the mid-muscle belly passive stiffness of the BFlh was significantly

higher in the injured legs ($p = 0.009$, $d = 0.7$) compared with the controls. Additionally, the distal MTJ stiffness was much higher in the previously injured legs compared with controls ($p < 0.001$, $d = 1.6$).

Conclusions

Outcomes support the importance of BFlh properties related to stiffness, and fascicle length for injury susceptibility in athletes. Future prospective studies should determine whether the higher stiffness in the injured athletes is a cause or consequence of the HSI. Physical therapy and rehabilitation programmes after HSI should focus on BFlh muscle properties i.e., elasticity and fascicle length for reducing re-injury and increasing sports performance.

1. Introduction

Hamstring strain injuries (HSI) are common in sports with requirements for high-speed running. A further problem with HSI is the high and severe reoccurrence of HSI [1], which could indicate difficulty in rehabilitating the initial HSI effectively. HSI incidence has increased compared with earlier epidemiologic data in various sports [1]. For example, 24% of all injuries have been recorded as HSI in soccer nowadays [2]. Hence, scientists have focused on identifying risk factors and developing an optimal prevention strategy for HSI over the last two decades.

The late swing phase of running is often described as the most vulnerable time for HSI [3–5]. During the late swing phase of running, the hamstrings produce eccentric contraction to control the antagonists and decelerate the tibia [6]. At this moment, the biceps femoris long head (BFlh) undergoes the greatest elongation among the hamstrings by reaching 110% of its length [7]. Due to this elongation, HSI generally occur when the muscle fascicles can not resist the increased tensile force [3]. In particular, HSI occur frequently in the BFlh among the hamstrings [8], with BFlh distal musculotendinous junction (MTJ) injuries being more common [9], recurrent, severe and require longer rehabilitation than other HSI [9, 10].

Besides various risk factors [11], research has identified increased passive hamstring muscle stiffness [12] and shorter BFlh fascicle lengths [13] as structural risk factors for HSI [11]. However, it was suggested that the use of trigonometric equation [14] or manual linear extrapolation [15, 16] techniques overestimate the BFlh fascicle length in comparison to the extended field of view (EFOV)/panoramic ultrasound scanning technique [17]. The former techniques were used in previous retrospective studies comparing BFlh fascicle lengths between athletes with and without injury history [18–22]. Specifically, trigonometric equations techniques (+0.5 to +1.9 cm), and manual linear extrapolation technique (+0.33 cm) overestimate BFlh fascicle lengths [17]. Additionally, a further study [12] found passive muscle stiffness is a risk factor for HSI. However, an unspecific technique (free oscillation) was used that measures the passive stiffness of all muscle and tendon units of all knee flexors. Thus, this technique does not provide specific information for the BFlh, i.e., not providing specific measurements of stiffness of the MTJ and other locations of the BFlh. The recent technological advancements allow scientists to explore the passive stiffness of a specific part of individual muscles via ultrasound-based shear-wave elastography [23]. In this way, specific information on BFlh muscle stiffness in athletes who returned to play from HSI can provide new insights into the hamstring injury rehabilitation process.

To the authors' knowledge, no retrospective study compared the BFlh fascicle length via the EFOV technique or passive stiffness of muscle belly and distal MTJ of the BFlh in athletes with and without HSI history altogether. Exploring these structural parameters of the BFlh can bring new insights into the rehabilitation and prevention strategies for HSI.

Therefore, this study aimed to compare the BFlh fascicle length via the EFOV technique, passive stiffness of the mid-belly and MTJ of the BFlh via ultrasound-based shear wave elastography between previously injured legs and uninjured contralateral legs of athletes with HSI history, and both legs of athletes without HSI history. The hypotheses of this study were considered as follows: 1) previously injured legs will have shorter fascicles in the BFlh compared to contralateral uninjured legs and both legs of the athletes without HSI due to possible decreased exertion at the beginning of rehabilitation [22], reduced involvement in sports [24], and/or neural inhibition after the HSI [24–29]; 2) previously injured legs will have higher stiffness in the BFlh after the injury compared with contralateral uninjured legs and both legs of the athletes without HSI due to possible contractures or scars in the muscle [24, 26, 30–35].

2. Methodology

2.1. Research design

This study was designed as a retrospective comparative case-control study. The injured legs of the athletes with HSI history constituted the injured group (n = 19). The uninjured legs of the athletes with HSI formed the internal control group (n = 19). Both legs of the athletes without HSI and lower extremity injuries participating in the same sports were used as the external control group (n = 38). Ethical approval was obtained from the Health Research Ethics Committee of Universitas 'Aisyiyah Yogyakarta (Approval No. 2843/KEP-UNISA/V/2023). Before the study, participants read and signed a written informed consent according to the Declaration of Helsinki [36]. Indonesian authors (second, third, fourth and fifth authors) of this study communicated with the participants in Indonesian languages. The recruitment and data collection of the study started on the 10th of May 2023 and finished on the 30th of June 2023.

2.2. Participants

Athletes who returned to sports after an HSI were included in the injured group. In this study, athletes were defined as people partaking professionally and semi-professionally in sports. The inclusion criteria for the injured group were: a) male athlete between 18–45 years old; b) returning to pre-level sports competition after HSI in the BFlh. Criteria for the control group were: a) male athlete between 18 and 45 years old, b) being without a known lower extremity injury history, c) performing the same sports as the injured group (e.g., if one professional football player is included in the injured group, one professional football player from the same competition level participated to the study in the control group.)

2.3. Sample size

A priori sample size calculation was made using the F test, one-way ANOVA option in the G*Power (Version 3.1) [37] for two groups, 0.74 effect size [22], 0.05 alpha level, 0.95 statistical power. In total, a sample size of 26 was required for the study (13 previously injured players, 13 controls). However, this study compared 19 athletes for the injured group and 19 athletes for the uninjured group. Therefore, 19 injured legs were compared to 19 contralateral uninjured legs and 38 legs of the control group to reach a high statistical power. The achieved power of the study was determined as 0.96 for the fascicle length (effect size: 0.62 (injured leg vs. uninjured legs of the control group), two groups, 0.05 alpha level and 38 sample size (total

legs)); the power of 0.97 for mid-muscle belly stiffness (effect size: 0.70 (injured leg vs uninjured legs of the control group), two groups, 0.05 alpha level and 38 sample size (total legs)); and power of 1.00 for the distal MTJ stiffness measurements of the BFlh (effect size: 1.6 (injured leg vs uninjured legs of the control group), two groups, 0.05 alpha level and 38 sample size (total legs)) via G*Power (Version 3.1) [37].

2.4. Measurement procedures

Before starting the measurements, a medical examination was performed by a medical team consisting of an orthopaedic surgeon, a medical doctor, and a physiotherapist. Physical characteristics (height (cm), body mass (kg), body mass index (BMI)), injury history, and playing history (years) of the participants were recorded. The preferred kicking leg of a ball was accepted as the dominant leg.

The BFlh fascicle length was measured via the EFOV technique via b-mode ultrasound (LOGIQ P8, General Electric Healthcare, Wauwatosa, WI). The mid-belly and distal MTJ passive muscle stiffness measurements of the BFlh were completed via ultrasound-based shear wave elastography (SWE) (LOGIQ P8, General Electric Healthcare, Wauwatosa, WI). The BFlh fascicle length and passive stiffness measurements were performed twice for each dominant and non-dominant leg by totally removing the ultrasound probe (L3-12-RS: Wideband Linear Array Probe, 2 to 11 MHz, width: 51.2 mm, depth: 40 to 80 mm) from the skin between the measurements on the same measurement session. The average results of the two measurements were accepted as the outcomes. Reliability analyses were performed for the outcomes of BFlh fascicle length, mid-belly and distal MTJ passive stiffnesses of the BFlh between these two measurements for all the legs of all the athletes. The measurements were performed by the first author, who is highly experienced with the BFlh fascicle length, passive muscle stiffness and morphology measurements of the BFlh [16, 38, 39], alongside at least one of the medical team. The fascicle length digitisations were directly performed using the ultrasound machine's measure function, and the results were recorded during the measurement sessions. Likewise, the ultrasound machine directly provided passive muscle stiffness results, which were recorded during the measurement sessions.

2.4.1. The BFlh fascicle length measurement via the EFOV technique. The participants laid down on a standard medical bed in a prone position when the hamstring was at the passive resting position. Firstly, the proximal and distal MTJ were determined by monitoring via the ultrasound probe [17]. Then, the path of the BFlh muscle belly was monitored [17]. Afterwards, the ultrasound probe was continuously moved parallel to the BFlh muscle orientation, starting from the distal MTJ towards the proximal MTJ around the ischial tuberosity at a constant speed. This process was performed a few times for each participant until determining the correct path to visualise the BFlh fascicles. Then, the first measurement followed the visible path within the ultrasound gel on the skin. During the measurements, the assessor continuously adjusted the ultrasound probe to make the fascicles and aponeuroses of the BFlh visible based on different anatomical features of the BFlh (Fig 1). Then, four visible fascicles starting around the BFlh's mid-belly were digitised using the ultrasound machine's measure function. The four fascicle length values were averaged and accepted as the BFlh fascicle length. Subsequently, this process was repeated after totally removing the ultrasound probe from the skin after about two minutes of a waiting period. The mean value of the two measurements was accepted as the BFlh fascicle length of the measured leg.

2.4.2. The BFlh mid-belly and distal MTJ passive stiffness measurements. Participants kept their position on a medical bed as described in the fascicle length measurement section for both mid-muscle belly and distal MTJ stiffness measurements of the BFlh. The ultrasound
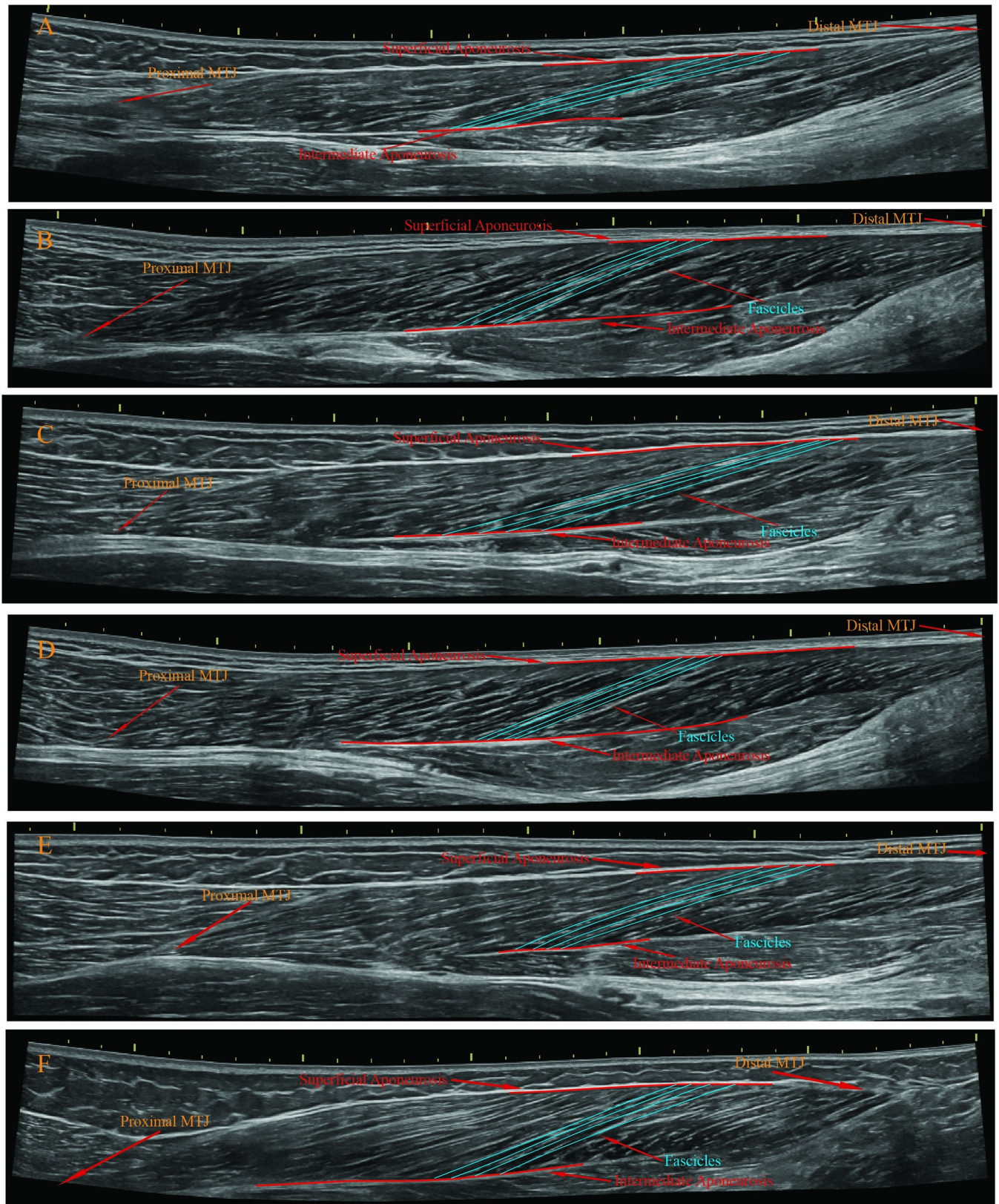
Fig 1. A-F: Random examples of the biceps femoris long head fascicle length measurements via panoramic ultrasound scanning in various participants.

probe was placed on the 50% distance between the proximal MTJ and Distal MTJ on the muscle belly [39]. The probe was arranged parallel to the muscle fascicle orientations as described in the BFlh fascicle length measurement section. At the same time, the superficial and intermediate aponeuroses were visible, and they were parallel to each other as much as they could be at the location [39]. A slight and equal transducer pressure was applied during the measurements [39]. The region of interest (ROI) was selected on the centre of the BFlh muscle, and an elastogram with a 1 cm diameter was taken from the ROIs [39] (Fig 2). For the distal MTJ stiffness measurements of the BFlh, the participants kept the same position in the mid-muscle belly passive muscle stiffness of the BFlh. The ultrasound probe was oriented parallel to the BFlh muscle orientation, and the distal MTJ was visualised. The distal MTJ was determined by following the previous relevant research [40, 41]. An elastogram with a 0.25 cm diameter was taken from the closest place to the distal MTJ, where the 0.25 cm elastogram chamber fits into the triangle of the distal MTJ (Fig 3). The measurements were replicated by totally removing the ultrasound probe from the skin, and the mean value of these two measurements was accepted as the mid-belly passive muscle stiffness of the BFlh.

2.5. Statistical analyses

The BFlh fascicle length, mid-belly and distal MTJ passive stiffness results complied with parametric test assumptions (Levene's test) and were compared between the injured legs, uninjured contralateral legs, and both legs of the control group via one-way analysis of variance (ANOVA). Additionally, the one-way ANOVA was also used to compare the participants' physical characteristics and total years of performing sports to confirm no significant differences between comparison groups. Tukey posthoc tests were performed to assess statistical significance between grouped results using SPSS software (IBM SPSS Statistics, version 29). The alpha level was set as 0.05 for the statistical significance. Cohen's *d*-effect sizes [42] were calculated for all parameters. Effect sizes for our sample consisting of highly trained athletes were interpreted as follows: < 0.25, trivial; 0.25–0.50, small; 0.50–1.00, moderate; > 1.00, large [43]. For intra-rater reliability analyses, the intraclass correlation coefficients (ICC, two-way mixed, absolute agreement on single measurements) were calculated for each outcome. The reliability results were classified as follows: < 0.50, low reliability; 0.50–0.75, moderate reliability; 0.75–0.90, high reliability; > 0.90, very high reliability [16].

3. Results

The injured group consisted of 8 professional runners, 8 semi-professional football players, 2 semi-professional basketball players, and 1 semi-professional cyclist. The control group consisted of the athletes who exactly matched with the injured group (8 professional runners, 8 semi-professional football players, 2 semi-professional basketball players, and 1 semi-professional cyclist). There were no significant differences between the participants' physical characteristics and total years of performing sports between the groups (Table 1).

In the injured group, ten participants injured their dominant legs and nine participants injured their non-dominant legs. Four injuries were located around proximal MTJ, eight were located around distal MTJ, and seven were situated around the mid-muscle belly of the BFlh. Among the injured athletes, fourteen athletes experienced the HSI for the first time (mean time to injury = 9 ± 11.8 months ago), and five athletes experienced the HSI twice (mean time to first injury = 3.9 ± 2.1 years ago; mean time to last injury = 12.4 ± 14.8 months ago). In the injured group, three athletes stated that they have received a structured physiotherapy program; three athletes received massage; four athletes mentioned they received ice compression, one of the four athletes mentioned he swam in addition to the ice compression during the
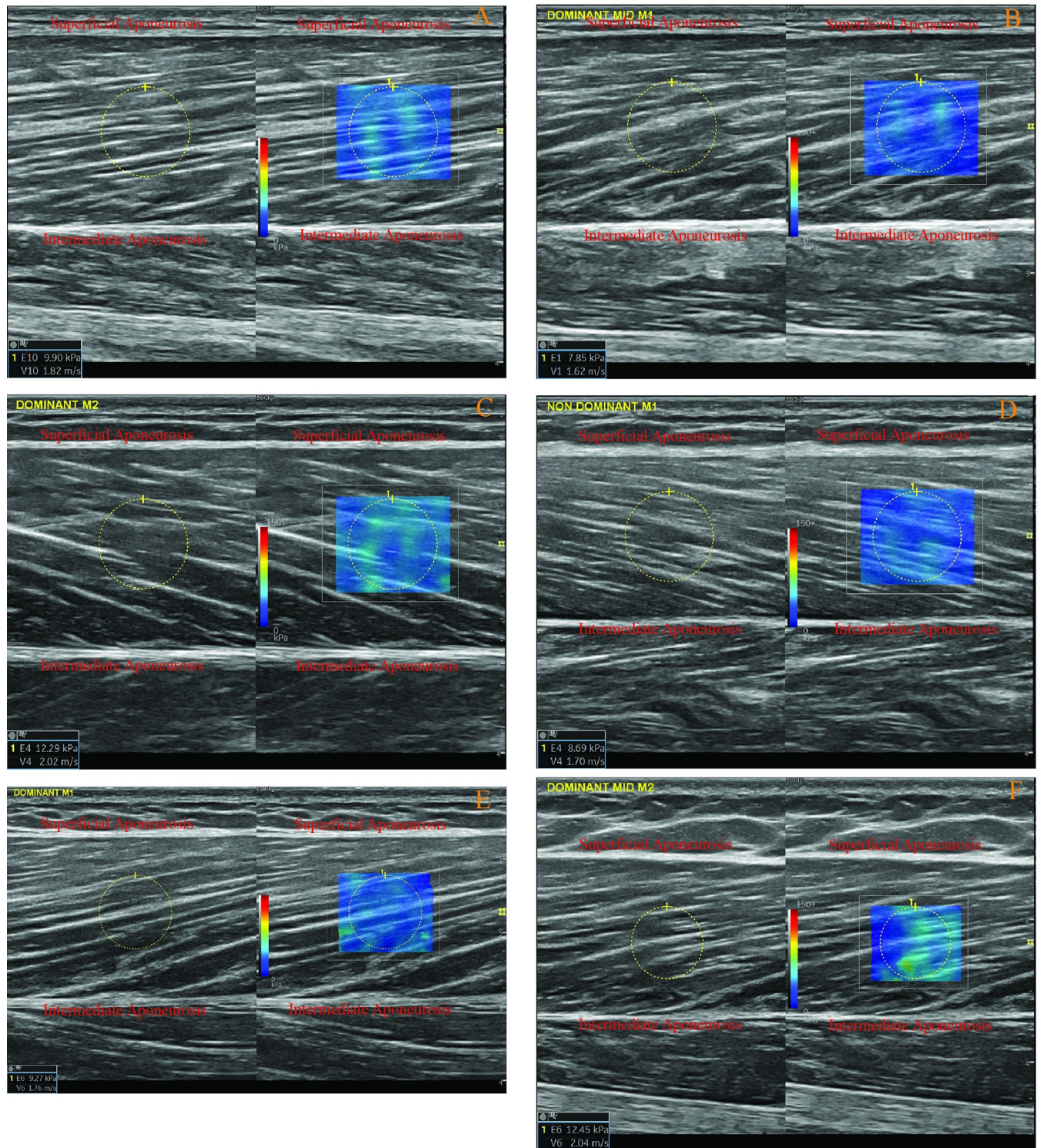
Fig 2. A-F: Random examples of the measurements of mid-muscle belly passive stiffness of the biceps femoris long head via ultrasound-based shear-wave elastography in various participants. The different image orientations, whether from left to right or vice versa, result from the 180° differences in the grip positions of the ultrasound probes during each measurement, which do not affect the outcomes.

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Fig 3. A-F: Random examples of the measurements of distal musculotendinous junction passive stiffness of the biceps femoris long head via ultrasound-based shear-wave elastography in various participants. The different image orientations, whether from left to right or vice versa, result from the 180˚ differences in the grip positions of the ultrasound probes during each measurement, which do not affect the outcomes.
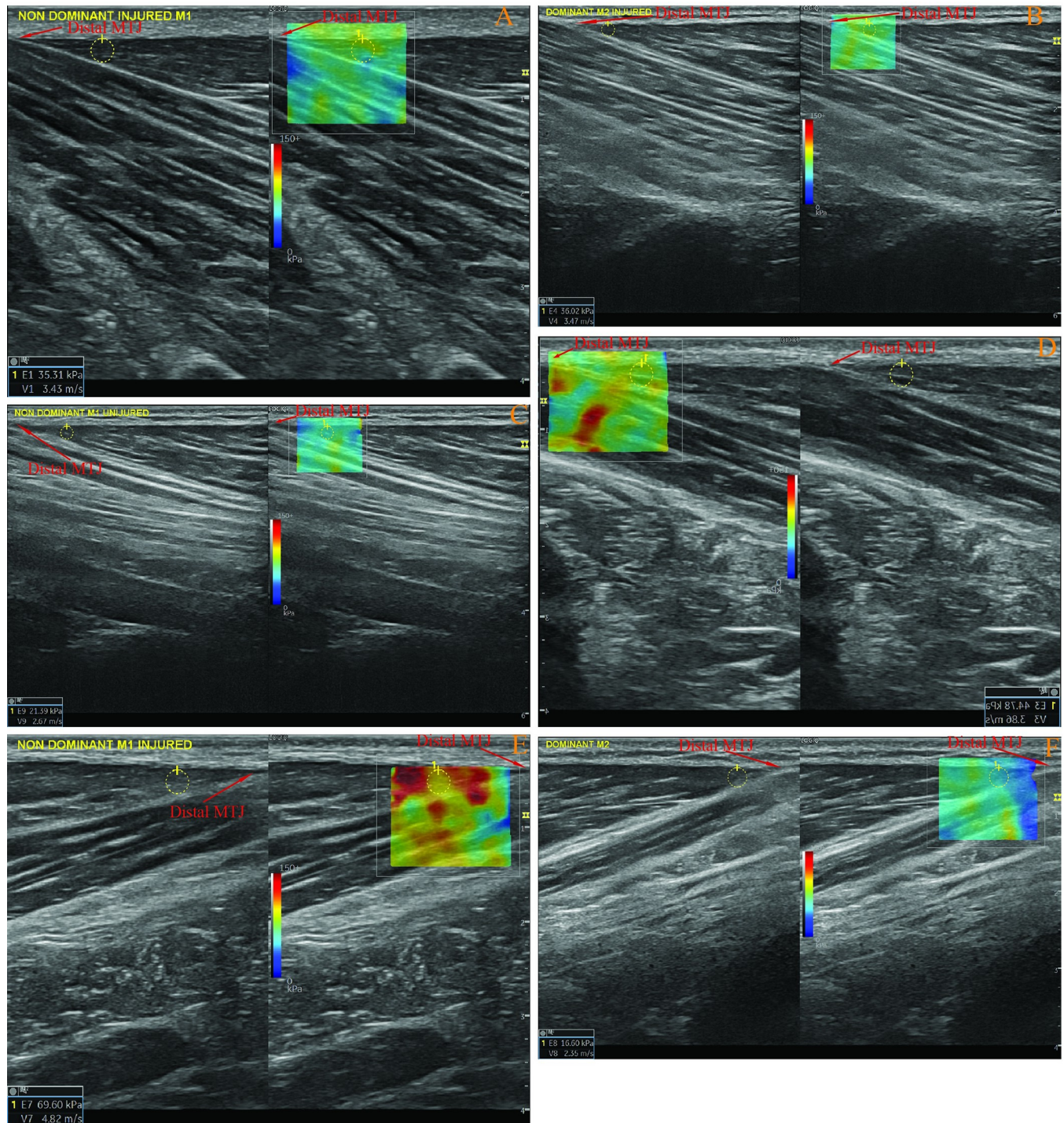
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Table 1. Participants' physical characteristics and total years of performing sport.

	Injured group (n = 19)	Control group (n = 19)	*p*-value for Levene's test	*the p*-value for the mean difference
Age (years)	29.3 ± 6.5	26.4 ± 7.3	0.504	0.195
Height (cm)	171.2 ± 5.7	171.6 ± 6	0.844	0.825
Weight (kg)	68.9 ± 9.2	66.2 ± 8.6	0.958	0.358
BMI (kg/m^2)	23.5 ± 2.9	22.5 ± 2.7	0.747	0.262
Total years of performing the sport	8.7 ± 5.5	8.4 ± 5.3	0.661	0.870

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healing process; two athletes pointed out they used warm compression during the healing process; eight athletes mentioned they did not receive any rehabilitation and waited for the natural healing process.

All the measurements were performed twice except for one participant for the fascicle length and distal MTJ muscle stiffness measurements due to a human error in recording the results during the measurement sessions. Therefore, these measurements could not be included in the reliability analysis. However, the single measurements were accepted as the valid fascicle length and distal MTJ values in the primary analyses to compare injured and uninjured athletes. The reliability results of the measurements were as follows: Fascicle length (n = 75 legs), ICC = 0.982, 95% CI [0.971, 0.988]; mid-belly muscle stiffness (n = 76 legs), ICC = 0.973, 95% CI [0.955, 0.988], distal MTJ stiffness (n = 75 legs), ICC = 0.995, 95% CI [0.993, 0.997].

BFlh fascicle length measurements in the injured legs compared with the uninjured contralateral legs showed no significant difference (Mean difference (MD) = -0.43 ± 0.70 cm, $p = 0.719$). However, BFlh fascicle lengths of the injured legs in comparison with the legs of the control group revealed a trend to be shorter with a medium effect size despite its statistical nonsignificance ($d = 0.62$, MD = -1.10 ± 2.93 cm, $p = 0.067$) (Table 2). Moreover, the difference in the BFlh fascicle lengths between the uninjured contralateral legs of the injured athletes and both legs of the control group were not significantly different (MD = -0.67 ± 2.9 cm, $p = 0.362$) (Table 2). For the mid-belly passive muscle stiffness of the BFlh between the injured leg and control group legs, a significantly higher stiffness was detected for the injured legs (MD = 3.29 ± 6.09 KPa, $p = 0.009$) (Table 2). However, within injured athletes, the comparison of injured and uninjured legs showed no difference (MD = 2.17 ± 6.71, $p = 0.198$). Moreover, the passive stiffness of the BFlh distal MTJ was significantly higher in the injured legs

Table 2. Differences in the BFlh fascicle length, Mid-muscle belly stiffness and distal MTJ stiffness between the groups.

	Injured thighs (n = 19) (mean ± SD)	Uninjured contralateral thighs (n = 19) (mean ± SD)	Control thighs (n = 38) (mean ± SD	Mean Difference Cohen's *d* effect size (Injured thighs vs. uninjured contralateral thighs) (mean ± SD, ES)	Mean Difference and Cohen's *d* effect size (Injured thighs vs. control thighs) (mean ± SD, ES)	Mean Difference (Uninjured contralateral thighs vs. control thighs) (mean ± SD, ES)
Fascicle length (cm)	8.03 ± 2	8.46 ± 1.86	9.13 ± 1.52	-0.43 ± 0.7 $p = 0.719$, **$d = 0.22$**	-1.1 ± 2.93 $p = 0.067$, **$d = 0.62$**	-0.67 ± 2.9 $p = 0.362$, **$d = 0.39$**
Passive stiffness of mid-muscle belly (kPa)	13.2 ± 6.4	11.03 ± 3.5	9.91 ± 1.81	2.17 ± 6.71 $p = 0.198$, **$d = 0.37$**	3.29 ± 6.09 $p^* = 0.009$, **$d = 0.7$**	1.12 ± 3.22 $p = 0.557$; **$d = 0.4$**
Passive stiffness of distal musculotendinous junction (kPa)	31.95 ± 17.39	20.98 ± 15.12	11.46 ± 5	10.97 ± 14.46 $p^* = 0.017$, **$d = 0.67$**	20.49 ± 19.56 $p^{**} < 0.001$, **$d = 1.6$**	9.52 ± 17.66 $p^* = 0.017$, **$d = 0.85$**

Abbreviations. BFlh, biceps femoris long head; ES, effect size; MTJ, musculotendinous junction; SD, standard deviation. *, statistically significant (p < 0.05); **, statistically significant (p < 0.001).

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compared with the contralateral uninjured legs (10.97 ± 14.46, $p = 0.017$). These findings were consistent in further comparisons between the injured legs and both legs of the control group (MD = 20.49 ± 19.56, $p < 0.001$), and between the contralateral uninjured legs and both legs of the control group (MD = 9.52 ± 17.66, $p = 0.017$) (Table 2).

4. Discussion

The structural parameters of human skeletal muscles have traditionally been given great attention by studies focusing on sports performance [44–67] and recently by research conducted on musculoskeletal injuries [12, 13, 16, 38, 68–76]. Likewise, this study has retrospectively examined athletes with and without HSI to compare changes in the muscle structure-related parameters of the BFlh muscle after an HSI. BFlh fascicle length was measured via b-mode panoramic ultrasound scanning, plus mid-muscle belly and distal muscle belly stiffness by ultrasound-based shear wave elastography.

This study employed a panoramic ultrasound scanning to measure the BFlh fascicle length which is methodologically different to former relevant retrospective studies [18–22] comparing athletes with and without HSI history. Most of these retrospective studies [18, 20–22] used the trigonometric equation technique, except for one study employing the manual linear extrapolation technique [19] for estimating the BFlh fascicle length. By using panoramic ultrasound scanning, all the lengths of BFlh fascicles could be monitored and calculated without an estimation of invisible parts, which may reduce an overestimation of the BFlh fascicles [17]. Only one former retrospective study [77] compared injured and uninjured legs of previously injured athletes using panoramic ultrasound scanning, however, they did have an external control (i.e., uninjured matched athletes). Our study did not detect significant differences between BFlh fascicle length despite being appropriately powered (0.96 statistical power). There were trivial and moderate effect sizes for the differences between the limbs of the injured group (MD = -0.43 ± 0.70 cm, $d = 0.22$) and between the injured leg of the injured group and legs of the controls (MD = -1.10 ± 2.93 cm, $d = 0.62$). This suggests that differences in BFlh between groups may be relevant for HSI susceptibility.

The previous retrospective studies found shorter BFlh fascicle lengths of the injured leg compared to the control ranging between -1.74 cm to -0.47 cm. In agreement, our study found a decrement of BFlh fascicle length (about -1.10 cm) in the injured legs of the athletes. Findings and the BFlh ultrasound assessment methods of the previous studies are presented in Table 3.

Table 3. Differences in the BFlh fascicle lengths between athletes with and without hamstring strain injury history in the previous retrospective studies.

Study	Participants	Injured leg vs. uninjured contralateral leg mean difference (cm) and ES	Injured leg vs. control group mean difference (cm) and ES	Ultrasound assessment method
de Lima-E-Silva et al. [18]	20 injured football players vs. 60 uninjured football players	0.11 cm ($p = 0.91$, $d = 0.04$)	-1.80 cm ($p = 0.02^*$, $d = 0.59$)	Trigonometric equation method
Nin et al. [19]	5 injured athletes (mix) vs. 10 uninjured athletes (mix)	-0.41 cm (p = NG, $d = -0.37$)	-0.58 cm (p = NG, $d = -0.67$)	Manual Linear Extrapolation method
Pimenta et al. [20] (Baseline values)	12 injured football players vs. 20 uninjured football players:	-1.74 cm ($p = 0.012^*$, d = NG)	-0.80 cm ($p = 0.014^*$, d = NG)	Linear Extrapolation method via trigonometric equation for invisible part
Timmins et al. [21]	12 injured Australian footballers vs. 18 uninjured Australian footballers	-0.47 cm (p = NG, d = NG)	-0.73 cm (p = NG, d = NG)	Trigonometric equation method
Timmins et al. [22]	16 previously injured athletes vs. 20 recreationally active controls	-1.54 cm ($p < 0.001^*$, $d = -0.74$)	-0.47 cm (The control group was accepted as the average results of both limbs (p = NG, d = NG)	Trigonometric equation method

Abbreviations. BFlh, biceps femoris long head; ES, effect size; NG, not given; *, statistically significant (p < 0.05).

A recent meta-analytic study [26] indicated moderately shorter BFlh fascicle lengths of injured legs of the athletes (effect size = 0.57), which is similar to our present research (effect size = 0.62). Variability of results in the literature might have multifactorial causes including athlete age [47, 78, 79], ethnicity [80], sports disciplines [81], rehabilitation process [82–84], abd ultrasound measurement methods [17, 38]. However, due to the nature of the retrospective study, longitudinal trials are needed to understand the differences in athletes after HSI.

A previous study [26] suggested that neural inhibition following the injury [24–29] and reduced exertion may cause BFlh fascicle shortening at the early phase of rehabilitation [22]. Together with the former, decreased participation in sports [24] are a possible underlying mechanism for the shortening of BFlh fascicle lengths after injury. Future studies are needed to monitor the architecture of the hamstrings before the HSI, during rehabilitation, and after returning to play for a better understanding of the mechanisms behind the architectural alterations after the HSI. Based on the findings of this study, it may be suggested that rehabilitation programs should include exercises that can lead to fascicle lengthening in the hamstrings of both legs of previously injured athletes to minimise the possibility of microscopic damage of the fascicles during eccentric contraction [25, 26, 85].

To the authors' knowledge, this study was the first retrospective study comparing the passive distal MTJ stiffness of the BFlh between athletes with and without an HSI history. Our study observed significantly higher stiffness in the previously injured BFlh muscles at the mid-muscle belly (MD = 3.29 ± 6.09 kPa, $p = 0.009$, $d = 0.70$) and distal MTJ compared to the controls (MD = 20.49 ± 19.56 kPa, $p < 0.001$, $d = 1.60$) with moderate and large effect sizes, respectively. A previous retrospective study [19] pointed out larger stiffness ($d = 1.28$) at the muscle belly of the BFlh in HSI athletes compared to controls. Our study also detected greater mid-belly stiffness, though only moderately higher ($d = 0.70$), in the athletes with HSI history. Additionally, we detected large ($d = 1.60$) differences at the distal MTJ of the BFlh between the injured muscle and controls. Additionally, we foundmoderately higher stiffnesses at the distal MTJ of this injured leg compared to the contralateral legs ($d = 0.67$). The larger stiffness at the distal MTJ ($d = 1.60$) compared to the mid-part ($d = 0.70$) of the injured BFlh can be caused by distal MTJ injuries which were the most frequent in our sample (8 of 19 athletes). Distal MTJ injuries were pointed out as common [9], more recurrent and severe, and requiring longer rehabilitation [9, 10]. Reasons for higher stiffness in the injured legs compared with the controls might be explained by potential scarring of tissue around the injury location [24, 26, 30–34]. Tissue changes (i.e., mechanical properties) would affect the passive tension and stiffness of the BFlh structure towards higher values [86]. Moreover, sixteen out of nineteen of the injured athletes in our study did not receive a structured physiotherapy program including eccentric training and static stretching. Such structured rehabilitation may help to prevent muscle shortening [39] and improve muscle elasticity [87] during the healing process. Higher stiffness after lower extremity injuries is not uncommon. A previous study detected considerable increments in hamstrings' passive muscle stiffness for both injured and uninjured legs after anterior cruciate ligament reconstructions compared to healthy controls [35]. Another study [34] observed significant increases in stiffness within the first 6 weeks and 6 months after a tendon injury. Of our participants, ten were injured within less than six months, with seven of them injured approximately six weeks (1–2 months) before the measurement day (Supporting Information S1). Measurements closer to the injury occurrence may have contributed to the differences in the distal MTJ stiffness results.

Moreover, it is documented in the literature that hamstring muscle stiffness results may be influenced by age [88, 89], genetics [88, 90], sex [91], sports profession [39, 92], pelvic tilt type [93], hip and knee positions [94], injury and scar status [32–34], and operator of the shear-wave elastography [95]. Besides reasons based on the characteristics and history of the

individual, stiffness results of the BFlh vary between different ultrasound brands and versions even at similar measurement positions in healthy participants. Baseline or reliability values of the BFlh passive muscle belly stiffness at resting appeared as 4.5 kPa [96], 9.91 kPa (this study), 10.54–15.72 [97], 10.8 kPa [98], 11.3–11.7 kPa [99], 11.57 kPa, [100], 14.43–16.27 kPa [101], 15.74–19.01 kPa [102], 16.47–19.87 kPa [41], 16.9–24.7 kPa [39]. A future study should compare differences in the BFlh stiffness values on the same sample between commercially available devices to determine the comparability of results in the literature.

Outcomes suggest rehabilitation programmes should consider establishing muscle elasticity after the HSI. In one study, researchers found a four-week static stretching program was effective in maintaining elasticity of the BFlh [87]. However, the effectiveness of other rehabilitation modalities are not well-documented in the literature. Future studies should investigate the effectiveness of rehabilitation modalities on the stiffness of hamstrings. Ultrasound-based shear-wave elastography could be a useful tool to monitor muscle stiffness during the rehabilitation process in the injured area.

The first limitation of this study is its retrospective cross-sectional design which does not allow conclusions towards cause or effect of the measured parameters for the HSI. Another limitation of the retrospective design is not having detailed information about the participants' rehabilitation process, which may lead to difficulties in interpreting the results. Additionally, the absence of retrospective information regarding the severity of injuries in the players with a history of HSI is acknowledged as a limitation in this study. Moreover, this study only applied intra-rater reliability assessments, which is another confounding factor. The measurements could not blinded due to the multiple required tasks of the assessor during the data collection. Furthermore, another confounding factor of this study was the BFlh fascicle length measurement method. There is no gold standard measurement method for BFlh fascicle length in the literature [17]. Despite this, panoramic ultrasound scanning does not overestimate the BFlh fascicle length compared to trigonometric equations and manual linear extrapolation methods. A recent study [103] stated that ultrasound assessment can overestimate increases in serial sarcomere numbers by about 5%. On the other hand, shear-wave elastography values can be influenced by assessment depths and the size of the ROIs and elastograms [104]. Despite the potential advantages of measuring a specific location within a muscle, not assessing the entire muscle and tendon structures can be another drawback of ultrasound-based shear-wave studies, such as ours. Therefore, future studies should aim to provide standardised measurement approaches for measuring the skeletal muscles and tendons at their entire muscle volumes by providing specific location and overall volume stiffness information.

Initially, it was also planned to measure the proximal MTJ stiffness of the BFlh as an additional study outcome. However, the shear waves of the ultrasound machine could not reach a full ROI colour distribution at the proximal MTJ of the BFlh. This issue might have been because: a) the shear waves of our ultrasound machine were not durable enough to pass through gluteus maximus muscle fascicles, which have a different orientation than the BFlh, for reaching the proximal MTJ of the BFlh; b) the higher depth of the proximal MTJ compared the distal MTJ, c) the technical abilities of the ultrasound machine, or d) another reason which may be identified by future studies. Consequently, we decided not to continue proximal MTJ stiffness measurements after a few trials, and we noted this issue as a limitation of this study.

5. Conclusions

This study has used relatively advanced measurement methods for measuring the BFlh fascicles and stiffness, e.g. panoramic ultrasound scanning and shear-wave elastography. Results showed significantly higher mid-muscle belly and distal MTJ stiffness values in previously

injured athletes compared to the controls. Despite being only a trend, moderately shorter fascicles were observed in the injured players compared to the controls. Physical therapy and rehabilitation programmes should aim to improve BFlh muscle elasticity and fascicle length after the HSI. Future prospective studies should investigate whether higher stiffness is a cause or consequence of HSI.

Supporting information

S1 Data.
(PDF)

Author Contributions

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References

1. Opar DA, Williams MD, Shield AJ. Hamstring strain injuries: factors that lead to injury and re-injury. Sports Med. 2012; 42(3):209–26. https://doi.org/10.2165/11594800-000000000-00000 PMID: 22239734

2. Ekstrand J, Bengtsson H, Waldén M, Davison M, Khan KM, Hägglund M. Hamstring injury rates have increased during recent seasons and now constitute 24% of all injuries in men's professional football: the UEFA Elite Club Injury Study from 2001/02 to 2021/22. British Journal of Sports Medicine. 2022: bjsports-2021-105407. https://doi.org/10.1136/bjsports-2021-105407 PMID: 36588400

3. Liu H, Garrett WE, Moorman CT, Yu B. Injury rate, mechanism, and risk factors of hamstring strain injuries in sports: A review of the literature. Journal of Sport and Health Science. 2012; 1(2):92–101. https://doi.org/10.1016/j.jshs.2012.07.003

4. Kenneally-Dabrowski CJB, Brown NAT, Lai AKM, Perriman D, Spratford W, Serpell BG. Late swing or early stance? A narrative review of hamstring injury mechanisms during high-speed running. Scand J Med Sci Sports. 2019; 29(8):1083–91. https://doi.org/10.1111/sms.13437 PMID: 31033024

5. Chumanov ES, Heiderscheit BC, Thelen DG. Hamstring musculotendon dynamics during stance and swing phases of high-speed running. Med Sci Sports Exerc. 2011; 43(3):525–32. https://doi.org/10.1249/MSS.0b013e3181f23fe8 PMID: 20689454

6. Kujala UM, Orava S, Järvinen M. Hamstring injuries. Current trends in treatment and prevention. Sports medicine (Auckland, NZ). 1997; 23(6):397–404. https://doi.org/10.2165/00007256-199723060-00005 PMID: 9219322

7. Thelen D, Chumanov E, Hoerth D, Best T, Swanson S, Li L, et al. Hamstring Muscle Kinematics during Treadmill Sprinting. Medicine and science in sports and exercise. 2005; 37:108–14. https://doi.org/10.1249/01.mss.0000150078.79120.c8 PMID: 15632676

8. Koulouris G, Connell DA, Brukner P, Schneider-Kolsky M. Magnetic resonance imaging parameters for assessing risk of recurrent hamstring injuries in elite athletes. Am J Sports Med. 2007; 35(9):1500–6. https://doi.org/10.1177/0363546507301258 PMID: 17426283

9. Entwisle T, Ling Y, Splatt A, Brukner P, Connell D. Distal Musculotendinous T Junction Injuries of the Biceps Femoris: An MRI Case Review. Orthop J Sports Med. 2017; 5(7):2325967117714998. https://doi.org/10.1177/2325967117714998 PMID: 28795071

10. Brukner P, Connell D. 'Serious thigh muscle strains': beware the intramuscular tendon which plays an important role in difficult hamstring and quadriceps muscle strains. British Journal of Sports Medicine. 2016; 50(4):205–8. https://doi.org/10.1136/bjsports-2015-095136 PMID: 26519522

11. Green B, Bourne MN, van Dyk N, Pizzari T. Recalibrating the risk of hamstring strain injury (HSI): A 2020 systematic review and meta-analysis of risk factors for index and recurrent hamstring strain injury in sport. British Journal of Sports Medicine. 2020; 54(18):1081. https://doi.org/10.1136/bjsports-2019-100983 PMID: 32299793

12. Watsford ML, Murphy AJ, McLachlan KA, Bryant AL, Cameron ML, Crossley KM, et al. A prospective study of the relationship between lower body stiffness and hamstring injury in professional Australian rules footballers. Am J Sports Med. 2010; 38(10):2058–64. https://doi.org/10.1177/0363546510370197 PMID: 20595555

13. Timmins RG, Bourne MN, Shield AJ, Williams MD, Lorenzen C, Opar DA. Short biceps femoris fascicles and eccentric knee flexor weakness increase the risk of hamstring injury in elite football (soccer): a prospective cohort study. Br J Sports Med. 2016; 50(24):1524–35. https://doi.org/10.1136/bjsports-2015-095362 PMID: 26675089

14. Kellis E, Galanis N, Natsis K, Kapetanos G. Validity of architectural properties of the hamstring muscles: correlation of ultrasound findings with cadaveric dissection. J Biomech. 2009; 42(15):2549–54. https://doi.org/10.1016/j.jbiomech.2009.07.011 PMID: 19646698

15. Potier TG, Alexander CM, Seynnes OR. Effects of eccentric strength training on biceps femoris muscle architecture and knee joint range of movement. Eur J Appl Physiol. 2009; 105(6):939–44. https://doi.org/10.1007/s00421-008-0980-7 PMID: 19271232

16. Yagiz G, Dayala VK, Williams K, Owen JA, Kubis H-P. Alterations in biceps femoris long head fascicle length, Eccentric hamstring strength qualities and single-leg hop distance throughout the ninety minutes of TSAFT90 simulated football match. PLOS ONE. 2022; 17(12):e0278222. https://doi.org/10.1371/journal.pone.0278222 PMID: 36490247

17. Franchi MV, Fitze DP, Raiteri BJ, Hahn D, Spörri J. Ultrasound-derived Biceps Femoris Long Head Fascicle Length: Extrapolation Pitfalls. Med Sci Sports Exerc. 2020; 52(1):233–43. https://doi.org/10.1249/MSS.0000000000002123 PMID: 31403609

18. de Lima-E-Silva FX, Oliveira GS, Medeiros TM, Dornelles MP, Ribeiro-Alvares JBA, Baroni BM. Flexibility, strength, and fascicle length of football players with and without history of hamstring strain injury in the prior season. Science and Medicine in Football. 2020; 4(4):322–8. https://doi.org/10.1080/24733938.2020.1751871

19. Nin DZ, Pain MTG, Lim YH, Kong PW. Hamstring Muscle Architecture and Viscoelastic Properties: Reliability and Retrospective Comparison Between Previously Injuries and Uninjured Athletes. Journal of Mechanics in Medicine and Biology. 2021; 21(01):2150007. https://doi.org/10.1142/S021951942150007X

20. Pimenta R, Antunes H, Lopes T, Veloso A. Do Repeated Sprints Affect the Biceps Femoris Long Head Architecture in Football Players with and without an Injury History?-A Retrospective Study. Biology (Basel). 2023; 12(1). https://doi.org/10.3390/biology12010096 PMID: 36671788

21. Timmins RG, Bourne MN, Hickey JT, Maniar N, Tofari PJ, Williams MD, et al. Effect of Prior Injury on Changes to Biceps Femoris Architecture across an Australian Football League Season. Medicine &

Science in Sports & Exercise. 2017;49(10). https://doi.org/10.1249/MSS.0000000000001333 PMID: 28548976

22. Timmins RG, Shield AJ, Williams MD, Lorenzen C, Opar DA. Biceps femoris long head architecture: a reliability and retrospective injury study. Med Sci Sports Exerc. 2015; 47(5):905–13. https://doi.org/10.1249/MSS.0000000000000507 PMID: 25207929

23. Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An KN. Validation of shear wave elastography in skeletal muscle. J Biomech. 2013; 46(14):2381–7. https://doi.org/10.1016/j.jbiomech.2013.07.033 PMID: 23953670

24. Sanfilippo JL, Silder AMY, Sherry MA, Tuite MJ, Heiderscheit BC. Hamstring Strength and Morphology Progression after Return to Sport from Injury. Medicine & Science in Sports & Exercise. 2013;45 (3). https://doi.org/10.1249/MSS.0b013e3182776eff PMID: 23059864

25. Fyfe JJ, Opar DA, Williams MD, Shield AJ. The role of neuromuscular inhibition in hamstring strain injury recurrence. Journal of electromyography and kinesiology. 2013; 23(3):523–30. https://doi.org/10.1016/j.jelekin.2012.12.006 PMID: 23402871

26. Kellis E, Sahinis C. Is Muscle Architecture Different in Athletes with a Previous Hamstring Strain? A Systematic Review and Meta-Analysis. Journal of Functional Morphology and Kinesiology. 2022 https://doi.org/10.3390/jfmk7010016 PMID: 35225902

27. Nagano Y, Higashihara A, Edama M. Change in muscle thickness under contracting conditions following return to sports after a hamstring muscle strain injury—a pilot study. Asia-Pacific Journal of Sports Medicine, Arthroscopy, Rehabilitation and Technology. 2015; 2(2):63–7. https://doi.org/10.1016/j.asmart.2015.01.001 PMID: 29264242

28. Sole G, Milosavljevic S, Nicholson H, Sullivan SJ. Selective strength loss and decreased muscle activity in hamstring injury. journal of orthopaedic & sports physical therapy. 2011; 41(5):354–63. https://doi.org/10.2519/jospt.2011.3268 PMID: 21289455

29. Sole G, Milosavljevic S, Nicholson H, Sullivan SJ. Altered muscle activation following hamstring injuries. British Journal of Sports Medicine. 2012; 46(2):118–23. https://doi.org/10.1136/bjsm.2010.079343 PMID: 21393256

30. Best TM, Hunter KD. Muscle injury and repair. Physical medicine and rehabilitation clinics of North America. 2000; 11(2):251–66. PMID: 10810760

31. Purslow PP. The structure and functional significance of variations in the connective tissue within muscle. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2002; 133 (4):947–66. https://doi.org/10.1016/s1095-6433(02)00141-1 PMID: 12485685

32. DeJong H, Abbott S, Zelesco M, Spilsbury K, Martin L, Sanderson R, et al. A Novel, Reliable Protocol to Objectively Assess Scar Stiffness Using Shear Wave Elastography. Ultrasound Med Biol. 2020; 46 (7):1614–29. https://doi.org/10.1016/j.ultrasmedbio.2020.03.003 PMID: 32386847

33. Tsai WY, Hsueh YY, Chen PY, Hung KS, Huang CC. High-Frequency Ultrasound Elastography for Assessing Elastic Properties of Skin and Scars. IEEE Trans Ultrason Ferroelectr Freq Control. 2022; 69(6):1871–80. https://doi.org/10.1109/TUFFC.2022.3154235 PMID: 35201987

34. Frankewycz B, Henssler L, Weber J, Silva N, Koch M, Jung EM, et al. Changes of Material Elastic Properties during Healing of Ruptured Achilles Tendons Measured with Shear Wave Elastography: A Pilot Study. Int J Mol Sci. 2020; 21(10). https://doi.org/10.3390/ijms21103427 PMID: 32408704

35. Kuszewski MT, Gnat R, Szlachta G, Kaczyńska M, Knapik A. Passive stiffness of the hamstrings and the rectus femoris in persons after an ACL reconstruction. Phys Sportsmed. 2019; 47(1):91–5. https://doi.org/10.1080/00913847.2018.1527171 PMID: 30247085

36. Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013; 310(20):2191–4. https://doi.org/10.1001/jama.2013.281053 PMID: 24141714

37. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007; 39(2):175–91. https://doi.org/10.3758/bf03193146 PMID: 17695343

38. Yagiz G, Akaras E, Kubis H-P, Owen JA. Heterogeneous effects of eccentric training and nordic hamstring exercise on the biceps femoris fascicle length based on ultrasound assessment and extrapolation methods: A systematic review of randomised controlled trials with meta-analyses. PLOS ONE. 2021; 16(11):e0259821. https://doi.org/10.1371/journal.pone.0259821 PMID: 34752499

39. Yagiz G, Shida N, Kuruma H, Furuta M, Morimoto K, Yamada M, et al. Rugby Players Exhibit Stiffer Biceps Femoris, Lower Biceps Femoris Fascicle Length to Knee Extensors, and Knee Flexors to Extensors Muscle Volume Ratios Than Active Controls. International Journal of Sports Physiology and Performance. 2023; 18(9):1030–7. https://doi.org/10.1123/ijspp.2023-0077 PMID: 37495222

40. Freitas S, Marmeleira J, Valamatos MJ, Blazevich A, Mil-Homens P. Ultrasonographic Measurement of the Biceps Femoris Long-Head Muscle Architecture. Journal of Ultrasound in Medicine. 2017;37. https://doi.org/10.1002/jum.14436 PMID: 29027683

41. Vatovec R, Marušič J, Marković G, Šarabon N. Effects of Nordic hamstring exercise combined with glider exercise on hip flexion flexibility and hamstring passive stiffness. Journal of Sports Sciences. 2021; 39(20):2370–7. https://doi.org/10.1080/02640414.2021.1933350 PMID: 34074227

42. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.

43. Rhea MR. Determining the magnitude of treatment effects in strength training research through the use of the effect size. J Strength Cond Res. 2004; 18(4):918–20. https://doi.org/10.1519/14403.1 PMID: 15574101

44. Abe T, Fukashiro S, Harada Y, Kawamoto K. Relationship between sprint performance and muscle fascicle length in female sprinters. J Physiol Anthropol Appl Human Sci. 2001; 20(2):141–7. https://doi.org/10.2114/jpa.20.141 PMID: 11385937

45. Abe T, Kojima K, Stager JM. Skeletal muscle mass and muscular function in master swimmers is related to training distance. Rejuvenation Res. 2014; 17(5):415–21. https://doi.org/10.1089/rej.2014.1563 PMID: 24797514

46. Abe T, Loenneke JP, Thiebaud RS. Morphological and functional relationships with ultrasound measured muscle thickness of the lower extremity: a brief review. Ultrasound. 2015; 23(3):166–73. https://doi.org/10.1177/1742271X15587599 PMID: 27433253

47. Akima H, Kano Y, Enomoto Y, Ishizu M, Okada M, Oishi Y, et al. Muscle function in 164 men and women aged 20–84 yr. Med Sci Sports Exerc. 2001; 33(2):220–6. https://doi.org/10.1097/00005768-200102000-00008 PMID: 11224809

48. Brechue WF, Abe T. The role of FFM accumulation and skeletal muscle architecture in powerlifting performance. European Journal of Applied Physiology. 2002; 86(4):327–36. https://doi.org/10.1007/s00421-001-0543-7 PMID: 11990746

49. Freilich RJ, Kirsner RL, Byrne E. Isometric strength and thickness relationships in human quadriceps muscle. Neuromuscul Disord. 1995; 5(5):415–22. https://doi.org/10.1016/0960-8966(94)00078-n PMID: 7496175

50. Fukunaga T, Miyatani M, Tachi M, Kouzaki M, Kawakami Y, Kanehisa H. Muscle volume is a major determinant of joint torque in humans. Acta Physiol Scand. 2001; 172(4):249–55. https://doi.org/10.1046/j.1365-201x.2001.00867.x PMID: 11531646

51. Fukunaga T, Roy RR, Shellock FG, Hodgson JA, Day MK, Lee PL, et al. Physiological cross-sectional area of human leg muscles based on magnetic resonance imaging. J Orthop Res. 1992; 10(6):928–34. https://doi.org/10.1002/jor.1100100623 PMID: 1403308

52. Fukunaga T, Roy RR, Shellock FG, Hodgson JA, Edgerton VR. Specific tension of human plantar flexors and dorsiflexors. J Appl Physiol (1985). 1996; 80(1):158–65. https://doi.org/10.1152/jappl.1996.80.1.158 PMID: 8847297

53. Ikai M, Fukunaga T. Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. Int Z Angew Physiol. 1968; 26(1):26–32. https://doi.org/10.1007/BF00696087 PMID: 5700894

54. Ikebukuro T, Kubo K, Okada J, Yata H, Tsunoda N. The relationship between muscle thickness in the lower limbs and competition performance in weightlifters and sprinters. Japanese Journal of Physical Fitness and Sports Medicine. 2011; 60(4):401–11.

55. Kumagai K, Abe T, Brechue W, Ryushi T, Takano S, Mizuno M. Sprint performance is related to muscle fascicle length in male 100-m sprinters. Journal of applied physiology (Bethesda, Md: 1985). 2000; 88:811–6. https://doi.org/10.1152/jappl.2000.88.3.811 PMID: 10710372

56. Lieber RL. Skeletal muscle structure and function. Inplications for Rehabilitation and Sports Medicine. Maryland, USA: Williams and Wilkins; 1992.

57. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. Muscle Nerve. 2000; 23(11):1647–66. https://doi.org/10.1002/1097-4598(200011)23:11<1647::aid-mus1>3.0.co;2-m PMID: 11054744

58. Mangine GT, Fukuda DH, LaMonica MB, Gonzalez AM, Wells AJ, Townsend JR, et al. Influence of gender and muscle architecture asymmetry on jump and sprint performance. J Sports Sci Med. 2014; 13(4):904–11. PMID: 25435784

59. Mangine GT, Fukuda DH, Townsend JR, Wells AJ, Gonzalez AM, Jajtner AR, et al. Sprinting performance on the Woodway Curve 3.0TM is related to muscle architecture. European Journal of Sport Science. 2015; 15(7):606–14. https://doi.org/10.1080/17461391.2014.969322 PMID: 25334069

60. Maughan RJ, Watson JS, Weir J. Strength and cross-sectional area of human skeletal muscle. J Physiol. 1983; 338:37–49. https://doi.org/10.1113/jphysiol.1983.sp014658 PMID: 6875963

61. Moreau NG, Simpson KN, Teefey SA, Damiano DL. Muscle architecture predicts maximum strength and is related to activity levels in cerebral palsy. Phys Ther. 2010; 90(11):1619–30. https://doi.org/10.2522/ptj.20090377 PMID: 20847035

62. Narici MV, Landoni L, Minetti AE. Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. European Journal of Applied Physiology and Occupational Physiology. 1992; 65(5):438–44. https://doi.org/10.1007/BF00243511 PMID: 1425650

63. Nasirzade A, Ehsanbakhsh A, Ilbeygi S, Sobhkhiz A, Argavani H, Aliakbari M. Relationship between sprint performance of front crawl swimming and muscle fascicle length in young swimmers. J Sports Sci Med. 2014; 13(3):550–6. PMID: 25177181

64. Nasirzadeh A, Sadeghi H, Sobhkhiz A, Mohammadian K, Nikouei A, Baghaian M, Fatahi A. Multivariate analysis of 200-m front crawl swimming performance in young male swimmers. Acta of bioengineering and biomechanics / Wroclaw University of Technology. 2015;17. https://doi.org/10.5277/ABB-00160-2014-03

65. Nimphius S, McGuigan MR, Newton RU. Changes in muscle architecture and performance during a competitive season in female softball players. J Strength Cond Res. 2012; 26(10):2655–66. https://doi.org/10.1519/JSC.0b013e318269f81e PMID: 22847524

66. Shephard RJ, Bouhlel E, Vandewalle H, Monod H. Muscle mass as a factor limiting physical work. J Appl Physiol (1985). 1988; 64(4):1472–9. https://doi.org/10.1152/jappl.1988.64.4.1472 PMID: 3378982

67. Yagiz G, Akaras E, Kubis H-P, Owen JA. The Effects of Resistance Training on Architecture and Volume of the Upper Extremity Muscles: A Systematic Review of Randomised Controlled Trials and Meta-Analyses. Applied Sciences. 2022; 12(3):1593.

68. Hides J, Frazer C, Blanch P, Grantham B, Sexton C, Mendis MD. Clinical utility of measuring the size of the lumbar multifidus and quadratus lumborum muscles in the Australian football league setting: A prospective cohort study. Physical Therapy in Sport. 2020; 46:186–93. https://doi.org/10.1016/j.ptsp.2020.09.007 PMID: 32966952

69. Hides JA, Brown CT, Penfold L, Stanton WR. Screening the lumbopelvic muscles for a relationship to injury of the quadriceps, hamstrings, and adductor muscles among elite Australian Football League players. J Orthop Sports Phys Ther. 2011; 41(10):767–75. https://doi.org/10.2519/jospt.2011.3755 PMID: 21891873

70. Hides JA, Stanton WR. Can motor control training lower the risk of injury for professional football players? Medicine & Science in Sports & Exercise. 2014; 46(4):762–8. https://doi.org/10.1249/MSS.0000000000000169 PMID: 24056268

71. Hides JA, Stanton WR. Predicting football injuries using size and ratio of the multifidus and quadratus lumborum muscles. Scandinavian Journal of Medicine & Science in Sports. 2017; 27(4):440–7. https://doi.org/10.1111/sms.12643 PMID: 28271614

72. Hides JA, Stanton WR, Mendis MD, Franettovich Smith MM, Sexton MJ. Small Multifidus Muscle Size Predicts Football Injuries. Orthopaedic Journal of Sports Medicine. 2014; 2(6):2325967114537588. https://doi.org/10.1177/2325967114537588 PMID: 26535339

73. Jeon JY, Kang HW, Kim DY, Kim YT, Lee DY, Lee D-O. Relationship between calf muscle cross-sectional area and ankle fracture. Foot and Ankle Surgery. 2020. https://doi.org/10.1016/j.fas.2020.11.006 PMID: 33303352

74. Lindström M, Strandberg S, Wredmark T, Felländer-Tsai L, Henriksson M. Functional and muscle morphometric effects of ACL reconstruction. A prospective CT study with 1 year follow-up. Scandinavian Journal of Medicine & Science in Sports. 2013; 23(4):431–42. https://doi.org/10.1111/j.1600-0838.2011.01417.x PMID: 22107159

75. Mangine GT, Hoffman JR, Gonzalez AM, Jajtner AR, Scanlon T, Rogowski JP, et al. Bilateral differences in muscle architecture and increased rate of injury in national basketball association players. J Athl Train. 2014; 49(6):794–9. https://doi.org/10.4085/1062-6050-49.3.60 PMID: 25322345

76. Shida N, Yagiz G, Yamada T. The Effects of Exergames on Muscle Architecture: A Systematic Review and Meta-Analysis. Applied Sciences. 2021; 11(21):10325. https://doi.org/10.3390/app112110325

77. Avrillon S, Hug F, Guilhem G. Bilateral differences in hamstring coordination in previously injured elite athletes. Journal of Applied Physiology. 2020; 128(3):688–97. https://doi.org/10.1152/japplphysiol.00411.2019 PMID: 32027546

78. Landi F, Cruz-Jentoft AJ, Liperoti R, Russo A, Giovannini S, Tosato M, et al. Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study. Age and Ageing. 2013; 42(2):203–9. https://doi.org/10.1093/ageing/afs194 PMID: 23321202

79. Wilkinson DJ, Piasecki M, Atherton PJ. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. Ageing Res Rev. 2018; 47:123–32. https://doi.org/10.1016/j.arr.2018.07.005 PMID: 30048806

80. Abe T, Brown JB, Brechue WF. Architectural characteristics of muscle in black and white college football players. Med Sci Sports Exerc. 1999; 31(10):1448–52. https://doi.org/10.1097/00005768-199910000-00014 PMID: 10527318

81. Abe T, Kumagai K, Brechue WF. Fascicle length of leg muscles is greater in sprinters than distance runners. Med Sci Sports Exerc. 2000; 32(6):1125–9. https://doi.org/10.1097/00005768-200006000-00014 PMID: 10862540

82. Agres AN, Arampatzis A, Gehlen T, Manegold S, Duda GN. Muscle Fascicles Exhibit Limited Passive Elongation Throughout the Rehabilitation of Achilles Tendon Rupture After Percutaneous Repair. Front Physiol. 2020; 11:746. https://doi.org/10.3389/fphys.2020.00746 PMID: 32792966

83. Hickey JT, Timmins RG, Maniar N, Rio E, Hickey PF, Pitcher CA, et al. Pain-Free Versus Pain-Threshold Rehabilitation Following Acute Hamstring Strain Injury: A Randomized Controlled Trial. Journal of Orthopaedic & Sports Physical Therapy. 2020; 50(2):91–103. https://doi.org/10.2519/jospt.2020.8895 PMID: 32005093

84. Whiteley R, Hickey JT, Vermeulen R, Timmins R, Best TM, Rio E, Opar D. Biceps Femoris Fascicle Lengths Increase after Hamstring Injury Rehabilitation to a Greater Extent in the Injured Leg. Translational Sports Medicine. 2022; 2022:5131914. https://doi.org/10.1155/2022/5131914

85. Brockett CL, Morgan DL, Proske U. Predicting hamstring strain injury in elite athletes. Medicine & Science in Sports & Exercise. 2004; 36(3):379–87. https://doi.org/10.1249/01.mss.0000117165.75832.05 PMID: 15076778

86. Nakao G, Kodesho T, Kato T, Yokoyama Y, Saito Y, Ohsaki Y, et al. Relationship between shear elastic modulus and passive muscle force in human hamstring muscles using a Thiel soft-embalmed cadaver. J Med Ultrason (2001). 2023; 50(3):275–83. https://doi.org/10.1007/s10396-023-01317-8 PMID: 37170041

87. Ichihashi N, Umegaki H, Ikezoe T, Nakamura M, Nishishita S, Fujita K, et al. The effects of a 4-week static stretching programme on the individual muscles comprising the hamstrings. J Sports Sci. 2016; 34(23):2155–9. https://doi.org/10.1080/02640414.2016.1172725 PMID: 27113325

88. Eby SF, Cloud BA, Brandenburg JE, Giambini H, Song P, Chen S, et al. Shear wave elastography of passive skeletal muscle stiffness: influences of sex and age throughout adulthood. Clin Biomech (Bristol, Avon). 2015; 30(1):22–7. https://doi.org/10.1016/j.clinbiomech.2014.11.011 PMID: 25483294

89. Kennedy P, Barnhill E, Gray C, Brown C, van Beek EJR, Roberts N, Greig CA. Magnetic resonance elastography (MRE) shows significant reduction of thigh muscle stiffness in healthy older adults. Geroscience. 2020; 42(1):311–21. https://doi.org/10.1007/s11357-019-00147-2 PMID: 31865527

90. Miyamoto N, Miyamoto-Mikami E, Hirata K, Kimura N, Fuku N. Association analysis of the ACTN3 R577X polymorphism with passive muscle stiffness and muscle strain injury. Scand J Med Sci Sports. 2018; 28(3):1209–14. https://doi.org/10.1111/sms.12994 PMID: 29032593

91. McPherson AL, Nagai T, Schilaty ND, Hale R, Hewett TE, Bates NA. High school male basketball athletes exhibit greater hamstring muscle stiffness than females as assessed with shear wave elastography. Skeletal Radiol. 2020; 49(8):1231–7. https://doi.org/10.1007/s00256-020-03397-w PMID: 32123955

92. Avrillon S, Lacourpaille L, Hug F, Le Sant G, Frey A, Nordez A, Guilhem G. Hamstring muscle elasticity differs in specialized high-performance athletes. Scand J Med Sci Sports. 2020; 30(1):83–91. https://doi.org/10.1111/sms.13564 PMID: 31593612

93. Nakamura M, Hasegawa S, Umegaki H, Nishishita S, Kobayashi T, Fujita K, et al. The difference in passive tension applied to the muscles composing the hamstrings—Comparison among muscles using ultrasound shear wave elastography. Man Ther. 2016; 24:1–6. https://doi.org/10.1016/j.math.2016.03.012 PMID: 27317500

94. Miyamoto N, Hirata K, Kanehisa H. Effects of hamstring stretching on passive muscle stiffness vary between hip flexion and knee extension maneuvers. Scand J Med Sci Sports. 2017; 27(1):99–106. https://doi.org/10.1111/sms.12620 PMID: 26669626

95. Paluch Ł, Nawrocka-Laskus E, Wieczorek J, Mruk B, Frel M, Walecki J. Use of Ultrasound Elastography in the Assessment of the Musculoskeletal System. Pol J Radiol. 2016; 81:240–6. https://doi.org/10.12659/PJR.896099 PMID: 27279926

96. Freitas SR, Radaelli R, Oliveira R, Vaz JR. Hamstring Stiffness and Strength Responses to Repeated Sprints in Healthy Nonathletes and Soccer Players With Versus Without Previous Injury. Sports Health. 2023:19417381231175474. https://doi.org/10.1177/19417381231175474 PMID: 37254837

97. Lee Y, Kim M, Lee H. The Measurement of Stiffness for Major Muscles with Shear Wave Elastography and Myoton: A Quantitative Analysis Study. Diagnostics. 2021 https://doi.org/10.3390/diagnostics11030524 PMID: 33804273

98. Alfuraih AM, Alhowimel A, Alghanim S, Khayat Y, Aljamaan A, Alsobayel HI. The Association between Tensiomyography and Elastography Stiffness Measurements in Lower Limb Skeletal Muscles. Sensors. 2022 https://doi.org/10.3390/s22031206 PMID: 35161950

99. Ham S, Kim S, Choi H, Lee Y, Lee H. Greater Muscle Stiffness during Contraction at Menstruation as Measured by Shear-Wave Elastography. Tohoku J Exp Med. 2020; 250(4):207–13. https://doi.org/10.1620/tjem.250.207 PMID: 32238619

100. Liu J, Wang K, Wu J, Miao H, Qian Z, Ren L, Ren L. In Vivo Assessment of Lower Limb Muscle Stress State Based on Shear Wave Elastography. IEEE Access. 2020; 8:122185–96. https://doi.org/10.1109/ACCESS.2020.3007145

101. Cornelson SM, Ruff AN, Wells C, Sclocco R, Kettner NW. Sonographic measures and sensory threshold of the normal sciatic nerve and hamstring muscles. J Ultrasound. 2022; 25(1):47–57. https://doi.org/10.1007/s40477-020-00552-w PMID: 33515412

102. Šarabon N, Kozinc Ž, Podrekar N. Using shear-wave elastography in skeletal muscle: A repeatability and reproducibility study on biceps femoris muscle. PLOS ONE. 2019; 14(8):e0222008. https://doi.org/10.1371/journal.pone.0222008 PMID: 31469888

103. Hinks A, Franchi MV, Power GA. Ultrasonographic measurements of fascicle length overestimate adaptations in serial sarcomere number. Experimental Physiology. 2023;n/a(n/a). https://doi.org/10.1113/EP091334 PMID: 37608723

104. Dulgheriu T, Muntean D, Solomon C, Dudea SM. Comparison of Ultrasound Elastography Values Between: Different Machines, Transducers, Acquisition Depths, ROI Diameters and Examiners, on a Biological Tissue in vitro Study- Preliminary Results. Ultrasound in Medicine & Biology. 2022; 48:S27. https://doi.org/10.1016/j.ultrasmedbio.2022.04.088