Hamstring Muscle Stiffness During Isometric Contractions Until Task Failure in Footballers With and Without Injury History

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Abstract

Martínez-Serrano, A, Radaelli, R, Trindade de Freitas, T, Alcaraz, PE, and Freitas, SR. Hamstring muscle stiffness during isometric contractions until task failure in footballers with and without injury history. *J Strength Cond Res* XX(X): 000–000, 2024—Despite various proposed prevention strategies, the incidence of hamstring injuries in modern soccer is still elevated. Recent research has focused on exploring how muscle tissue stiffness behaves under fatigue conditions as a potential risk factor. This study aimed to examine the active stiffness of biceps femoris long head (BFlh) and semitendinosus (ST) muscles using ultrasound-based shear wave elastography (SWE) during a knee flexors' submaximal contraction until exhaustion in highly trained national-level male footballers, comparing previously injured and noninjured limbs. A case-control study was performed including 94 highly trained male footballers. Using SWE, the passive and active stiffness of the BFlh and ST were assessed at rest and during a knee flexors' submaximal isometric contraction at 40% of maximal voluntary isometric contraction (MVIC) until exhaustion. Differences in stiffness patterns between previously injured and noninjured limbs were analyzed, along with passive muscle stiffness, knee flexors' MVIC, and endurance capacity. No statistically significant differences in the active stiffness of BFlh and ST between previously injured and noninjured limbs throughout the contraction task were found ($p > 0.05$; 0–100% contraction time). Similarly, there were no statistically significant differences in BFlh (mean difference [mean diff.] = 0.2 kPa; $p > 0.05$) and ST (mean diff. = 0.9 kPa; $p > 0.05$) passive stiffness, knee flexors' MVIC (mean diff. = −8.5 Nm; $p > 0.05$), or time to exhaustion (mean diff. = 6.95 seconds; $p > 0.05$). Load-sharing between the BFlh and ST did not change significantly throughout the contraction ($p > 0.05$; 0–100% contraction time). These results suggest that players with a history of hamstring injuries may retain similar mechanical properties and coordination strategies as noninjured players.

Key Words: soccer, biceps femoris, mechanical, performance, shear wave elastography

Introduction

Skeletal muscle injuries are currently under the spotlight of football medicine because of the financial burden and loss of individual/collective performance they imply (17,26). Among the different locations of muscle injuries, the hamstrings remain one of the most frequently injured muscle groups, with hamstring strain injuries (HSIs) accounting for 4–13% of all football-related injuries and presenting a recurrence rate of 4–68% (14). Despite different prevention strategies proposed in the scientific literature (47), the incidence of hamstring injuries has not decreased over 18 seasons in Union of European Football Associations (UEFA) Champions League teams (16). Specifically, the biceps femoris long head (BFlh) seems to be the most affected muscle in footballers with the semitendinosus (ST) being less affected (25,28). Although the nature of HSIs has been suggested to be multifactorial (9), injury history has been identified as the most consistent risk factor for HSIs across studies, whereas neuromuscular fatigue has been shown to potentially increase injury susceptibility (23). Thus, research efforts should be conducted to identify modifiable risk factors that could allow reducing HSI incidence.

Recent studies have suggested that the distribution of force among synergistic hamstring muscles (i.e., load-sharing) may play a role in hamstring injury occurrence (2,20,48). It is important to note that different methodological approaches could be used to assess and infer load-sharing (1,5,6,51). Through the use of T2 relaxation quantification with magnetic resonance imaging, previous research found that previously injured amateur footballers had a more symmetrical metabolic response between biceps femoris, ST, and semimembranosus (SM) after a prone leg curl exercise performed until exhaustion, compared with the noninjured controls, suggesting that part of the ST activity was replaced by more synergistic activity of BF and SM (48). Changes in muscle activation have also been observed in recreationally active populations, where subjects with a history of HSIs showed decreased BFlh activation (estimated through magnetic resonance

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imaging [MRI] T2 relaxation quantification) after performing 2 popular hamstring exercises such as the single-leg Roman chair hold (52) or the Nordic hamstring exercise (7). These findings might explain the presence of neuromuscular inhibition (45), which could require the compensation of other synergistic muscles. Moreover, players who suffer a hamstring injury usually present structural changes in the muscle such as the formation of nonfunctional scar tissue (13), persistent muscle atrophy (49), or fascicle shortening (30) that could modify the mechanical properties of the muscle during contraction. However, the assessment of the metabolic response through functional magnetic resonance imaging is limited to pre-post measurements taken at rest and, consequently, does not consider the effects of the fatigue developed during the active contraction. Also, T2 relaxation reflects metabolic activity, and not a mechanical parameter, which may be more relevant for injury mechanism understanding.

In this regard, ultrasound-based shear wave elastography (SWE) is a technique that allows to assessment of the skeletal muscle mechanical properties (e.g., muscle stiffness) in both passive (3,31,33) and active conditions (39–42) and to infer the load-sharing strategy between the hamstring muscles (39,40). Interestingly, Freitas et al. (20) analyzed the differences in the BFlh/ST active stiffness pattern of noninjured and hamstring-injured limbs of elite football players during a knee flexion (KF) submaximal isometric contraction until exhaustion at 20% of the maximal isometric voluntary contraction (MVIC). The authors found that, at the start of the contraction, previously injured limbs had a lower absolute BFlh active stiffness and, consequently, a lower BFlh/ST ratio. Moreover, a tendency (although without reaching statistical significance at $p < 0.05$) was found for a steeper increase in the BFlh/ST ratio in injured limbs, which suggests a greater relative activity of the BFlh (compared with ST) throughout the development of fatigue. Similar conclusions (through the use of surface electromyography, magnetic resonance imaging, and dynamometry) were reported by Avrillon et al. (2), where the contribution of BF torque at 20% of MVIC in a nonfatigued state was found to be lower in the hamstring-injured limb of elite male sprinters and long jumpers. These findings may corroborate the muscle inhibition that has been suggested to occur after the injury occurrence (11,22,45) and could play a major role in modifying the load-sharing strategies among hamstring muscles. Notably, both studies identified muscle imbalances in nonfatigued states at low-intensity isometric contractions; thus, the observation at higher contraction intensity remains to be explored. At 20% of knee flexors' MVIC, the BFlh/ST active stiffness ratio is known to be lower than 40% of MVIC in healthy individuals, together with a higher strength endurance time of 20% (39). However, this has not been explored in experienced footballers, as well as the behavior during an isometric contraction until exhaustion in those with previous injuries. As nontraumatic hamstring injury in footballers often involves fast movements that require rapid muscle contractions with high levels of muscle activation (38), it is important to examine if a KF isometric task performed at a higher contraction intensity could provide better outcomes that could differentiate injured from noninjured players.

The aim of this study was to analyze the active stiffness patterns of the BFlh and ST muscles using SWE during a submaximal contraction at 40% of KF MVIC until exhaustion, in both previously injured and noninjured limbs of highly trained national-level male footballers. In addition, we investigated potential differences in passive stiffness, knee flexors' MVIC, and endurance capacity between the limbs. We hypothesized twofold: (a) the BFlh active stiffness levels of previously hamstring-injured limbs would be decreased in nonfatigued states (i.e., lower BFlh/ST ratio), and the BFlh/ST ratio would have a higher increase in the presence of fatigue, and (b) no differences would be observed for muscles' passive stiffness, knee flexors' MVIC, and endurance capacity between injured and noninjured limbs.

Methods

Experimental Approach to the Problem

A case-control study design was conducted. Players performed one experimental testing session at the start of the 2022/2023 preseason, with comparisons made between previously injured (cases) and noninjured (controls) limbs. The study's general procedures are schematized in Figure 1. Briefly, general demographic, anthropometric, and clinical lower-limb history information of the players was collected on their arrival at the laboratory. Two researchers identified both limbs' BFlh and ST localization through ultrasound, determined their muscles' region of interest, and measured their passive stiffness using SWE. Then, players were familiarized with the equipment and performed a brief specific warm-up (i.e., ~10 KF submaximal unilateral contractions at 50% perceived maximal intensity), followed by knee flexors' MVIC testing of both dominant and nondominant limbs (assessed with random order). The fatigue task consisting of a KF submaximal isometric contraction at 40% of MVIC until failure was then performed. Both limbs were tested in a random order, and the active stiffness of BFlh and ST muscles was recorded throughout the contraction. After the active stiffness measurements of each limb, knee flexors' MVIC was reassessed to identify the level of neuromuscular fatigue.

Subjects

Subjects' recruitment was performed through the researchers' personal contacts, which were directed to the health and performance departments of the teams. The sample size required to compare the shear moduli of BFlh and ST between injured and noninjured limbs was calculated on G*Power software (Version 3.1; Universität Düsseldorf) with an estimation of 24 total subjects, assuming a statistical power of 0.95, an effect size (ES) f of 0.39, alpha error probability of 0.05, 2 groups (injured and noninjured), 2 measurements (BFlh and ST), and a correlation among repeated measures of 0.5. Following previously published data (15) that reported that around 20–30% of the study sample would be found to have suffered a hamstring injury in the past 2 years, efforts were made to recruit a minimum of 90 male footballers. Players were asked to avoid any physical exercise 72 hours before the testing session, and they were excluded from testing if they had a clinical injury at the time. Hamstring strain injuries were defined according to the Munich Consensus Statement (43). The injured group included players' limbs that sustained HSIs within 2 years of the testing session and were free from any major surgeries in the lower limb. On the other hand, the noninjured group included players' limbs that were free from HSIs or any major surgeries in the lower limbs. The contralateral noninjured limbs of the injured group were also excluded from the latter. For data analysis, only field players were included. The flow chart of the sample screening can be seen in Figure 2. To perform group comparisons, a similar number of noninjured limbs were selected to match the sample size of injured limbs using a matched pairs design, considering similar characteristics as the injured counterparts (i.e., age, height, body mass, playing experience, limb dominance, affected limb right or left, and knee flexors' MVIC relative to body mass). Matching accuracy was

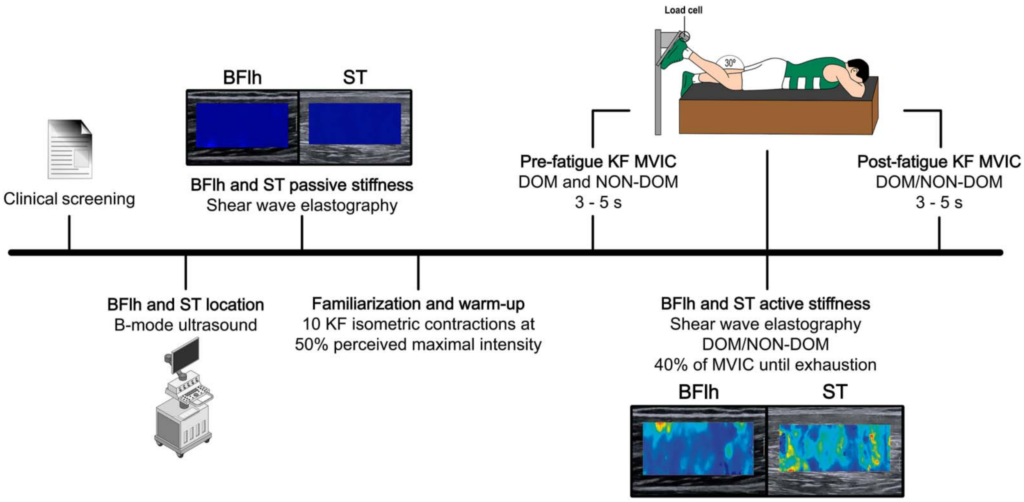

Figure 1. Study general procedures taken by the footballers. Representative sonograms of a subject's biceps femoris long head (BFlh) and ST are shown both in passive and active conditions (elastogram scale: 0–800 kPa). BFlh = biceps femoris long head; DOM = dominant limb; KF = knee flexion; MVIC = maximal voluntary isometric contraction; NON-DOM = nondominant limb; ST = semitendinosus.

determined using an independent-samples T-test, and the descriptive information is shown in Table 1. Informed consent was given by the players before data collection and their signed documents were obtained. The study was conducted under the principles outlined in the Declaration of Helsinki and approved by the Ethics Council of Faculty of Human Kinetics—CEFMH—approval number: #11/2022.

Procedures

Clinical Screening. Information about players' playing experience, playing position, limb dominance, number of injuries, injured limbs, date of injury, injury location, time to return to play, injury mechanism, injury context, and number of major surgeries in the lower limb was collected through an anamnesis enquiry.

Knee Flexor Maximal Voluntary Isometric Contraction. Players were positioned prone, with the hips in a neutral position (secured with a strap), and the knees flexed at ~30° in custom-built equipment as shown in Figure 1. The foot was positioned in a foot holder at a 90° ankle angle and strapped to avoid the internal/external rotation of the tibia. The foot holder contained a force transducer (Model STC; Vishay Precision, Malvern, PA) measuring at 1 kHz to collect the linear force perpendicular to the leg orientation. Force was amplified (Model UA73.202; Sensor Techniques, Cowbridge, United Kingdom), digitally converted

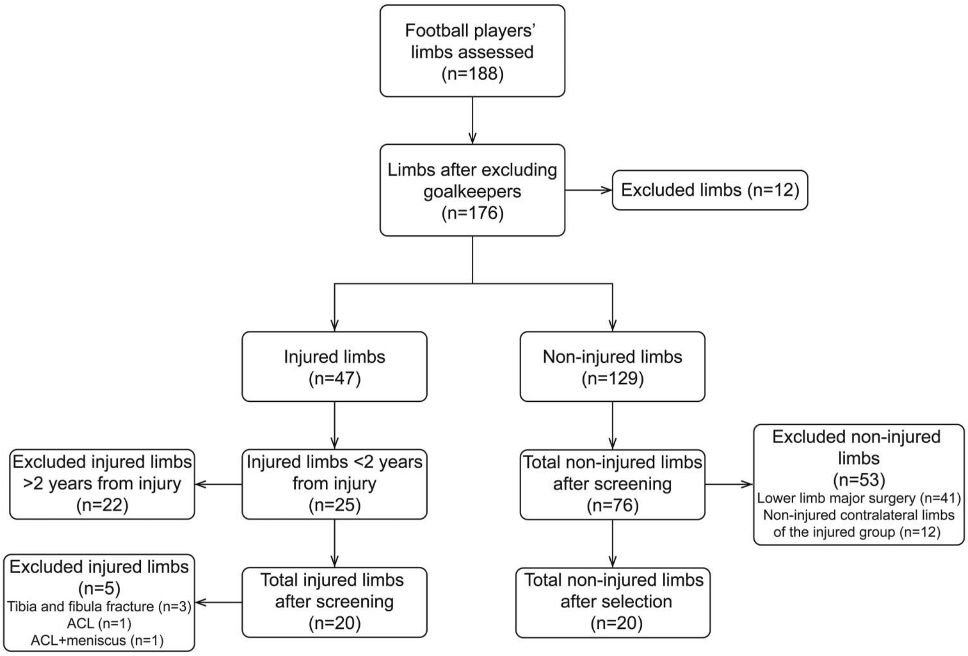

Figure 2. Flow chart of the sample screening.

Table 1

Demographic and clinical characteristics of footballers with hamstring-injured and noninjured limbs.*

	Noninjured (n = 20)	Injured (n = 20)
Age (y)	23.5 ± 3.6	24.0 ± 4.3
Height (cm)	181.7 ± 6.5	183.8 ± 5.7
Body mass (kg)	76.3 ± 7.2	79.7 ± 6.8
Playing experience (y)	16.5 ± 4.4	16.2 ± 5.5
Playing division: 2nd; 3rd (n)	11; 9	5; 15
Centre back (n)	8	1
Full back (n)	0	3
Wingers (n)	6	5
Midfielder (n)	4	5
Offensive midfielder (n)	0	0
Forward (n)	2	6
Dominant; nondominant limbs (n)	9; 11	9; 11
Right; left limbs (n)	12; 8	12; 8
Unilateral hamstring injuries (n)		14
Previous HSIs: 1; 2; 3 (n)		14; 4; 2
Injury mechanism: sprint; stretching; COD; unknown (n)		13; 3; 1; 3
Injury context: match; training (n)		9; 11
Match injury incident: ST-1st; END-1st; ST-2nd; END-2nd (n)		1; 2; 1; 5
Time between testing and injury (mo)		12.4 ± 7.1
Time between injury and RTP (wk)		5.5 ± 4.7

*COD = change of direction; END-1st = last 15 min of the first half; END-2nd = last 15 min of the second half; HSIs = hamstring strain injuries; RTP = return-to-play; ST = semitendinosus; ST-1st = first 15 min of the first half; ST-2nd = last 15 min of the second half.

(USB-230 Series; Measurement Computing, Corp., Norton, MA), and recorded using the DAQami software (v.4.1; Measurement Computing, Corp.). Knee flexion torque was estimated by multiplying the perpendicular distance between the center of the force transducer and the femoral lateral condyle. Players performed two 3- to 5-second duration MVICs in both limbs with a 1-minute rest between repetitions. The researchers gave verbal encouragement to ensure maximal effort during the test. If the difference between MVICs was higher than 5%, more repetitions were performed. The highest peak force value within the 5% difference range was used for analysis.

Fatigue Task. After a 5-minute passive rest remaining lying prone, players performed a sustained submaximal isometric KF at 40% of MVIC until failure, with visual feedback on the torque production as well as a visual delimited line that corresponded to their 40% of MVIC. The dominant and nondominant limbs were tested in a random order. The test stopped when the player was unable to produce force at the determined intensity. The researchers gave verbal encouragement to the players throughout the test to maintain torque at the determined intensity.

Passive and Active Muscle Stiffness. Two identical ultrasound systems (Aixplorer, v10; Supersonic Imagine, Aix-en-Provence, France) in B-mode, connected to a linear transducer array (SL10-2, 2–10 MHz; Vermon, Tours, France) were used to identify the location of the largest cross-sectional areas of the BFlh and ST muscles at ~55% of the distal-to-proximal femur length. Once the region of interest was identified, a 3D-printed plastic cast with the shape of the probe was attached to the skin using biadhesive tape to ensure a stable and precise recording of the muscles' stiffness. The cast was oriented according to the fascicle direction. Then, a 30-second recording in SWE mode (musculoskeletal preset; penetrate mode; smoothing level 5; persistence off; scale: 0–800 kPa; imaging frequency of 0.7–1.1 Hz) of both muscles was taken to assess passive muscle stiffness. During the recording, players were instructed to remain completely relaxed. This was ensured by visually inspecting the elastogram window. Data collection from both ultrasound systems started simultaneously using a pedal switch. Active stiffness of BFlh and ST was continuously assessed during the fatigue task with the same settings used in the passive measurement. As the maximum recording time for SWE videos was 60 seconds, clips were taken continuously until the end of the fatigue task (less than 5-second intervals because of ultrasound processing time).

Data Analysis. The highest peak torque obtained during the knee flexors' MVIC trials before (pre-test) and after (post-test) the fatigue task was used to calculate the fatigue index (i.e., the percentage of KF MVIC torque loss after the fatigue task). Time to exhaustion was determined by the total time (s) performed by each subject in the fatigue task. Shear modulus data from the passive and active assessments were analyzed using customized Matlab routines (version R2022a; The MathWorks, Inc., Natick, MA; available at https://cimt.uchile.cl/mcerda/). Each video clip was extracted from the ultrasound software and converted into .avi format. The Matlab routine allowed users to manually select the largest rectangular region of interest in the elastogram window of the video and convert the pixels containing elastogram measurements into elastic moduli values based on the recorded scale (0–800 kPa). These values were then averaged to obtain a representative muscle value and exported to individual Excel files. It is important to consider that the values displayed in the elastogram windows of the Aixplorer's ultrasound scanner represent Young's modulus (E) of the medium. Therefore, to estimate the shear moduli (μ) of the muscles, these values were divided by 3 following the formula: $\mu = E/3$ (4). To ensure that the values were stable at the desired contraction intensity, a manual inspection of the data was performed by excluding the initial 5 seconds of the contraction onset. Using custom routines in Matlab (version R2022a; The MathWorks, Inc.), data outliers from each muscle data set were (a) excluded using the interquartile method; (b) interpolated using a fourth-order polynomial function (to impute missing data due to the processing time between ultrasound clips); (c) smoothed using a Savitzky-Golay filter; (d) normalized as a percentage from 1 to 100% contraction time; and (e) standardized for uniform length of 100 data points representing the full range of contraction from 1 to 100%. In addition, the BFlh/ST ratio was calculated at each data point during the fatigue task. This ratio was obtained by dividing the active stiffness values of the BFlh by the corresponding stiffness values of the ST at each percentage of the contraction duration.

Statistical Analyses

Statistical analysis of discrete data was performed on JASP (version 0.17.1.0), whereas the analysis of continuous data was performed using spm1d (version M.0.4.10, SPM1D package, spm1d.org), i.e., an open-source package for one-dimensional statistical parametric mapping (SPM) in Matlab (version R2022a; The MathWorks, Inc.). Data distribution and homogeneity were confirmed using the Shapiro-Wilk and Levene's tests, respectively. The following statistical approaches were used: a 2-way repeated-measures analysis of variance (ANOVA) (limb [injured, noninjured] × time [pre, post]) for knee flexors' MVIC, a 2-way repeated-measures ANOVA (limb [injured, noninjured] × muscle [BFlh, ST]) for muscles' passive stiffness and muscles' slopes, an independent-samples T-tests for fatigue index, and Mann-Whitney *U* test for time to exhaustion and BFlh/ST ratio slope. Regarding continuous data, a 2-way SPM ANOVA (limb [injured, noninjured] × muscle [BFlh, ST]) was performed to analyze muscles' active stiffness differences between limbs throughout the contraction. A post hoc SPM paired sample 2-tailed *t*-test with Bonferroni's correction was performed if significant main or interaction effects were found. Moreover, an independent-samples 2-tailed *t* test was used to examine BFlh/ST ratio differences between limbs.

Snedecor's *F*-values and degrees of freedom were calculated from the repeated-measures ANOVA. Cohen's *d* and its 95% confidence interval (CI) were used for ES calculations of multiple comparisons and were interpreted as trivial (<0.2), small (0.2–0.59), moderate (0.6–1.19), large (1.2–1.99), very large (2–4), and near perfect (>4) (27). Data are presented as mean ± *SD*. For group comparisons, the mean differences and their 95% CIs were calculated. The alpha level was set at $p < 0.05$.

Results

Sample

Four teams accepted to collaborate, and 94 highly trained national-level (37) male footballers competing in the second (n = 50 players) and third (n = 44 players) professional divisions (age = 24.4 ± 3.8 years; height = 180.9 ± 6.5 cm; body mass = 76.7 ± 6.9 kg; football playing experience = 17.0 ± 4.5 years) participated in the study. As shown in Figure 2, 148 limbs were excluded, and a total of 40 lower limbs (i.e., 20 with and 20 without previous hamstring injury) were considered for analysis.

Injury Data

Descriptive sporting and clinical data from the injured and noninjured limbs can be found in Table 1, where no differences were found for any quantitative variable ($p > 0.05$).

Biceps Femoris Long Head and Semitendinosus Active Stiffness

Descriptive data of the active stiffness of BFlh and ST of the injured and noninjured limbs as well as the slope during the fatigue task can be found in Figure 3. No significant main effects for limb factor ($p > 0.05$) or limb × muscle interaction ($p > 0.05$) were found. However, a significant main effect for muscle factor ($p < 0.001$) was noted from 0 to 100% contraction time with the ST muscle showing higher active stiffness compared with BFlh throughout the full contraction duration ($p < 0.001$) (see Figure, Supplemental Digital Content 1, http://links.lww.com/JSCR/A589, which demonstrates the results of the SPM analysis). The slope analysis revealed no significant effects for muscle factor (F[1,19] = 2.707; p = 0.116), limb factor (F[1,19] = 0.060; p = 0.809), or limb × muscle interaction (F[1,19] = 0.004; p = 0.948).

Biceps Femoris Long Head/ST Ratio

No significant differences were found throughout the contraction between the BFlh/ST ratio of injured and noninjured limbs ($p > 0.05$) (Figure 4A, B). Biceps femoris long head/ST ratio slope was similar between injured and noninjured limbs (p = 0.547; ES = 0.24 [−0.38 to 0.86]) (Figure 4C).

Passive Stiffness, Knee Flexors' Maximal Voluntary Isometric Contraction, and Fatigue Task Results

Table 2 shows the descriptive strength performance results and BFlh and ST passive stiffness outcomes of injured and noninjured limbs. Considering muscles' passive stiffness, significant main effects for muscle factor (F[1,19] = 7.485; p = 0.013), but not for limb factor (F[1,19] = 0.370; p = 0.550) or muscle × limb interaction, (F[1,19] = 0.022; p = 0.885) were found. Biceps femoris long head showed lower passive stiffness levels compared with ST (mean diff. = −0.66 kPa [−1.16 to −0.15 kPa]; p = 0.013; ES = −0.54 [−0.98 to −0.10]).

Regarding knee flexors' MVIC, no significant effects were found for limb factor (F[1,19] = 1.032; p = 0.322) or time × limb interaction (F[1,19] = 1.242; p = 0.279). However, a significant time factor effect was noted (F[1,19] = 46.034; $p < 0.001$) showing a significant knee flexors' MVIC decrease after the fatigue task (mean diff. = 17.95 Nm [12.41–23.49 Nm]; $p < 0.001$; ES = 0.62 [0.34–0.90]).

No significant differences were found in fatigue index (mean diff. = −1.96% [−9.01 to 5.09%]; p = 0.577; ES = −0.18 [−0.80 to 0.44]) or time to exhaustion (mean diff. = −6.95 seconds [−34.15 to 20.25 seconds]; p = 0.608; ES = −0.16 [−0.78 to 0.46]) between the injured and noninjured limbs.

Discussion

In this study, we examined the load-sharing dynamics of the BFlh and ST (through the active stiffness quantification with SWE) in both healthy and previously hamstring-injured limbs of highly trained footballers. In addition, we evaluated the muscles' passive mechanical properties and the knee flexors' maximal and endurance strength performance. Contrary to initial hypotheses, the study's findings revealed that at 40% of MVIC, the passive and active mechanical characteristics, alongside the load-sharing capacities of the BFlh and ST muscles in previously injured players, were not compromised. Similarly, no evident differences in force generation and endurance capacities between injured and noninjured limbs were observed.

During the fatigue task, our analysis showed that active stiffness levels of the BFlh and ST muscles were consistent across

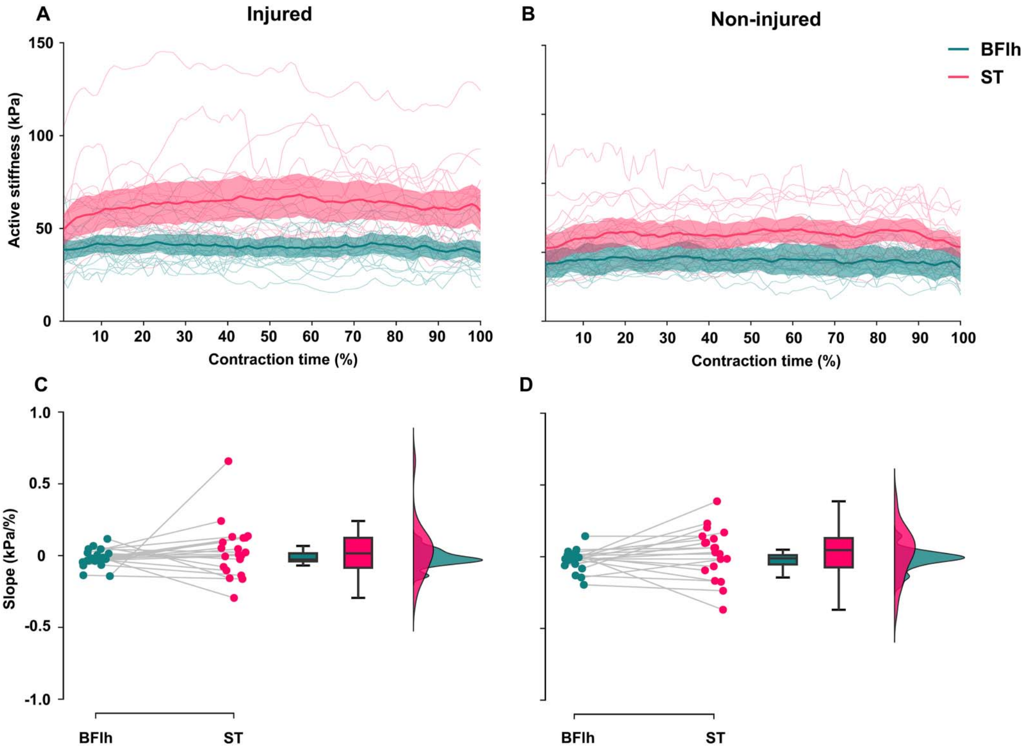

Figure 3. Injured (A) and noninjured (B) mean active stiffness (solid line) of biceps femoris long head (BFlh) and semitendinosus (ST) during the knee flexors' submaximal contraction until exhaustion. Shaded areas represent the upper and lower 95% confidence interval. Individual data points can be seen as shaded lines. C, D) show the between-muscle comparisons of injured and noninjured limbs slope, respectively.

injured and noninjured limbs, a result that diverges from the findings of Freitas et al. (20) yet aligns with those in another study by the same research group (21). Despite applying a similar methodological procedure but with a higher contraction intensity (i.e., 40% of MVIC) than that used by Freitas et al. (20) (i.e., 20% of MVIC), no significant differences in BFlh active stiffness across limbs were detected during the fatigue task. These findings might be partly explained by the different training statuses between the samples (elite vs. highly trained football players) and the higher contraction intensity used. Martínez-Serrano et al. (36) investigated the same contraction intensity (i.e., 40% of MVIC) in recreationally active individuals and concluded that to induce changes in the shear modulus of the BFlh and ST, longer contraction durations at reduced intensities were required. This could elucidate the lack of discernible differences between injured and noninjured limbs, given that the average contraction duration for all limbs was 102.9 ± 42.1 seconds, even shorter than the duration observed in the abovementioned study (153.1 ± 40.0 seconds). Notably, Evangelidis et al. (18) demonstrated that intermittent contractions at 50% of MVIC for a total duration of 494 seconds in healthy individuals could elicit active stiffness alterations in the ST and BFlh muscles. Hence, future research aiming to identify differences between injured and noninjured players under isometric conditions may consider longer muscle contraction durations, although the efficiency of testing sessions, particularly in preseason assessments of full squads, could be affected. Nevertheless, incomplete knowledge surrounding near-maximal isometric contractions or explosive dynamic contractions in SWE measurements is still present, mainly because of the saturation of the elastogram (8) and the low sample rate of the ultrasound devices (24). This technological constraint hinders the understanding of the mechanical properties of the muscle under more realistic conditions. Finally, another contributing factor could be the level of neuromuscular fatigue induced by the fatigue protocol. In our study, the overall strength loss was 12.4 ± 10.9%, whereas previous research has documented reductions in KF torque typically ranging from ~4 to ~8% (20,40). Notwithstanding, despite having a lower fatigue index, they observed within-muscle stiffness changes during the contraction until exhaustion at 20% of MVIC, suggesting that the level of neuromuscular fatigue might not substantially influence muscle active stiffness changes compared to increased intensity levels.

The evaluation of load-sharing patterns revealed similar BFlh/ST ratios between injured (0.72 ± 0.3 ratio) and noninjured (0.78 ± 0.28 ratio) limbs throughout the contraction duration, which contradicts our initial hypothesis. Particularly, load-sharing strategies appeared highly individualized, as evidenced by the distinct BFlh/ST ratios during contraction in injured (coefficient of variation [CV] = 42.3%) and noninjured limbs (CV = 35.5%), suggesting that players might have adopted diverse strategies to sustain torque production at the predetermined intensity, mainly by altering the ST behavior rather than the BFlh. Moreover, it is important to note that between 40 and 60% of knee flexors' MVIC, the change in the shear modulus of these muscles stabilizes, probably because of the increased participation of other synergistic muscles (e.g., SM, gastrocnemius, biceps femoris short head, gracilis, and sartorius) (39). Because of experimental limitations, this hypothesis could not be directly verified. Thus, future research may explore the contribution of these synergistic muscles as well as including hip extension contractions.

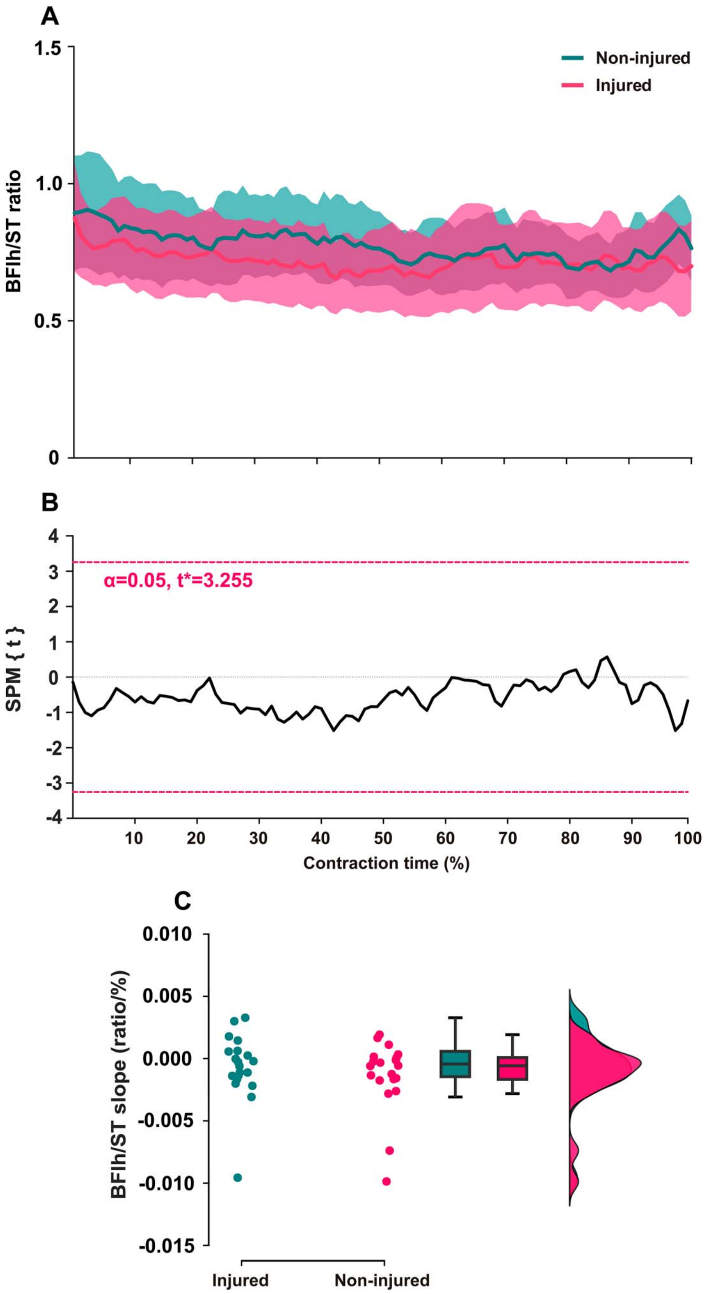

Figure 4. A) Mean (solid line) biceps femoris long head/semitendinosus (BFlh/ST) ratio during the knee flexors' submaximal contraction until exhaustion in injured and noninjured limbs. Shaded areas represent the upper and lower 95% confidence interval. B) Statistical parametric mapping (SPM) analysis, i.e., t-statistics (SPM{t}), of the differences in BFlh/ST ratio between injured and noninjured limbs. The pink dashed lines represent the critical thresholds. C) Between limb comparison of BFlh/ST ratio slope.

On the other hand, inconsistent evidence regarding the differences in passive neuromechanical properties measured through SWE in previously injured hamstrings has been found. Some studies reported increased passive stiffness in the muscle belly of injured limbs (54) and increased passive stiffness exclusively in the BFlh fascia of injured limbs (29), whereas others observed no differences (21). Our findings indicate similar passive stiffness between injured and noninjured limbs as Freitas et al. (21), although with lower absolute values (mean BFlh = 4.3–4.5 kPa and mean ST = 4.5–4.7 kPa). Interestingly, our data suggest higher overall passive stiffness in the ST compared with the BFlh (BFlh = 6.7 ± 1.3 kPa and ST = 7.4 ± 1.1 kPa), which diverges from previous research suggesting that the ST typically exhibits the lowest passive stiffness values within the hamstring group irrespective of hip and knee position (34,51) and athlete training status or sport specialization (3). The underlying causes remain unclear; however, the specific location of the injury may be a contributing factor, which was not evaluated in this study as the clinical data were derived from self-reported information.

Table 2

Performance metrics and passive stiffness of noninjured and injured limbs.*†

	Noninjured (n = 20)	Injured (n = 20)
Relative knee flexors' MVIC (Nm·kg^{-1})	1.9 ± 0.3	1.9 ± 0.3
PRE knee flexors' MVIC (Nm)	146.9 ± 20.4	155.4 ± 33.2
POST knee flexors' MVIC (Nm)	131.3 ± 28.2	135.1 ± 32.5
Fatigue index (%)	11.3 ± 12.2	13.2 ± 9.7
Time to exhaustion (s)	106.4 ± 39.2	99.5 ± 45.6
Passive BFlh stiffness (kPa)	6.8 ± 1.1	6.6 ± 1.45
Passive ST stiffness (kPa)	7.5 ± 1.2	7.29 ± 1.1

*BFlh = biceps femoris long head; MVIC = maximal voluntary isometric contraction; ST = semitendinosus.

†Data presented as mean ± *SD*.

Maximal force production capabilities of the knee flexors were similar between injured and noninjured limbs, consistent with the hypothesis that muscle inhibition could predominantly occur in dynamic actions, especially under eccentric contractions (10,22). Although some studies present conflicting evidence, it is pertinent to note that such investigations were either conducted with recreationally active males (11) or identified differences exclusively at longer muscle lengths (44). Consequently, it could be assumed that knee flexors' weakness under isometric conditions becomes meaningful only at lengthened positions, similar to those experienced during the early stance and late swing phase of a sprint, where hamstring injuries are more susceptible to occur (12,46). Ultimately, no significant difference in knee flexors' endurance between limbs with and without a history of HSIs was found. Freckleton et al. (19) and Lord et al. (35) found contradictory results, showing reduced endurance capacities in the injured limb; however, both studies used different endurance tests compared with this study. This underscores the importance of considering the specific functional demands and biomechanical positions when assessing muscle strength and rehabilitation outcomes in hamstring injury management.

This study faces several limitations that require consideration when interpreting its findings. The absence of detailed injury reports from medical records and reliance on players' self-reports introduces potential gaps in our understanding of the precise type and location of injuries. Such details, including the specific muscle injury location (proximal, medial, or distal) and the injury site (myofascial, myotendinous junction/muscular, or tendinous), are critical for fully comprehending the passive and active mechanical properties of the BFlh and ST muscles. Future research should, therefore, incorporate in-depth assessments of injury location through gold-standard imaging (i.e., MRI), considering the specific characteristics of individual injuries to determine variations in stiffness between limbs more accurately. Last, our findings revealed heterogeneous responses in BFlh and ST muscle stiffness during the fatigue task, challenging the hypothesis of uniform load-sharing patterns between injured and noninjured limbs. This variability may be attributable to the contributions of synergistic muscles in torque production (33) and the potential coactivation of antagonist muscles at higher contraction intensities (53). However, the complexity of our experimental setup and resource constraints excluded direct measurements of these factors. As a result, caution should be taken in interpreting these findings, emphasizing the need for further research to elucidate the mechanical differences in athletes with a history of hamstring injuries, incorporating a more detailed examination of contributing factors to muscle function and injury effects.

Practical Applications

This study analyzed the load-sharing dynamics and mechanical properties of the BFlh and ST muscles during a submaximal isometric contraction until exhaustion at 40% of MVIC in highly trained footballers with and without a history of hamstring injuries. Contrary to initial expectations, our findings revealed no significant compromise in the mechanical characteristics or load-sharing capacities of the BFlh and ST in previously injured limbs at 40% of MVIC. This consistency extended to maximal force generation and endurance capacities across injured and noninjured limbs. The mechanical parameters examined may suggest that at submaximal intensities, previously injured limbs could potentially retain or recover their functional capacities similar to noninjured counterparts. This could be attributed to effective rehabilitation processes that restore muscle function to near preinjury levels or to the intrinsic ability of the muscle tissues to adapt and compensate after injury. Importantly, this might also indicate that the detection of subtle yet clinically significant differences in muscle mechanical properties may require assessments at higher intensities or under fast dynamic conditions that more closely mimic the demands placed on these muscles.

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