applied sciences

MDPI

Article

Effects of Repeated Sprints on Hamstring Active Shear Modulus Pattern and Neuromuscular Parameters in Football Players with and without Hamstring Strain Injury History—A Retrospective Study

Ricardo Pimenta [1,*], Tomás Lopes [2], Paula Bruno [1] and António Veloso [1]

[1] CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Estrada da Costa, 1499-002 Cruz Quebrada-Dafundo, Portugal
[2] Department of Biochemistry, King's College London, Strand, London WC2R 2LS, UK
* Correspondence: rjl.pimenta@gmail.com; Tel.: +351-918-852-877

Featured Application: Hamstring strain injuries occur particularly in the later stages of a football match, suggesting that fatigue may play an important role. Moreover, sprinting is the principal mechanism to sustain a hamstring strain injury since it demands the hamstrings muscle group. After the injury, sprint performance and strength might be reduced, and muscle tissue properties can be altered even after the player returns to competition. Therefore, the aim of the present study is to compare the effects of (i) a repeated sprint protocol on the sprint performance, hamstrings shear modulus pattern, and neuromuscular parameters between players with and without previous hamstring strain injury; and (ii) between limbs with hamstring strain injury and their healthy contralateral limbs on the hamstrings shear modulus pattern and neuromuscular parameters. It should be noted that the biceps femoris long head muscle is the most affected muscle, and it has been reported that a higher contribution of the biceps femoris long head could possibly explain the greater injury rate on this muscle.

Abstract: The aim of the present study is to compare the effects of a (i) repeated sprint protocol on the sprint performance, hamstrings shear modulus pattern, and neuromuscular parameters between players with and without previous hamstring strain injury (HSI); and (ii) between limbs with HSI and their healthy contralateral limbs on the hamstrings shear modulus pattern and neuromuscular parameters. One-hundred-and-five professional and semiprofessional football field players were invited to participate in this study during the pre-season 2021/2022 (June–July), resulting in a sample size of 210 limbs with 46 sustaining HSI in the previous 2 years. No differences were seen between previously injured and healthy control players regarding their sprint performance, hamstrings shear modulus pattern, and neuromuscular parameters, except for the early rate of torque development (0–50 ms) with previously injured limbs in the biceps femoris long head (BFlh) displaying higher rates than their contralateral muscle (injured: 496.93 ± 234.22 Nm/s; contralateral 422.72 ± 208.29 Nm/s; $p = 0.005$; $\eta^2_p = 0.469$). Overall, the present study provides evidence for no differences regarding sprint performance, hamstrings load sharing pattern, and major neuromuscular parameters between players with previous HSI in the last 2 years and healthy control players. Therefore, the results can possibly suggest that the duration between injury and screening could recover the differences between injured-control and injured-contralateral groups.

Keywords: shear wave elastography; sprints; fatigue; peak torque; rate of torque development; tissue properties; football

Citation: Pimenta, R.; Lopes, T.; Bruno, P.; Veloso, A. Effects of Repeated Sprints on Hamstring Active Shear Modulus Pattern and Neuromuscular Parameters in Football Players with and without Hamstring Strain Injury History—A Retrospective Study. *Appl. Sci.* **2023**, *13*, 3099. https://doi.org/10.3390/app13053099

Academic Editors: Marco Parente and Silvestro Roatta

Received: 6 January 2023
Revised: 7 February 2023
Accepted: 26 February 2023
Published: 27 February 2023

1. Introduction

Hamstring strain injuries (HSI) are the most common muscle injuries in football and can be responsible for a long absence from playing with a high recurrence rate (12–33%) [1–3]. They have been increasing annually [4], with sprint being the most common mechanism for sustaining an HSI [5,6]. A recent study indicated that the injury rate increased for 12% from the 2001/2002 season to the 2021/2022 season, constituting 24% of all injuries in men's professional football [7], which implies a significant financial cost for clubs. A previous study demonstrated that the average cost for having a professional first team player injured for 1 month is approximately €500,000 in European football leagues [8]. Due to these implications, HSI has drawn the attention of sports scientists and researchers. However, until today, only a recent (same season) and previous HSI are the undisputed risk factors for future injury [9–12], with age also having high relevance [12]. Indeed, various studies have identified age as a strong risk factor for HSI, demonstrating a lower HSI frequency for football players below 23 years of age [2,12,13], and a nearly four-fold increase after this age [14]. Apart from the cumulative exposure to mechanical loads being correlated with increased HSI [12], aging is also accompanied by structural changes in skeletal muscle that are believed to contribute to the higher risk of HSI observed, including increased non-muscle connective tissue, reduction in cross-sectional area, change in fiber type populations, and decreased fiber number [12,15].

Another possible risk factor that has been suggested for sustaining a HSI is fatigue [2]. Previous studies of physical demands in football have shown that fatigue is developed towards the end of a game, where the amount of high-intensity running and technical performance is lowered [16–18]. There is also evidence that fatigue changes the neuromuscular coordination mechanism and causes adaptations of the control strategy over synergistic muscles, i.e., a load sharing strategy [19]. Moreover, previous studies have reported, using magnetic resonance T2 relaxometry, that changing the load sharing strategy between the hamstring muscles may be associated with HSI [20,21], showing that players with a history of injury had a more homogeneous load distribution (i.e., with a decrease in the semitendinosus (ST) and an increase in the biceps femoris (BF) load), greater metabolic activity, and lower hamstring muscle endurance in knee flexion (KF) dynamic contractions [20,21].

It has also been reported that the change in the active shear modulus (i.e., valued during muscle contraction) measured by shear wave elastography is correlated with changes in torque production, and that it is possible to know which muscle contributes more for a specific task, thus detecting the load sharing strategy [22]. Indeed, Mendes et al. (2020) reported a decrease in active ST shear modulus and a lack of changes in BFlh active shear modulus during an isometric knee contraction until failure, which led to an increase in the BFlh/ST active shear modulus ratio, and consequently a higher BFlh and lower ST contribution in the fatigue task. However, when evaluating the same protocol in professionals with and without football players, there were no differences between limbs over the course of the fatigue task for BFlh, ST, and BFlh/ST active stiffness, with similar knee flexors endurance between limbs [23].

It should be noted that single-joint tasks (knee flexions) have a low functional relevance when compared to an activity such as sprinting. Indeed, HSI occurs often during high-speed running [6,24], and the majority affects the BFlh [9,25,26]. Moreover, after a HSI, players showed a decrease in acceleration performance and a substantially lower sprint speed at return to play [27], as well as a higher drop in speed during repeated sprint tests [28]. Three studies suggest that the workload of the knee flexors had a lower contribution of ST [20,21,29] and greater relative [29] contribution of the BFlh muscle to torque production in the KF task, thus increasing the BFlh injury risk. However, in our previous study, no differences were seen in the load sharing pattern using the same protocol in healthy individuals without HSI, only impacting the neuromuscular system with a decrease of peak torque (PT) and rate of torque development (RTD) in the early phase (0–50 ms; 50–100 ms) after the sprint protocol (under review). Although, it is unknown if any differences exist in the load sharing strategy and neuromuscular parameters between football players with

previous HSI compared to players without HSI after repeated sprinting. Moreover, in relation to the neuromuscular parameters, it is expected that the early phase of RTD will be impacted since injured athletes could possibly have limited time to generate torque to stabilize a perturbed joint.

The present study aims at comparing the effects of a repeated sprint protocol on the sprint performance, hamstrings shear modulus pattern, and neuromuscular parameters between players with and players without previous HSI. We hypothesized that a lower sprint performance, a greater relative contribution of BFlh, and consequently a higher BFlh/ST ratio, and a greater decrease in the neuromuscular parameters would be seen for players with a previous HSI. Secondarily, a comparison between limbs with HSI and their contralateral limbs on the hamstrings shear modulus pattern and neuromuscular parameters was performed, in order to ascertain whether or not the muscle injury would influence the hamstring load sharing pattern and neuromuscular parameters.

2. Materials and Methods

Several clubs were invited to participate in the present study, specifically clubs near to the area of the University of Lisbon. The clubs were invited using oral presentations or e-mail to reach the maximum number of football players, which resulted in 105 football field players of 10 Portuguese professional (2 teams first division, 2 teams second division, and 3 teams third division) and semiprofessional teams (3 fourth division), which participated in this retrospective study during the pre-season 2021/2022 (June–July), resulting in a sample size of 210 limbs. All participants read and signed an informed consent form prior to participating in the study. The Ethical Committee at the Faculty of Human Kinetics at the University of Lisbon approved the study (#5/2021). Participants were instructed to avoid any strenuous activities 24 h before the test to minimize confounding factors.

Potential participants were excluded from this study if they had:

- History of serious knee or hip injuries in the past year (e.g., anterior cruciate ligament tear, medial collateral ligament, femoroacetabular shock, groin injuries that required surgery, and/or other serious injuries that could compromise the performance in the sprint and in the maximal voluntary isometric contractions (MVIC) or the shear wave measurement);
- History of lower back complaints or current complaints in the same region;
- Less than 5 years of experience in competitive football;
- Electronic implants, foreign bodies (ferromagnetic) close to the thigh region.

2.1. Dynamometry

The knee flexor torque was measured at a sampling rate of 1000 Hz using custom-made equipment (Figure 1). Participants were placed in the prone position, with the hips in neutral anatomical position, knees flexed at 30° (0° = full extension) with the ankle in 15° plantar flexion, as previously reported [29]. This position allows for the assessment of muscle shear modulus with minimal passive tension [30]. Both feet were fixed in a foot holder containing a force transducer (Model STC, Vishay Precision, Malvern, PA, USA; Figure 1) at the heel level to collect the linear force perpendicular to the leg orientation and with the ankle at 90°. Force data were amplified (Model UA73.202, Sensor Techniques, Cowbridge, UK), digitally converted (USB-230 Series, Measurement Computing Corporation Norton, MA, USA), recorded using the DAQami software (v4.1, Measurement Computing Corporation, Norton, MA, USA), and multiplied by the perpendicular distance between the force transducer center and the femoral lateral condyle in order to estimate the knee torque. Visual feedback of force production was provided to individuals during the assessments. The neuromuscular parameters assessed and analyzed were PT and RTD, where separate RTD intervals were defined: 0–50 ms, 50–100 ms, 150–200 ms, and time until maximum RTD (TU-RTDmax).

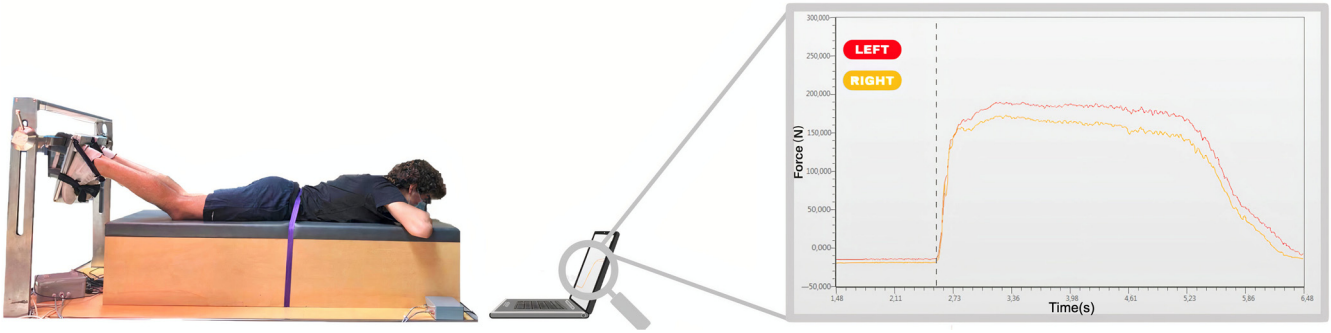

Figure 1. Experimental setup used to assess the knee flexors' peak torque and rate of torque development with 30° of knee flexion and neutral hip. DAQami layout measuring force (N) in the y-axis and time (s) in the x-axis, with the red line corresponding to the left limb and the yellow to the right limb, and the dashed line corresponding to the onset of force production (defined as the instant where the torque value reached three standard deviations above the baseline value).

2.2. Sprint Performance

Sprint performance was evaluated by a 10 × 30 m repeated sprint protocol using a two-point stance, with participants positioned 1 m behind the photocells. The average sprint speed was recorded using four photocells and data was processed by the Chronojump software (version: 2.1.1-16, Chronojump Boscosystem, Barcelona, Spain).

2.3. Shear Wave Elastography

Hamstrings shear modulus was assessed using two similar ultrasound scanners (Aixplorer, v11; Supersonic Imagine, Aix-en-Provence, France; Aixplorer, v12; Supersonic Imagine, Aix-en-Provence, France) in SWE mode (musculoskeletal preset, penetrate mode, smoothing level 5, opacity 100%, scale: 0–800 kPa for active (i.e., during contraction), coupled with a linear transducer array (SL10-2, 2–10 MHz. Super Linear, Vermon, Tours, France)). The SWE procedures were detailed in the previous paper with a similar test protocol [31]. The transducer was placed to align with the muscle fascicles orientation, and to perform minimal pressure during the measurements. To verify data quality, elastography map areas were measured.

2.4. Protocol

Participants visited the Centro de Alto Rendimento Jamor indoors facility, where wind and temperature had no effect on sprint performance and shear modulus assessment, respectively. Lower limb injury history was registered by a physiotherapist. Injury history was obtained through interview and using musculoskeletal injury information recorded in the club's medical department, in which injury diagnosis was based on MRI, ultrasound, or palpation examination. After the interview, both limbs were tested simultaneously for neuromuscular and muscle shear modulus parameters. Then, individuals were asked to perform 10 submaximal KFs at a self-perceived low intensity to prepare and familiarize with the equipment for the maximum voluntary isometric contraction (MVIC) evaluation, which consisted of two 3-s trials with 30 s of recovery between trials. Although expert recommendations suggest five trials to be performed during RTD testing [32], performing only two trials has shown high reliability and concurrent validity [33]. Based on the highest PT on the tested limb, individuals familiarized themselves with the 20% of MVIC through trials using visual feedback. Subsequently, the active shear modulus was then measured twice for each muscle at 20% of MVIC. Each trial lasted ~30 s. After active shear modulus measures, a standardized warm-up protocol for sprinting was performed, composed of 5 min running on a treadmill at 2 m/s and 3 × 30-s sets of low, medium, and high skipping. A previous study (data not published) demonstrated that this warm up protocol did not impact the hamstring active shear modulus pattern. Immediately after the warm-up, a 10 × 30-m repeated sprint task was performed. Then, post-task active shear

modulus measurements were conducted followed by two MVIC trials. The order of the measurements in each muscle was randomized.

2.5. Data Processing

Shear wave elastography data were processed using automated MATLAB routines (The Mathworks Inc., Natick, MA, USA) [29]. For the shear modulus calculation, each clip exported from Aixplorer's software was sequenced in .jpeg images. Image processing converted each pixel of the color map into a value of the Young's modulus based on the recorded color scale. The largest ROI in the elastogram window was determined by avoiding aponeuroses and tissue artifacts (e.g., vessels), and the values were averaged to obtain a representative muscle value. Within each trial, the most stable Young's modulus values over ~20 s in the active condition were averaged and divided by 3 to better represent the muscle shear elastic modulus [34]. The shear modulus of each muscle was considered for analysis. In addition, the BFlh/ST ratio was calculated and interpreted as a load sharing parameter. Neuromuscular parameters, such as PT, 0–50 ms, 50–100 ms, 150–200 ms RTD, and TU-RTDmax, were determined using automated MATLAB routines (The Mathworks Inc., Natick, MA, USA). The onset of force production was defined by visual detection and using a mathematical algorithm (threshold-based method) [35,36]. In brief, after selecting the onset by visual detection, the threshold-based model verified this within specific time frame conditions, identifying the point where the torque value reached three standard deviations above the baseline value. It should be noted that the visual detection merely supports the detection of the onset by the mathematical model, serving as an indication of which approximate time frame to search for the onset, with the model then searching around (before and after) the visually detected onset for a more accurate onset. In case a more accurate onset would be detected by the algorithm in relation to the visual detection, this would then be selected as the final onset.

2.6. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 27.0 (IBM Corporation, Armonk, NY, USA). Normality of the data distribution was confirmed using the Shapiro–Wilk test. Using the pre- and post-task data from both repetitions, the standard error of measurement (SEM) active shear modulus for each muscle was determined and used to interpret whether the effects of the protocols were within this measurement error.

The effect of the sprint task and its interaction with injury was examined by conducting a 2 × 10 mixed randomized groups and repeated-measures ANCOVA [injury × sprint] for the variable average sprint speed, using age as covariate.

For the effect of the sprint task in all previously injured limbs vs. control group on active shear modulus for each muscle, BFlh/ST ratio, and all neuromuscular parameters and their interaction on injury was conducted using a 2 × 2 mixed randomized groups and repeated-measures ANCOVA (injury (injury and control) × instant (pre and post)), with age as covariate. In this comparison, all the contralateral limbs of the previously injured players were excluded, and a randomized selection was performed for the limb selected in the healthy control players. All the ANCOVA assumptions were verified and in general accepted (only some cases of severe outliers were excluded from analysis).

A comparison between the BFlh previously injured limbs and control group (players with the same age without injury) for the active shear modulus, all neuromuscular parameters, BFlh/ST ratio, and their interaction with injury, was worked out using a two-way mixed randomized groups and repeated-measures ANOVA (injury (injury and control) × instant (pre and post)). The limb chosen to perform a pair with the BFlh previously injured limb was random and stratified using the rand function in Excel with only counting the same side (limb).

To compare all the previously injured limbs with their contralateral limb for the active shear modulus, BFlh/ST ratio, and all neuromuscular parameters and their interaction on

injury, a two-way repeated-measures ANOVA (injury (injury and control) × instant (pre and post)) was performed.

For the comparison between the BFlh previously injured limbs with the contralateral limb for the active shear modulus, BFlh/ST ratio, and all neuromuscular parameters and their interaction with injury, a two-way repeated-measures ANOVA (injury (injury and control) × instant (pre and post)) was conducted. Data are presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

3. Results

Of the 210 limbs evaluated, the total of HSIs in the last 2 years were 46 limbs: 37 were without recurrence, seven had one recurrence, and only one had two recurrences. The period between injury and testing was 12.03 ± 6.6 months (range: 2–19 months). The time loss for limbs with only one injury was 24.1 ± 17.1 days and for the limbs with recurrence it was 22.2 ± 15.4 days. Six players (n = 12) who had injuries in both limbs were excluded in all the statistical comparisons since sprint performance, and therefore load sharing patterns, could differ between players with one previously injured limb and both previously injured limbs. Therefore, 34 previously injured limbs were analyzed, with the most affected muscle being the BFlh with 14 injuries, followed by the SM with six, and the ST with three, while 11 injuries could not be attributed to a specific muscle.

Large elastography map areas were obtained (BFlh: 5.5 ± 0.6 cm^2; BFsh: 5.6 ± 0.7 cm^2; SM: 4.7 ± 0.9 cm^2; and ST: 5.7 ± 0.6 cm^2) for the shear modulus. Additionally, the elastogram window filling was very high (BFlh: 98.8 ± 2.5%; BFsh: 98.3 ± 2.6%; SM: 97.1 ± 3.5%; and ST: 99.6 ± 0.9%) for the shear modulus. In pre-task conditions, the shear modulus SEM of each muscle was: BFlh: 4.33 kPa, BFsh: 9.03 kPa, SM: 4.75 kPa, and ST: 5.98 kPa. After the sprint task, the active shear modulus SEM was: BFlh: 6.15 kPa, BFsh: 9.33 kPa, SM: 4.96 kPa, ST: 7.12 kPa.

3.1. Sprint Performance

A significant effect in the average sprint speed was seen for both groups of players (fastest: 7.07 ± 0.33 m/s; slowest: 6.68 ± 0.33 m/s; $p < 0.001$; $\eta^2_p = 0.084$) and for the interaction between sprint and age ($p = 0.031$; $\eta^2_p = 0.031$); however, no significant interaction was found between injury and sprint ($p = 0.509$; $\eta^2_p = 0.008$). A significant effect was found for age ($p = 0.002$; $\eta^2_p = 0.284$); however, no significant differences were seen between players with and without injury history ($p = 0.284$; $\eta^2_p = 0.012$) (Figure 2).

3.2. Shear Modulus and Mechanical Parameters

3.2.1. Previously Injured Players vs. Healthy Control Group

In relation to the comparison of previously injured players vs. healthy control group with age as a covariate (Table 1) in the active shear modulus, the BFlh (pre: 31.73 ± 10.43 kPa; post: 31.99 ± 12.28 kPa; $p = 0.012$; $\eta^2_p = 0.064$) and SM (pre: 35.62 ± 11.18 kPa; post: 35.56 ± 11.39 kPa; $p = 0.050$; $\eta^2_p = 0.040$) showed a significant difference between instants. Regarding neuromuscular parameters, significant differences between instants was only seen for TU-RTDmax (pre: 0.077 ± 0.020 s; post: 0.074 ± 0.020 s; $p = 0.031$; $\eta^2_p = 0.048$) and, respectively, the interaction of instant with age ($p = 0.040$; $\eta^2_p = 0.043$).

3.2.2. BFlh Previously Injured Players vs. Healthy Control Group

The comparison between BFlh previously injured players vs. healthy control group (Table 2), showed significant differences pre vs. post only in the neuromuscular parameters, with a decrease in PT (pre: 135.9 ± 35.6 Nm; post: 124.7 ± 31.5 Nm; $p < 0.001$; $\eta^2_p = 0.447$), and RTD 50–100 ms (pre: 796.9 ± 233.9 Nm/s; post: 730.6 ± 213 Nm/s; $p = 0.002$; $\eta^2_p = 0.306$) observed between instants. Moreover, a significant interaction between injury and instant was seen for RTD 50–100 ms ($p = 0.043$; $\eta^2_p = 0.148$).

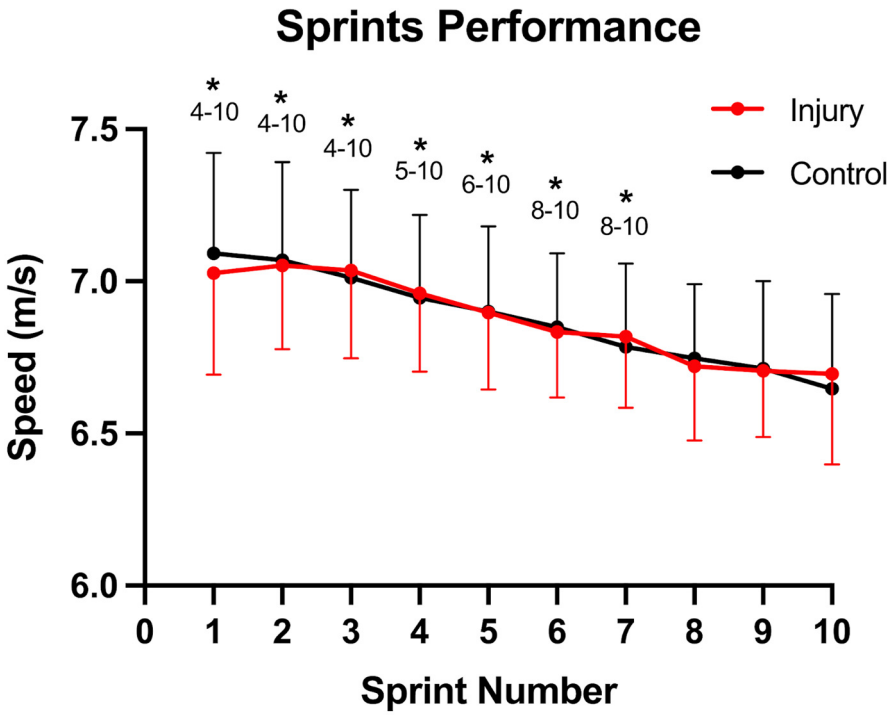

Figure 2. Average sprint speed values in the sprint performance of 10 sets × 30 m in players with no injury history (black) and players with injury history (red). Data are presented as mean ± standard deviation. * A significant difference between the current sprint and each subsequent sprint is indicated ($p < 0.05$).

Table 1. Acute effects of a sprint protocol in limbs with previous hamstring injury history (n = 34) vs. healthy control players (n = 65) using age as a covariate on the active shear modulus and neuromuscular parameters.

		Pre Sprint		Post Sprint		Instant (Pre vs. Post)		Injury (Injured vs. Pair)		Injury × Instant Interaction	
		Injured	Control	Injured	Control	p	η^2_p	p	η^2_p	p	η^2_p
Active (kPa)	**BFlh**	30.94 ± 9.85	32.52 ± 10.01	30.75 ± 11.60	33.24 ± 11.80	0.012	0.064	0.368	0.008	0.574	0.003
	BFsh	48.11 ± 16.61	52.23 ± 16.37	53.11 ± 16.08	54.82 ± 15.83	0.775	0.001	0.354	0.009	0.472	0.005
	SM	35.19 ± 10.84	36.05 ± 10.67	34.82 ± 11.06	36.31 ± 10.89	0.050	0.040	0.605	0.003	0.650	0.002
	ST	61.27 ± 17.33	59.39 ± 17.83	58.45 ± 16.52	58.96 ± 16.30	0.330	0.010	0.841	<0.001	0.330	0.010
Ratio	**BFlh/ST**	0.53 ± 0.27	0.65 ± 0.59	0.55 ± 0.26	0.62 ± 0.34	0.853	<0.001	0.261	0.013	0.421	0.007
Peak Torque (N·m)		139.09 ± 33.45	141.45 ± 25.84	127.27 ± 32.95	130.79 ± 25.16	0.055	0.038	0.660	0.002	0.571	0.003
RTD 0–50 (N·m/s)		514.57 ± 194.54	479.81 ± 193.93	484.47 ± 196.11	448.18 ± 178.15	0.870	<0.001	0.286	0.012	0.885	<0.001
RTD 50–100 (N·m/s)		807.81 ± 222.87	816.87 ± 209.56	730.42 ± 224.07	735.79 ± 197.19	0.259	0.013	0.981	0.001	0.996	<0.001
RTD 150–200 (N·m/s)		343.68 ± 147.67	366.03 ± 122.88	310.92 ± 94.95	344.10 ± 102.96	0.209	0.016	0.230	0.015	0.474	0.005
TU-RTDmax (s)		0.073 ± 0.022	0.080 ± 0.023	0.074 ± 0.023	0.076 ± 0.021	0.031	0.048	0.200	0.017	0.835	<0.001

Abbreviations: BFlh, biceps femoris long head; BFsh, biceps femoris short head; SM, semimembranosus; ST, semitendinosus; BFlh/ST ratio, biceps femoris long head ratio; RTD 0–50, rate torque development between 0–50 ms; RTD 50–100, rate torque development between 50–100 ms; RTD 150–200, rate torque development between 150–200 ms; TU-RTDmax, time until reach the maximum value of rate torque development (ms); p, p value; η^2_p, eta squared. Data are presented as mean ± standard deviation.

3.2.3. Previously Hamstring Injured Limbs vs. Contralateral Limb

With respect to the comparison involving previously hamstring injury limbs vs. the contralateral limb (Table 3), for the active shear modulus, only a significant interaction between sprint instants and limbs with or without injury history was seen for ST ($p = 0.003$; $\eta^2_p = 0.241$). For neuromuscular parameters, significant differences were seen for PT (pre: 138.7 ± 43.6 Nm; post: 126.7 ± 42.0 Nm; $p < 0.001$; $\eta^2_p = 0.548$) and RTD 50–100 ms (pre: 807.8 ± 224.7 Nm/s; post: 725.4 ± 208.1 Nm/s; $p = 0.002$; $\eta^2_p = 0.258$) between instants.

Furthermore, a significant injury and instant interaction was seen for RTD 150–200 ms (p = 0.045; η^2_p = 0.116).

Table 2. Acute effects of a sprint protocol in limbs with previous biceps femoris long head injury history (n = 14) vs. healthy control limbs (n = 14, individuals with the same age without any hamstring injury in the same side limb) on the active shear modulus and neuromuscular parameters values.

		Pre Sprint		Post Sprint		Instant (Pre vs. Post)		Injury (Injured vs. Pair)		Injury × Instant Interaction	
		Injured	Control	Injured	Control	p	η^2_p	p	η^2_p	p	η^2_p
Active (kPa)	**BFlh**	29.41 ± 9.92	29.65 ± 12.65	28.79 ± 11.11	31.80 ± 14.31	0.646	0.008	0.706	0.006	0.409	0.026
	BFsh	47.79 ± 10.03	53.71 ± 18.81	57.32 ± 16.90	54.91 ± 19.47	0.085	0.118	0.766	0.004	0.177	0.075
	SM	33.70 ± 9.52	35.01 ± 7.26	33.83 ± 9.17	37.63 ± 10.91	0.259	0.053	0.461	0.023	0.310	0.043
	ST	61.69 ± 18.18	56.52 ± 19.57	61.01 ± 17.21	54.73 ± 14.03	0.512	0.017	0.262	0.096	0.768	0.003
Ratio	**BFlh/ST**	0.50 ± 0.18	0.59 ± 0.33	0.48 ± 0.16	0.59 ± 0.26	0.817	0.002	0.223	0.056	0.870	0.001
Peak Torque (N·m)		139.35 ± 44.69	132.46 ± 23.29	131.12 ± 38.15	118.19 ± 22.91	<0.001	0.447	0.434	0.024	0.230	0.055
RTD 0–50 (N·m/s)		480.64 ± 235.17	496.54 ± 164.22	513.21 ± 240.58	449.06 ± 161.89	0.727	0.005	0.747	0.004	0.069	0.121
RTD 50–100 (N·m/s)		770.31 ± 268.89	823.65 ± 192.56	745.66 ± 245.47	715.54 ± 174.65	0.002	0.306	0.889	0.001	0.043	0.148
RTD 150–200 (N·m/s)		358.05 ± 150.08	304.66 ± 130.72	319.07 ± 101.33	286.72 ± 101.33	0.119	0.091	0.331	0.036	0.556	0.014
TU-RTDmax (s)		0.078 ± 0.022	0.073 ± 0.015	0.072 ± 0.021	0.070 ± 0.013	0.068	0.122	0.503	0.017	0.503	0.017

Abbreviations: BFlh, biceps femoris long head; BFsh, biceps femoris short head; SM, semimembranosus; ST, semitendinosus; BFlh/ST ratio, biceps femoris long head ratio; RTD 0–50, rate torque development between 0–50 ms; RTD 50–100, rate torque development between 50–100 ms; RTD 150–200, rate torque development between 150–200 ms; TU-RTDmax, time until reach the maximum value of rate torque development (ms); p, p value; η^2_p, eta squared. Data are presented as mean ± standard deviation.

Table 3. Acute effects of a sprint protocol in limbs with hamstring injury history (n = 34) vs. healthy contralateral limbs without injury history (n = 34) on active shear modulus and neuromuscular parameters values.

		Pre Sprint		Post Sprint		Instant (Pre vs. Post)		Injury (Injured vs. Pair)		Injury × Instant Interaction	
		Injured	Contralateral	Injured	Contralateral	p	η^2_p	p	η^2_p	p	η^2_p
Active (kPa)	**BFlh**	30.22 ± 10.32	29.80 ± 11.88	30.84 ± 12.91	29.56 ± 12.63	0.891	0.001	0.618	0.008	0.636	0.007
	BFsh	48.57 ± 16.57	50.64 ± 16.54	54.25 ± 15.31	49.90 ± 15.85	0.169	0.060	0.672	0.006	0.091	0.089
	SM	34.82 ± 9.48	35.62 ± 11.63	35.06 ± 9.38	35.39 ± 10.71	0.997	<0.001	0.760	0.003	0.775	0.003
	ST	61.84 ± 19.21	57.03 ± 15.85	58.28 ± 16.24	61.00 ± 16.14	0.910	<0.001	0.683	0.005	0.003	0.241
Ratio	**BFlh/ST**	0.54 ± 0.27	0.57 ± 0.23	0.56 ± 0.26	0.51 ± 0.21	0.578	0.010	0.848	0.001	0.146	0.065
Peak Torque (N·m)		139.09 ± 33.45	138.27 ± 29.38	127.27 ± 32.95	126.21 ± 28.19	<0.001	0.548	0.669	0.006	0.904	<0.001
RTD 0–50 (N·m/s)		514.57 ± 194.54	496.34 ± 220.05	484.47 ± 196.11	443.38 ± 172.61	0.085	0.087	0.086	0.087	0.205	0.048
RTD 50–100 (N·m/s)		807.81 ± 222.87	807.79 ± 243.06	730.42 ± 224.07	720.38 ± 206.99	0.002	0.258	0.786	0.002	0.578	0.009
RTD 150–200 (N·m/s)		343.68 ± 147.67	334.93 ± 119.66	310.92 ± 94.95	333.80 ± 102.60	0.216	0.038	0.593	0.009	0.045	0.116
TU-RTDmax (s)		0.073 ± 0.022	0.076 ± 0.022	0.74 ± 0.023	0.076 ± 0.020	0.978	<0.001	0.132	0.067	0.993s	<0.001

Abbreviations: BFlh, biceps femoris long head; BFsh, biceps femoris short head; SM, semimembranosus; ST, semitendinosus; BFlh/ST ratio, biceps femoris long head ratio; RTD 0–50, rate torque development between 0–50 ms; RTD 50–100, rate torque development between 50–100 ms; RTD 150–200, rate torque development between 150–200 ms; TU-RTDmax, time until reach the maximum value of rate torque development (ms); p, p value; η^2_p, eta squared. Data are presented as mean ± standard deviation.

3.2.4. BFlh Previously Injured Limbs vs. Contralateral Limb

Concerning the comparison involving BFlh previously injured limbs vs. the contralateral limb (Table 4), in the active shear modulus, the BFsh (pre: 49.44 ± 12.34 kPa; post: 55.19 ± 15.60 kPa; p = 0.034; η^2_p = 0.348) showed a significant difference between instants, while the ST demonstrated an injury and instant interaction (p = 0.036; η^2_p = 0.318). Finally, for neuromuscular parameters, a statistical difference was observed between instants for PT (pre: 138.5 ± 41.4 Nm; post: 130.8 ± 36.5 Nm; p = 0.010; η^2_p = 0.415) and TU-RTDmax (pre: 0.083 ± 0.022 s; post: 0.072 ± 0.019 s; p = 0.004; η^2_p = 0.477). RTD 0–50 ms also presented a significant difference between limbs with BFlh injury history and their contralateral with no injury history (previously injured: 496.93 ± 234.3 Nm/s; contralateral: 422.7 ± 208.3 Nm/s; p = 0.005; η^2_p = 0.465).

Table 4. Acute effects of a sprint protocol in limbs with biceps femoris long head injury history (n = 14) vs. healthy contralateral limbs (n = 14) on the active shear modulus and neuromuscular parameters.

		Pre Sprint		Post Sprint		Instant (Pre vs. Post)		Injury (Injured vs. Pair)		Injury × Instant Interaction	
		Injured	Contralateral	Injured	Contralateral	*p*	η^2_p	*p*	η^2_p	*p*	η^2_p
Active (kPa)	**BFlh**	29.41 ± 9.92	30.56 ± 14.16	28.79 ± 11.11	29.02 ± 16.47	0.628	0.019	0.798	0.005	0.592	0.023
	BFsh	47.32 ± 10.33	51.55 ± 18.05	58.84 ± 16.71	51.55 ± 18.38	0.034	0.348	0.684	0.016	0.068	0.272
	SM	32.82 ± 9.37	35.86 ± 12.21	33.07 ± 9.14	36.36 ± 11.93	0.635	0.021	0.251	0.118	0.920	0.001
	ST	61.52 ± 18.91	60.53 ± 13.41	60.67 ± 17.87	66.72 ± 14.24	0.120	0.189	0.636	0.019	0.036	0.318
Ratio	**BFlh/ST**	0.51 ± 0.18	0.54 ± 0.20	0.49 ± 0.17	0.46 ± 0.20	0.329	0.079	0.922	0.001	0.160	0.157
Peak Torque (N·m)		139.35 ± 44.69	137.63 ± 38.93	131.18 ± 38.15	130.39 ± 36.42	0.010	0.415	0.739	0.009	0.679	0.014
RTD 0–50 (N·m/s)		480.64 ± 235.17	394.91 ± 232.23	513.21 ± 240.57	450.53 ± 194.45	0.065	0.238	0.005	0.465	0.306	0.080
RTD 50–100 (N·m/s)		770.31 ± 268.89	725.57 ± 288.79	745.66 ± 245.47	724.34 ± 244.45	0.617	0.020	0.221	0.113	0.326	0.074
RTD 150–200 (N·m/s)		358.05 ± 150.08	373.25 ± 108.94	319.07 ± 101.33	342.59 ± 107.17	0.136	0.163	0.371	0.062	0.720	0.010
TU-RTDmax (s)		0.078 ± 0.022	0.088 ± 0.023	0.072 ± 0.021	0.073 ± 0.017	0.004	0.477	0.169	0.140	0.113	0.182

Abbreviations: BFlh, biceps femoris long head; BFsh, biceps femoris short head; SM, semimembranosus; ST, semitendinosus; BFlh/ST ratio, biceps femoris long head ratio; RTD 0–50, rate torque development between 0–50 ms; RTD 50–100, rate torque development between 50–100 ms; RTD 150–200, rate torque development between 150–200 ms; TU-RTDmax, time until reach the maximum value of rate torque development (ms); *p*, *p* value; η^2_p, eta squared. Data are presented as mean ± standard deviation.

4. Discussion

This study examined the active (i.e., at 20% of MVIC) shear modulus as well as neuromuscular parameters in hamstrings before and after repeated sprinting in professional football players with and without HSI. To the best of our knowledge, this is the first study to examine the acute effects of repeated sprints in the hamstring load sharing on the active shear modulus using SWE in football players with and without previous HSI. The main findings were as follows: no differences in the sprint performance, load sharing, and most neuromuscular parameters were seen between limbs with previous HSI and without previous HSI. Secondly, we verified with different analyses that no differences in load sharing and neuromuscular parameters were seen between BFlh previously injured players and healthy control group (same-side) with the same age and dominance. In addition, no differences in load sharing and neuromuscular parameters were seen between previously injured hamstring limbs and the contralateral limbs. Finally, only a significant difference in RTD 0–50 ms was seen between BFlh previously injured limbs and their contralateral limb. These findings contradict our initial hypothesis since it was expected that limbs with HSI history would have a different load sharing pattern shear modulus with a greater relative contribution of BFlh.

Contrary to our initial hypothesis, no differences in sprint performance were seen between players with previous HSI and healthy ones, which is also opposite to what some previous studies have reported. Mendiguchia et al. (2014) reported a decrease in acceleration performance at return to play [27], with Røksund et al. (2017) showing a 16% higher drop in speed during repeated sprint tests [28] in players with previous HSI. It is curious that Røksund et al. (2017) used an 8 × 20 m sprint with the same recovery time (30 s), and since the 16% decrease was calculated between the mean of the first two sprints minus the mean of the last two sprints, it was expected that a higher volume in the present study (10 × 30 m) would induce greater fatigue between the previously injured vs. healthy control players. One possible explanation could be the time difference between injury and testing, which is not possible to compare since Røksund et al. (2017) did not report the time of this period, which is essential for the comparison. Mendiguchia et al. (2014) showed a decrease in acceleration performance at return to play between players with previous HSI and healthy ones. However, it was normalized after two months of follow-up without any specific supplementary or preventive training, indicating that regular football play is sufficient to restore sprint acceleration performance, which is in concordance with our results. In our study, all the players have at least 2 months between sustaining the hamstring injury and performing the RSA protocol. Therefore, it is possible to suggest that even without recovery treatment, a greater time of exposure to load positively im-

pacted the sprint performance in players with previous his, which led to a non-significant difference between groups. Moreover, other previous studies reported no differences in the sprint performance between healthy controls players and players who had suffered from any lower-limb previous injury in either limb [37]. In addition, previous HSI has been shown to have no association with the change in the sprint performance throughout the off-season [38]. Therefore, all these studies support that evaluating players after 2–3 months of return to play after a HSI showed no differences between previously injured vs. healthy control players. Neuromuscular fatigue has been suggested to be a potential risk factor for muscle strain injury [2,9,39,40], indicating that insufficient strength exercise could represent a risk factor to sustain a new HSI, or that insufficient rehabilitation after an HSI may increase the risk of re-injury. Contrary to our initial hypothesis, no significant differences were seen in all of the neuromuscular parameters when comparing the previously injured vs. healthy controls players, and between all HSI and contralateral limbs. Only a significant difference was seen in RTD 0–50 ms in the comparison of previously injured limbs on BFlh vs. contralateral limbs. However, greater values were observed for football players with previous injury, which was in contrast to previous studies. Indeed, the current literature [41–44] demonstrates an inability of those with prior unilateral hamstring strain injury to completely activate the involved limb, despite full voluntary exertion. Furthermore, it has been suggested that a failure to fully voluntarily activate the previously injured muscle may result in a limited stimulus for eccentric strength gain and fascicle lengthening during rehabilitation [45]. Therefore, the greater values on the RTD 0–50 ms in the present study, in football players with previously injured limb on BFlh vs. the contralateral limbs, could be possibly explained by a specific rehabilitation protocol especially for neural factors. Moreover, the difference between studies should be considered, especially the different times of screening, since in our case, this ranged between 2–19 months whereas previous studies ranged between 4 weeks-12 months [43], 1–18.2 months [41], and 2–18 months [42,44]. Moreover, these differ studies in sample composition, with previous studies analyzing recreationally active males [41–44] and the present study assessing professional football players. Furthermore, it should be noted that there are no differences in the rate of torque development and onset of muscle activity in football players with and without HSI in a prospective study, suggesting no association with injury risk [46].

In respect to muscle stiffness and tissue properties, Watsford et al. (2010) showed a significantly greater leg and hamstring musculotendinous stiffness, in the pre-season in Australian Rugby players after a lateral hopping test, in players that then sustained an HSI during the season, suggesting that those who recorded relatively high bilateral hamstring musculotendinous stiffness or leg stiffness values may have a higher risk of sustaining a noncontact, soft tissue hamstring injury during the season [47]. Kawai et al. (2021) reported an increase in the passive stiffness in football players' limbs with previous BF injury when compared to the healthy limbs using MyotonPRO® and the vibration disappearance threshold [48]. Our results contradict this finding since no differences were seen; however, it must be considered the different states of measurements (active vs. passive) and that SWE measurements provide a proxy of localized tissue properties [34] rather than a global measure of joint stiffness or the possible contribution of other muscles [48]. It should be noted that stiffness is a functional property and shear modulus is a tissue property. Therefore, these comparisons need to be considered with caution since two materials can have the same shear modulus but different stiffness. Indeed, a previous study reported only a moderate correlation between SWE and MyotonPRO® in the gastrocnemius muscle and Achilles tendon [49].

In contrast to previous results by Schuermans et al. (2016, 2014), who quantified the T2 relaxometry during dynamic KFs and reported that players with a history of HSI had a decrease in the ST and an increase in the BF load during dynamic KFs [20,21], and to Mendes et al. (2020), who showed a decrease in the active ST shear modulus with a lack of changes in the BFlh active shear modulus during an isometric knee contraction until

failure, which led to an increase of BFlh/ST active shear modulus ratio, and consequently a higher BFlh and lower ST contribution in the fatigue task [29], our results showed no differences in the load sharing pattern in the active state. One of the possible explanations between the present study and previous studies [20,21,29] could be the specificity of the task. Schuerman's studies used dynamic KFs until exhaustion and Mendes used isometric contraction until exhaustion in healthy participants. Since repeated sprints involved not only mechanical factors but also cardiometabolic factors, the perception of fatigue could lead to different muscle fatigue states. Moreover, it was shown, in professional football players using the same protocol as Mendes et al. (2020), that no differences between limbs over the course of the fatigue task for the BFlh, ST, and BFlh/ST active shear modulus ratio, with similar knee flexor endurance between limbs, only reporting an inhibition of the BFlh shear modulus in previously injured players at the start of the task [23]. The results of the latter study are partly in concordance with ours, since no differences were found for the ST nor the BFlh muscle in both conditions (pre-post). Moreover, Freitas et al. (2021) reported a lower BFlh/ST ratio in all of these comparisons at the start of the fatigue task in limbs with previous injury, with the speculation of whether this parameter could be associated with the occurrence of his, as in a previous study [23]. The hypothesis is that players at higher risk present a lower BFlh/ST ratio mainly due to a decreased BFlh active shear modulus. However, it should be noted that Schuermans et al. (2016, 2014) reported that players with a history of HSI had a decrease in the ST and an increase in the BF load (measuring T2 relaxation), and no differences were seen in all the instants (pre-post) in the present study.

This study has some limitations. Firstly, previously injured players were chosen with an his specifically in the previous 2 years, during which the load sharing pattern and neuromuscular parameters could have sufficient time to be restored. Indeed, since players were subject to a rehabilitation process in the club, it is possible that this process could lead to no significant differences between the contralateral limb and healthy controls. However, due to the demand of repeated sprints, this type of protocol can be only performed when the player can return to play after the rehabilitation program and, therefore, could not be possible to detect differences; moreover, the aim of the present study was to determine whether the load sharing pattern differs between previously injured and healthy football players in the same condition (i.e., ability to play), as well as the necessity of having a robust sample size. Secondly, it must be considered that the present study examined the hamstring load sharing pattern in isometric contractions after repeated sprints, whereas the magnitude of the effects would be greater during the sprints themselves as eccentric contractions are more demanding. It should be considered that load sharing can be influenced by different contraction dynamics, as shown by changes in load sharing between the soleus and gastrocnemius in a classical animal study [50]. Finally, future researchers should attempt these measurements in eccentric contractions since, to this date, this methodology does not allow such measurements due to a low sampling rate. The time between injury and screening should be minimized to detect differences between groups (if possible); however, due to the demand of repeated sprints, this type of protocol can be only performed when the player can return to play and, therefore, could not detect the differences due to the rehabilitation process. To overcome such limitations, we encourage further studies to perform other approaches, such as a prospective study, to verify whether the hamstring shear modulus pattern can distinguish between previously injured players or limbs against healthy controls.

5. Conclusions

The present study provides evidence for no differences regarding sprint performance, hamstring load sharing pattern, and neuromuscular parameters between players with previous HSI in the last 2 years and healthy controls players, although an increase in the early rate of torque development was seen in previously injured BFlh limbs compared to their healthy counterpart. Therefore, the results possibly suggest that the duration between injury and screening could recover the differences between previously injured vs. healthy

control and previously injured vs. contralateral groups. In light of the limitations of the present research, future studies should minimize the duration between the injury and screening, which could be challenging since players have to be exposed to the fatigue task. Moreover, studies should include follow-up analyses to determine whether the mechanical load sharing pattern differs between previously injured players and healthy controls, indicating a possible predictive tool.

Author Contributions: R.P. contributed to the conception and design of the study, collected, and processed all the data, performed the statistical analysis and interpretation of the data, produced the first draft of the manuscript, reviewed the article critically for important intellectual content and edited subsequent versions of the manuscript. T.L. contributed to the data collection and process, and manuscript editing. P.B. contributed to the statistical analysis and interpretation of the results. A.V. participated in the interpretation of the data, editing, and reviewing of the manuscript for critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by CIPER–Centro Interdisciplinar para o Estudo da Performance Humana (unit 447) which is also supported by Fundação para a Ciência e Tecnologia (grant: UID/DTP/ UI447/2019). RP is funded by a scholarship from the University of Lisbon (number 793).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Faculty of Human Kinetics at University of Lisbon (#5/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the clubs and players for their participation, to Sport Lisboa e Benfica for lending one Aixplorer unit and to the Centro de Alto Rendimento Jamor for providing the sports facilities. Moreover, the authors also thank Márcio Goethel for providing the MATLAB code for the calculation of the rate of torque development, and Mauricio Cerda for the MATLAB code for the calculation of shear modulus values. This work is supported by CIPER–Centro Interdisciplinar para o Estudo da Performance Humana (unit 447) which is also supported by Fundação para a Ciência e Tecnologia (grant: UID/DTP/ UI447/2019). RP is funded by a scholarship from the University of Lisbon (number 793).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hägglund, M.; Waldén, M.; Ekstrand, J. Risk Factors for Lower Extremity Muscle Injury in Professional Soccer: The UEFA Injury Study. *Am. J. Sports Med.* **2013**, *41*, 327–335. [CrossRef]
2. Woods, C.; Hawkins, R.D.; Maltby, S.; Hulse, M.; Thomas, A.; Hodson, A.; Football Association Medical Research Programme. The Football Association Medical Research Programme: An Audit of Injuries in Professional Football—Analysis of Hamstring Injuries. *Br. J. Sport. Med.* **2004**, *38*, 36–41. [CrossRef]
3. Heiderscheit, B.C.; Sherry, M.A.; Silder, A.; Chumanov, E.S.; Thelen, D.G. Hamstring Strain Injuries: Recommendations for Diagnosis, Rehabilitation, and Injury Prevention. *J. Orthop. Sports Phys. Ther.* **2010**, *40*, 67–81. [CrossRef]
4. Ekstrand, J.; Waldén, M.; Hägglund, M. Hamstring Injuries Have Increased by 4% Annually in Men's Professional Football, since 2001: A 13-Year Longitudinal Analysis of the UEFA Elite Club Injury Study. *Br. J. Sport. Med.* **2016**, *50*, 731–737. [CrossRef]
5. Silder, A.; Thelen, D.G.; Heiderscheit, B.C. Effects of Prior Hamstring Strain Injury on Strength, Flexibility, and Running Mechanics. *Clin. Biomech.* **2010**, *25*, 681–686. [CrossRef] [PubMed]
6. Morrey, B.F. Acute First-Time Hamstring Strains During High-Speed Running: A Longitudinal Study Including Clinical and Magnetic Resonance Imaging Findings. *Yearb. Orthop.* **2008**, *2008*, 155–156. [CrossRef]
7. Ekstrand, J.; Bengtsson, H.; Waldén, M.; Davison, M.; Khan, K.M.; 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. *Br. J. Sport. Med.* **2023**, *57*, 292–298. [CrossRef] [PubMed]
8. Ekstrand, J. Keeping Your Top Players on the Pitch: The Key to Football Medicine at a Professional Level. *Br. J. Sport. Med.* **2013**, *47*, 723–724. [CrossRef]
9. Ekstrand, J.; Hägglund, M.; Waldén, M. Epidemiology of Muscle Injuries in Professional Football (Soccer). *Am. J. Sport. Med.* **2011**, *39*, 1226–1232. [CrossRef]

10. van Beijsterveldt, A.M.C.; van de Port, I.G.L.; Vereijken, A.J.; Backx, F.J.G. Risk Factors for Hamstring Injuries in Male Soccer Players: A Systematic Review of Prospective Studies. *Scand. J. Med. Sci. Sport.* **2013**, *23*, 253–262. [CrossRef]
11. Freckleton, G.; Pizzari, T. Risk Factors for Hamstring Muscle Strain Injury in Sport: A Systematic Review and Meta-Analysis. *Br. J. Sport. Med.* **2013**, *47*, 351–358. [CrossRef]
12. Green, B.; Bourne, M.N.; 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. *Br. J. Sport. Med.* **2020**, *54*, 1081–1088. [CrossRef]
13. Orchard, J.W. Intrinsic and Extrinsic Risk Factors for Muscle Strains in Australian Football. *Am. J. Sport. Med.* **2001**, *29*, 300–303. [CrossRef]
14. Gabbe, B.J.; Finch, C.F.; Bennell, K.L.; Wajswelner, H. Risk Factors for Hamstring Injuries in Community Level Australian Football. *Br. J. Sport. Med.* **2005**, *39*, 106–110. [CrossRef]
15. Kirkendall, D.T.; Garrett, W.E., Jr. The Effects of Aging and Training on Skeletal Muscle. *Am. J. Sport. Med.* **1998**, *26*, 598–602. [CrossRef] [PubMed]
16. Bangsbo, J.; Iaia, F.M.; Krustrup, P. Metabolic Response and Fatigue in Soccer. *Int. J. Sport. Physiol. Perform.* **2007**, *2*, 111–127. [CrossRef]
17. Mohr, M.; Krustrup, P.; Bangsbo, J. Match Performance of High-Standard Soccer Players with Special Reference to Development of Fatigue. *J. Sport. Sci.* **2003**, *21*, 519–528. [CrossRef]
18. Rampinini, E.; Impellizzeri, F.M.; Castagna, C.; Coutts, A.J.; Wisløff, U. Technical Performance during Soccer Matches of the Italian Serie A League: Effect of Fatigue and Competitive Level. *J. Sci. Med. Sport* **2009**, *12*, 227–233. [CrossRef] [PubMed]
19. Stutzig, N.; Siebert, T. Muscle Force Compensation among Synergistic Muscles after Fatigue of a Single Muscle. *Hum. Mov. Sci.* **2015**, *42*, 273–287. [CrossRef] [PubMed]
20. Schuermans, J.; Van Tiggelen, D.; Danneels, L.; Witvrouw, E. Biceps Femoris and Semitendinosus—Teammates or Competitors? New Insights into Hamstring Injury Mechanisms in Male Football Players: A Muscle Functional MRI Study. *Br. J. Sport. Med.* **2014**, *48*, 1599–1606. [CrossRef] [PubMed]
21. Schuermans, J.; Van Tiggelen, D.; Danneels, L.; Witvrouw, E. Susceptibility to Hamstring Injuries in Soccer: A Prospective Study Using Muscle Functional Magnetic Resonance Imaging. *Am. J. Sport. Med.* **2016**, *44*, 1276–1285. [CrossRef] [PubMed]
22. Bouillard, K.; Hug, F.; Guével, A.; Nordez, A. Shear Elastic Modulus Can Be Used to Estimate an Index of Individual Muscle Force during a Submaximal Isometric Fatiguing Contraction. *J. Appl. Physiol.* **2012**, *113*, 1353–1361. [CrossRef] [PubMed]
23. Freitas, S.R.; Mendes, B.; Firmino, T.; Correia, J.P.; Witvrouw, E.E.; Raúl Oliveira, E.; Vaz, J.R. Semitendinosus and Biceps Femoris Long Head Active Stiffness Response until Failure in Professional Footballers with vs. without Previous Hamstring Injury. *Eur. J. Sport Sci.* **2022**, *22*, 1132–1140. [CrossRef]
24. Brooks, J.H.M.; Fuller, C.W.; Kemp, S.P.T.; Reddin, D.B. Incidence, Risk, and Prevention of Hamstring Muscle Injuries in Professional Rugby Union. *Am. J. Sport. Med.* **2006**, *34*, 1297–1306. [CrossRef]
25. Koulouris, G.; Connell, D.A.; Brukner, P.; Schneider-Kolsky, M. Magnetic Resonance Imaging Parameters for Assessing Risk of Recurrent Hamstring Injuries in Elite Athletes. *Am. J. Sport. Med.* **2007**, *35*, 1500–1506. [CrossRef] [PubMed]
26. Crema, M.D.; Guermazi, A.; Tol, J.L.; Niu, J.; Hamilton, B.; Roemer, F.W. Acute Hamstring Injury in Football Players: Association between Anatomical Location and Extent of Injury-A Large Single-Center MRI Report. *J. Sci. Med. Sport* **2016**, *19*, 317–322. [CrossRef]
27. Mendiguchia, J.; Samozino, P.; Martinez-Ruiz, E.; Brughelli, M.; Schmikli, S.; Morin, J.-B.; Mendez-Villanueva, A. Progression of Mechanical Properties during on-Field Sprint Running after Returning to Sports from a Hamstring Muscle Injury in Soccer Players. *Int. J. Sport. Med.* **2014**, *35*, 690–695. [CrossRef]
28. Røksund, O.D.; Kristoffersen, M.; Bogen, B.E.; Wisnes, A.; Engeseth, M.S.; Nilsen, A.-K.; Iversen, V.V.; Mæland, S.; Gundersen, H. Higher Drop in Speed during a Repeated Sprint Test in Soccer Players Reporting Former Hamstring Strain Injury. *Front. Physiol.* **2017**, *8*, 25. [CrossRef] [PubMed]
29. Mendes, B.; Firmino, T.; Oliveira, R.; Neto, T.; Cruz-Montecinos, C.; Cerda, M.; Correia, J.P.; Vaz, J.R.; Freitas, S.R. Effects of Knee Flexor Submaximal Isometric Contraction until Exhaustion on Semitendinosus and Biceps Femoris Long Head Shear Modulus in Healthy Individuals. *Sci. Rep.* **2020**, *10*, 16433. [CrossRef]
30. Silder, A.; Whittington, B.; Heiderscheit, B.; Thelen, D.G. Identification of Passive Elastic Joint Moment-Angle Relationships in the Lower Extremity. *J. Biomech.* **2007**, *40*, 2628–2635. [CrossRef]
31. Mendes, B.; Firmino, T.; Oliveira, R.; Neto, T.; Infante, J.; Vaz, J.R.; Freitas, S.R. Hamstring Stiffness Pattern during Contraction in Healthy Individuals: Analysis by Ultrasound-Based Shear Wave Elastography. *Eur. J. Appl. Physiol.* **2018**, *118*, 2403–2415. [CrossRef] [PubMed]
32. Maffiuletti, N.A.; Aagaard, P.; Blazevich, A.J.; Folland, J.; Tillin, N.; Duchateau, J. Rate of Force Development: Physiological and Methodological Considerations. *Eur. J. Appl. Physiol.* **2016**, *116*, 1091–1116. [CrossRef] [PubMed]
33. Mentiplay, B.F.; Perraton, L.G.; Bower, K.J.; Adair, B.; Pua, Y.-H.; Williams, G.P.; McGaw, R.; Clark, R.A. Assessment of Lower Limb Muscle Strength and Power Using Hand-Held and Fixed Dynamometry: A Reliability and Validity Study. *PLoS ONE* **2015**, *10*, e0140822. [CrossRef] [PubMed]
34. Bercoff, J.; Tanter, M.; Fink, M. Supersonic Shear Imaging: A New Technique for Soft Tissue Elasticity Mapping. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **2004**, *51*, 396–409. [CrossRef]

35. Li, X.; Zhou, P.; Aruin, A.S. Teager–Kaiser Energy Operation of Surface EMG Improves Muscle Activity Onset Detection. *Ann. Biomed. Eng.* **2007**, *35*, 1532–1538. [CrossRef]
36. Solnik, S.; Rider, P.; Steinweg, K.; DeVita, P.; Hortobágyi, T. Teager–Kaiser Energy Operator Signal Conditioning Improves EMG Onset Detection. *Eur. J. Appl. Physiol.* **2010**, *110*, 489–498. [CrossRef] [PubMed]
37. Padulo, J.; Attene, G.; Ardigò, L.P.; Bragazzi, N.L.; Maffulli, N.; Zagatto, A.M.; Dello Iacono, A. Can a Repeated Sprint Ability Test Help Clear a Previously Injured Soccer Player for Fully Functional Return to Activity? A Pilot Study. *Clin. J. Sport Med.* **2017**, *27*, 361–368. [CrossRef]
38. Vicens-Bordas, J.; Esteve, E.; Fort-Vanmeerhaeghe, A.; Casals, M.; Bandholm, T.; Ishøi, L.; Opar, D.; Shield, A.; Thorborg, K. Performance Changes during the off-Season Period in Football Players—Effects of Age and Previous Hamstring Injury. *J. Sport. Sci.* **2020**, *38*, 2489–2499. [CrossRef]
39. Heiser, T.M.; Weber, J.; Sullivan, G.; Clare, P.; Jacobs, R.R. Prophylaxis and Management of Hamstring Muscle Injuries in Intercollegiate Football Players. *Am. J. Sport. Med.* **1984**, *12*, 368–370. [CrossRef]
40. Tw, W.; Worrell, T.W.; Perrin, D.H.; Gansneder, B.M.; Gieck, J.H. Comparison of Isokinetic Strength and Flexibility Measures between Hamstring Injured and Noninjured Athletes. *Clin. J. Sport Med.* **1991**, *1*, 213. [CrossRef]
41. Opar, D.A.; Williams, M.D.; Timmins, R.G.; Dear, N.M.; Shield, A.J. Rate of Torque and Electromyographic Development during Anticipated Eccentric Contraction Is Lower in Previously Strained Hamstrings. *Am. J. Sport. Med.* **2013**, *41*, 116–125. [CrossRef] [PubMed]
42. Opar, D.A.; Williams, M.D.; Timmins, R.G.; Dear, N.M.; Shield, A.J. Knee Flexor Strength and Bicep Femoris Electromyographical Activity Is Lower in Previously Strained Hamstrings. *J. Electromyogr. Kinesiol.* **2013**, *23*, 696–703. [CrossRef] [PubMed]
43. Sole, G.; Milosavljevic, S.; Nicholson, H.D.; Sullivan, S.J. Selective Strength Loss and Decreased Muscle Activity in Hamstring Injury. *J. Orthop. Sport. Phys. Ther.* **2011**, *41*, 354–363. [CrossRef]
44. Buhmann, R.; Trajano, G.S.; Kerr, G.; Shield, A. Voluntary Activation and Reflex Responses after Hamstring Strain Injury. *Med. Sci. Sport. Exerc.* **2020**, *52*, 1862–1869. [CrossRef] [PubMed]
45. Fyfe, J.J.; Opar, D.A.; Williams, M.D.; Shield, A.J. The Role of Neuromuscular Inhibition in Hamstring Strain Injury Recurrence. *J. Electromyogr. Kinesiol.* **2013**, *23*, 523–530. [CrossRef]
46. van Dyk, N.; Bahr, R.; Burnett, A.F.; Verhagen, E.; von Tiggelen, D.; Witvrouw, E. No Association between Rate of Torque Development and Onset of Muscle Activity with Increased Risk of Hamstring Injury in Elite Football. *Scand. J. Med. Sci. Sport.* **2018**, *28*, 2153–2163. [CrossRef]
47. Watsford, M.L.; Murphy, A.J.; McLachlan, K.A.; Bryant, A.L.; Cameron, M.L.; Crossley, K.M.; Makdissi, M. A Prospective Study of the Relationship between Lower Body Stiffness and Hamstring Injury in Professional Australian Rules Footballers. *Am. J. Sport. Med.* **2010**, *38*, 2058–2064. [CrossRef]
48. Kawai, T.; Takamoto, K.; Bito, I. Previous Hamstring Muscle Strain Injury Alters Passive Tissue Stiffness and Vibration Sense. *J. Bodyw. Mov. Ther.* **2021**, *27*, 573–578. [CrossRef]
49. Feng, Y.N.; Li, Y.P.; Liu, C.L.; Zhang, Z.J. Assessing the Elastic Properties of Skeletal Muscle and Tendon Using Shearwave Ultrasound Elastography and MyotonPRO. *Sci. Rep.* **2018**, *8*, 17064. [CrossRef]
50. Prilutsky, B.I.; Herzog, W.; Allinger, T.L. Force-Sharing between Cat Soleus and Gastrocnemius Muscles during Walking: Explanations Based on Electrical Activity, Properties, and Kinematics. *J. Biomech.* **1994**, *27*, 1223–1235. [CrossRef]