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Hamstrings on focus: Are 72 hours sufficient for recovery after a football (soccer) match? A multidisciplinary approach based on hamstring injury risk factors and histology

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ABSTRACT

This study aimed to assess acute and residual changes in sprint-related hamstring injury (HSI) risk factors after a football (soccer) match, focusing on recovery within the commonly observed 72-h timeframe between elite football matches. We used a multifactorial approach within a football context, incorporating optical and ultrastructural microscopic analysis of BFlh (biceps femoris long head) muscle fibres, along with an examination of BFlh fibre composition. Changes in sprint performance-related factors and HSI modifiable risk factors were examined until 3 days after the match (MD $_{+3}$) in 20 football players. BFlh biopsy specimens were obtained before and at MD $_{+3}$ in 10 players. The findings indicated that at MD $_{+3}$, sprint-related performance and HSI risk factors had not fully recovered, with notable increases in localized BFlh fibre disruptions. Interestingly, match load (both external and internal) did not correlate with changes in sprint performance or HSI risk factors nor with BFlh fibre disruption. Furthermore, our study revealed a balanced distribution of ATPase-based fibre types in BFlh, with type-II fibres associated with sprint performance. Overall, the results suggest that a 72-h recovery period may not be adequate for hamstring muscles in terms of both HSI risk factors and BFlh fibre structure following a football match.

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Hamstring injury risk factors; pelvic tilt; sprint kinetics; biceps femoris; biopsy

Introduction

Acute hamstring muscle injuries increase year by year, representing 24% of the total injuries in modern football (soccer) (Ekstrand et al., 2023), where congested fixture periods associated with a higher rate of muscle injuries are an increasingly common scenario (Bengtsson et al., 2013; Carling et al., 2016; Dupont et al., 2010). Specifically, tight schedules with just 72 h between matches are becoming more prevalent in elite football (Julian et al., 2021). Although different studies have considered the acute effects produced by a football match in a generic way (mainly a physiological perspective) (Silva et al., 2018), there is no study to date that offers a detailed analysis of the response and evolution of the different risk factors associated with the injury of greater prevalence. This injury is further characterized by its significant economic and performance impact within the context of contemporary professional football (Eliakim et al., 2020).

In modern football, sprinting is recognized as a crucial element in numerous match-winning actions (Faude et al., 2012). Nevertheless, sprinting, which induces excessive strain – the primary factor leading to tissue failure – has been identified as a predominant mechanism for hamstring muscle injuries in football (Faude et al., 2012). Therefore, from this basic standpoint, it seems logical to expect sprinting to be a key parameter in football from both a performance and an injury prevention perspective. The high incidence of hamstring injuries in sprinting tasks underscores recent findings emphasizing the substantial role of this muscle group in achieving high speed and/or

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acceleration goals (Morin et al., 2015; Thelen et al., 2006). Specifically, it has been suggested that the higher percentage of strain together with the greater mechanical demands suffered by the biceps femoris during maximum sprint efforts (Thelen et al., 2006) justify the high injury rate of this muscle (approximately 80%) compared to the other muscles that constitute the hamstring muscles (Ekstrand et al., 2023). This prevalence, as well as prolonging hamstring muscles' function loss for up to 72 h after a football match (Silva et al., 2018), suggests that match play may cause some biceps femoris tissue damage. However, to date, no direct evidence (histological) has been provided in this regard.

In this context, a new musculoskeletal hamstring screening protocol covering a wide spectrum of modifiable HSI risk factors (strength, range of motion [ROM], lumbo-pelvic control [LPC], and sprint mechanical properties) (Mendiguchia et al., 2012, 2021, 2022; Opar et al., 2012) was proposed (Lahti et al., 2022). This approach takes into account the multifactorial nature of HSI and allows the player to be classified according to their individual sprint mechanism-related HSI risk factor(s) profile. The utilization of a football-contextualized multifactorial approach, in conjunction with direct evidence (histological) of BFlh fibre structure, may enhance our understanding of the impact of a real football match on hamstring muscles.

The central question addressed in this study was whether a 72-h recovery period is sufficient for hamstring-related recovery: does this timeframe allow for the recovery of modifiable risk factors for hamstring strain injuries (HSIs)? Furthermore, does a football match lead to any degree of biceps femoris long head (BFlh) tissue damage, and if so, what is its status at the 72-h mark? Despite their significance, these fundamental questions have yet to receive adequate attention and remain unanswered. Therefore, the aim of this study was to analyse the time course and magnitude of acute and residual changes in sprint mechanism-related HSI risk factors following a football match by using a football-contextualized multifactorial approach (screening test battery) accompanied by an optical and ultrastructural microscopic analysis (i.e., histological) of BFlh fibre structure and composition.

Materials and methods

Participants

Twenty young, healthy, and active male amateur outfield football players (mean ± SD, age 20.4 ± 1.9 years; height 175.8 ± 6.8 cm; body mass 74.4 ± 12.6 kg) who had not suffered any myotendinous injuries for 6 months before the experiments volunteered to participate in the study. All participants were affiliated with regional senior or youth teams.

The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki), and approval was given by the Ethics Committee of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021). All the participants were informed of the purpose of the study, known risks, and possible hazards associated with the experimental protocol before recruitment, and each gave written consent.

According to a power analysis (G*Power v3.1) (effect size 0.3, power 0.8, α probability error 0.05, based on previous research) (Mohr et al., 2023) a sample size of 15 or 16 participants was required for a repeated-measures ANOVA, with five measurement time points, for within and within-between factors, respectively.

Experimental design

A prospective observational case series was conducted to examine changes in sprint performance-related factors and HSI modifiable risk factors up to 72 h following a friendly football match, using a football-contextualized multifactorial approach based on the screening test battery proposed by Lahti et al. (2022). Additionally, we analysed changes in general markers of muscle damage (i.e., creatine kinase (CK) and hamstring muscle soreness), as well as a general physical performance indicator (vertical jump). These assessments included all 20 players up to 3 days after the match (MD_{+3}). Moreover, BFlh biopsy samples were obtained from 10 players within the cohort 7 days before the match and at MD_{+3}. Participants were familiarized with all the assessment procedures before the study period. A schematic overview of the experimental design is shown in Figure 1.

Football match

Two friendly matches were organized in the afternoon at 16:00, following the current rules of the game set by the Fédération Internationale de Football Association (FIFA), with each match involving 10 players from the sample. Each match comprised two halves of 45 min plus additional time, separated by a 15-min halftime interval. During the matches, participants played in positions with which they were familiar. The matches were performed on an artificial grass field of play with official dimensions (touchline 99 m, goal line 52 m) against U-20 teams competing at the regional first division level.

Match external and internal load

Data logging to evaluate the external load was performed with a 10 Hz global positioning system (GPS) (WIMU PRO, RealTrack Systems, Almería, Spain) and its corresponding software v962 (SPRO, RealTrack Systems, Almería, Spain). The device contains inertial sensors (four 3D accelerometers, three 3D gyroscopes, one 3D magnetometer and one barometer) that collect data at 100 Hz. The validity and reliability of this device has been analysed for the collection of time-motion variables and is considered a suitable instrument for this purpose in football (Bastida Castillo et al., 2018). Once calibrated, the devices were fitted to the upper back using tight harnesses. The devices were placed in the Smart Station (RealTrack Systems, Almería, Spain) at the end of the match to transfer the data to the SPro software (RealTrack Systems, Almería, Spain). This software generated a database with external load variables: distance travelled (DT; m); high-speed running (HSR; distance covered above 21 km·h- 2023; m); number of high-intensity accelerations (ACC; >3 $m \cdot s^{-2}$; n) and decelerations (DEC; >3 $m \cdot s^{-2}$; n).

Each player's declared rate of perceived exertion (declared RPE) was collected ~30 min after the match using the 0–10

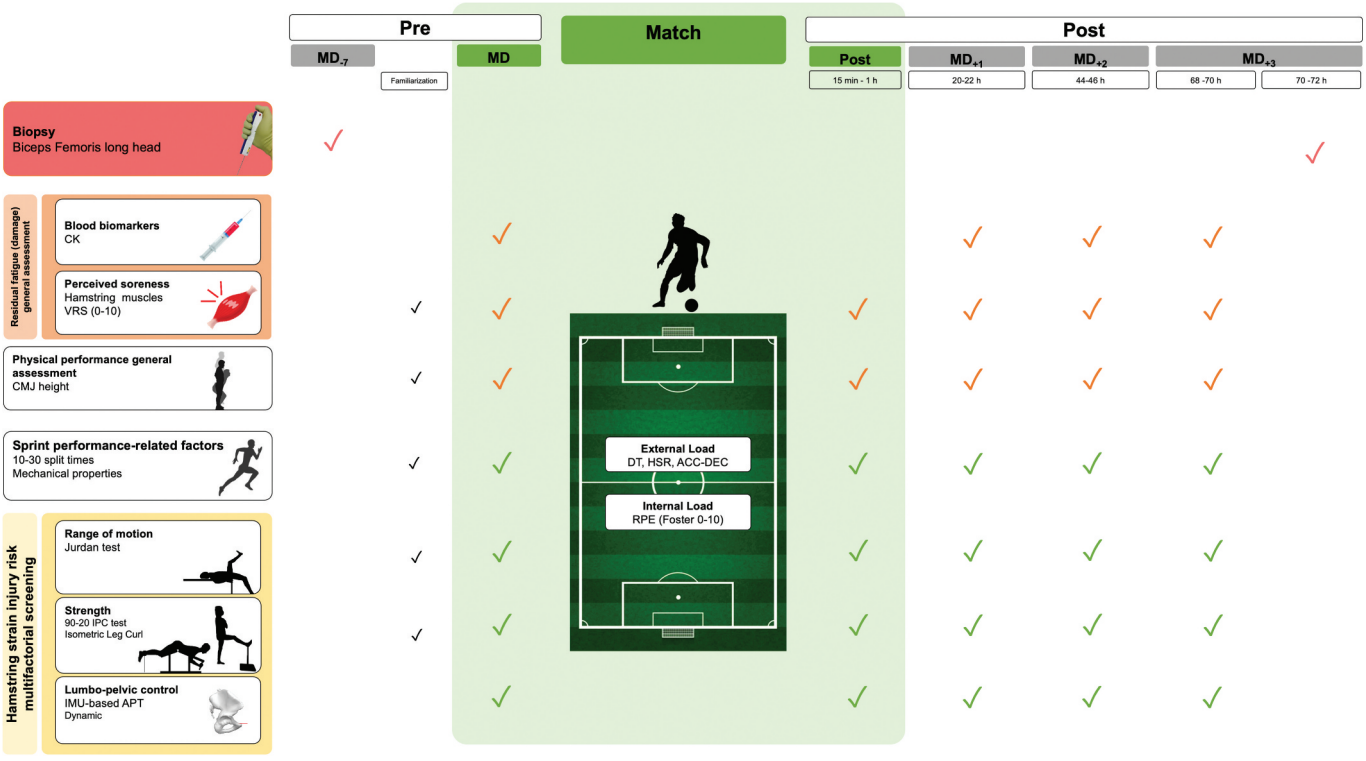


Figure 1. Schematic overview of the experimental design. ACC: high-intensity accelerations; APT: anterior pelvic tilt; CK: creatine kinase; CMJ: counter movement jump; DEC: high-intensity decelerations; DT: distance travelled; HSR: high-speed running (distance covered above 21 km·h- 2023; m); IMU: inertial motion unit; IPC: isometric posterior chain; MD: match day; RPE: rate of perceived exertion; VRS: visual rating scale.

point Borg rate of perceived exertion scale modified by (Foster et al., 2001) with its respective verbal anchors to collect subjective internal load estimations. The declared RPE was verbally collected exclusively by the same researcher (interpersonal communication) throughout the experiments, without peer influence, to mitigate bias in the individual rating process (Minett et al., 2022).

Blood sampling and processing

We obtained 5 mL of blood from the antecubital vein from the volunteers at each follow-up assessment (Figure 1). Blood samples were allowed to clot for 30 min in a tube (SST II Advance, Becton Dickinson Vacutainer Systems, UK) before being centrifuged at 3000 × *g* for 10 min at 4°C. Serum was aliquoted and stored at −80°C until needed for analysis of CK. Biochemical analysis of CK was performed in an Advia 2400 automatic device (Siemens Healthcare Diagnostics, Tarrytown, New York, USA) (Carmona et al., 2018).

Muscle soreness

A 10-point visual rating scale (VRS) quantified perceived hamstring muscle soreness for each limb (Carmona et al., 2018) while players were in the 90:20 isometric posterior chain strength test position (hip and knee flexion, respectively) (Matinlauri et al., 2019). Each number on the scale was accompanied by descriptive words for soreness, from 0, indicating no soreness, to 10, indicating intolerably intense soreness. We assessed muscle soreness in this extended position, as increased stiffness may cause reflex-mediated pain (Nosaka et al., 2002). In this position, the BFlh, with a larger hip extension than knee flexion moment arm, undergoes greater relative lengthening (Matinlauri et al., 2019), making this assessment more reflective of changes in BFlh reflex-mediated pain.

Vertical jump

The countermovement jump (CMJ) test was used to assess vertical jump performance as an indicator of lower limb explosive strength. Players self-selected the depth of the CMJ and were instructed to jump as high and as fast as possible, maintaining the hands-on-hips position until the final phase of the jump. A contact platform (Chronojump Boscosystem, Barcelona, Spain) was used to assess CMJ height (cm). The hardware was connected to a computer that displayed vertical jump height using a freely available software (2.0.2., Chronojump Boscosystem, Barcelona, Spain). This type of technology has proven its reliability and validity in other types of research with vertical jump tests (Pueo et al., 2020). Players performed three bilateral CMJs, and the best result was recorded and used for further analysis.

Sprint mechanical properties and performance

Instantaneous velocity over a 30 m sprint was measured by a linear velocity sensor (Race Analyser, Chronojump, Barcelona, Spain) set at 10 pulses per sample, implying a spatial accuracy of 3.032 cm (i.e., temporal precision of 4 µs between samples). The device, positioned at a height near the

players' gravity centre (GC), was held by a researcher seated 2 m behind the players, and featured a tether attached to a belt worn by the player around the waist. Players adopted a starting position and wore their usual football shoes. They were instructed to remain in this static starting position, and the researcher kept the tether tensioned with the device. Moreover, to achieve a faster and more reliable start, they were instructed to start at will whenever they wished. All tests were conducted on an outdoor football pitch with a synthetic grass surface. Field irrigation was standardized across repeated measures, and meteorological conditions, including wind speed, were consistent throughout the testing period. One sprint trial per subject was made and recorded for further analysis. The resulting data were then analysed using Chronojump software v 2.1.2–2 (Chronojump, Barcelona, Spain) to compute 10 and 30-m sprint times (DiPrampero et al., 2005; Samozino et al., 2016), in addition to sprint force-velocity spectra (F-V), using a simple field method validated by Samozino et al. (2016). In brief, this computation method relies on a macroscopic inverse dynamics analysis of the GC motion. Once the data were adjusted to the model, the modelled running velocity of each sprint was utilized to calculate the net anteroposterior ground reaction forces (GRFs), and sprint performance, defined by the 10- and 30-metre sprint times. Individual linear F-V relationships were then extrapolated to calculate theoretical relative horizontal maximal force (F0) (according to body mass, measured immediately before baseline measurements using a Pesperson mechanical scale with a 0.1 kg accuracy) and theoretical maximal horizontal velocity (V0) capabilities, as described elsewhere (Samozino et al., 2016).

Lumbo-pelvic control

Dynamic LPC was tested in parallel with sprint mechanical output and performance. A single triaxial inertial motion unit (IMU) (XSens DOT, Technologies B.V., Enschede, the Netherlands), attached with double-sided tape to the S1/L5 junction and overlapped with tape, was used, as previously described (Wada et al., 2020). The sensor range was ±2000°/s gyroscope, ±16 g accelerometer, and ±8 Gauss magnetometer. The internal sampling rate for strapdown integration and orientation through the extended Kalman filter was 800 Hz; the output sampling data frequency was chosen at 120 Hz. Anterior pelvic tilt (APT) was considered pitch, expressed in the IMU as the orientation angle in the sagittal plane with a root mean square error of 0.5° in a static inclination and 1° in a dynamic inclination. The single IMU sensor is supported as a valid tool to measure movements of the pelvis during sprinting (Wada et al., 2020).

For the dynamic LPC measurement, relevant raw data from the gyroscope and orientation units were first filtered by a fourth-order Butterworth low-pass filter with a cut-off frequency of 6 Hz. The start time of each sprint ($t = 0$) was detected using the value of 20°/s of the gyroscope in the sagittal plane as the threshold. This threshold accurately identifies sprint initiation by pinpointing the initial movement frame, excluding minimal movements and noise. It was refined through experimentation to achieve precise timing from a stationary position. Sprint times from 20 to 30 m of each subject were added to the start time in their trials. In that period, APT peaks were detected using Python software v 3.10.2, and the mean of the peaks was calculated (see Supplementary material 1 for syntax).

Force-generating capacity

The strength of the posterior chain muscles was measured as the maximal voluntary contraction (MVC) using the hands-on-chest 90° (hip flexion), 20° (knee flexion) isometric posterior chain (90:20 IPC) strength test. This test has previously been shown to have a "moderate to high" test – retest reliability (Matinlauri et al., 2019). The hamstring muscle strength was measured as the MVC using the prone isometric leg curl (ILC) strength test (Carmona et al., 2018), (for test reliability, please refer to the results section). For each test, players performed three isometric MVCs of 3–5 s (with each limb) with a short rest period (~30 s) between contractions. The force sensor (Force Sensor, Chronojump, Barcelona, Spain) sampling frequency was 160 Hz, and the MVC was then calculated as the average force in a 1 s window when a force plateau had been established (Carmona et al., 2018). The best of the three trials from each limb was recorded for further analyses.

Range of motion

To assess the interaction between the hip flexor and hamstring range of motion (ROM), the Jurdan test was used. A detailed description of the Jurdan test procedure is presented elsewhere (Lahti et al., 2020). This test has previously been shown to have an "excellent" test – retest reliability (Lahti et al., 2021). The test was recorded from each side using a CASIO Exilim EX-ZR 700 camera on a tripod situated at the height of the table and 3 m from the centre of the hip of the player on the table 2D angles were obtained using Kinovea software v 0.8.24 (a 2D motion analysis software that has previously been validated) (Puig-Diví et al., 2019) using the following anatomical landmarks: hip (greater trochanter), knee (medial epicondyle) and shin (inferior margin of the patellar tendon). The best of the three trials from each limb was recorded for further analyses.

Muscle biopsies

Skeletal muscle biopsies were obtained using a 12 G biopsy needle (Bard® Monopty® Disposable Core Biopsy Instrument, Bard Biopsy Systems, Tempe, USA), and the average muscle biopsy sample was 2.8 mm in diameter and 17 mm in length (~50 mg). Players were placed in the prone position, with their feet dangling over the edge of the table. Multifrequency 2–12 MHz ultrasound (Portable ultrasound system Viamo SV7, Canon Medical Systems S.A., Japan) was used to identify the area of origin of the hamstrings in the ischial tuberosity (IT) to locate the biceps femoris muscle (Balius et al., 2019). The longitudinal biopsy procedure was performed under local anaesthesia (2% Mepivacaine) to obtain tissue samples from the midsection of the BFlh muscle at a distance of 15 cm from the IT tuberosity and at a depth of 2 cm. For each subject (total $n = 10$ players),

two biopsies were collected from the dominant leg at different time points (Figure 1). Two samples were obtained in each procedure. Needle insertion was placed 5 cm above (proximal) the last insertion to avoid the affected tissue from earlier biopsies. Each muscle biopsy sample was divided into two pieces of equal size and processed for further histopathology analysis or electron microscopy evaluation.

Electron microscopy

Muscle biopsies were processed according to standard procedures outlined elsewhere (Dubowitz et al., 2013), with transversally oriented muscle fibres by fixation in glutaraldehyde (2.5%) in a 0.1 M sodium phosphate buffer (pH 7.4) for 3 days and washed three times for 10 min with 0.1 M phosphate buffer at 4°C. Samples were then immersed for fixation in a solution of 1% osmium tetroxide and 0.8% potassium ferrocyanide in 0.1 M phosphate buffer for 1.5 h at 4°C in the dark and washed three times for 10 min with 0.1 M phosphate buffer at 4°C. Tissue samples were then dehydrated with graded acetone solutions (for 10 min each with acetone 50%, acetone 70%, acetone 80% twice, acetone 90% twice, acetone 96% twice, and acetone 100% three times) and immediately processed for Spurr resin (Sigma–Aldrich Quimica SA, Madrid, Spain). Spurr resin infiltration went stepwise from Spurr/acetone 1:3, 1:1, and 3:1 (6–12 h each) to pure Spurr (overnight). Samples were included in moulds and placed in the oven at 60° for 3 days for resin polymerization (Dubowitz et al., 2013).

Semithin and ultrathin sections from resin-embedded samples were cut with a diamond knife on a Reichert-Jung ultramicrotome and mounted on nickel grids (200 mesh TB; Electron Microscopic Sciences, Fort Washington, MD) using an adhesive pen (David Sangyo). Sections were further contrasted in uranyl acetate (20 min) and lead citrate (90 s) before they were observed and photographed using transmission electronic microscopy (JEOL Model 1100, Boston, MA, USA) (Dubowitz et al., 2013).

Methods for myofibrillar disruption analysis were modified from Gibala et al. (1995). Fibres were scanned for sarcomeres with nonlinear Z lines and corresponding filaments that were not perpendicular. Areas with disrupted sarcomeres were classified as focal when fewer than three sarcomeres in both directions were affected, moderate when between three and nine sarcomeres were affected, and extreme if more than 10 sarcomeres in both directions had changes in morphology.

Histology and immunohistochemistry

Muscle tissue samples were frozen in isopentane cooled with liquid nitrogen and stored at −80°C. Cryostat sections were routinely handled and stained and reacted with techniques including haematoxylin and eosin (H&E), modified Gomori trichrome, reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), non-specific esterase and adenosine triphosphatase (ATPase) at pH 9.4. The percentage of the fibre types was counted in microphotographs, and the measurements were performed in a blinded fashion by the same observer to avoid bias in the results (Contreras-Muñoz et al., 2021). Three images were randomly selected within the muscle sample using a BX-61 microscope (Olympus) equipped with a DP72 camera (Olympus) and cellSens digital imaging software (version 1.9). For immunofluorescence analysis, frozen muscle biopsies were transversely sectioned (10 µm thick) using a cryotome (Leica Microsystems, Wetzlar, Germany) at −20°C; after 20 min at room temperature (RT), they were fixed with 4% paraformaldehyde for 7 min. They were then abundantly rinsed with phosphate-buffered saline (PBS) containing 0.5% Tween and blocked for 90 min with PBS containing 1% bovine serum albumin (BSA) and 8%–10% donkey serum at RT. After that, sections were directly incubated at 4°C overnight with primary antibodies against merosin and dystrophin using 4H8 (Enzo Life Sciences, Germany) and Dys2 (Novocastra, Newcastle, UK). After primary antibody incubation, sections were abundantly rinsed with PBS Tween Triton and then incubated for 3 h at RT in the dark with 1:500 dilution secondary antibodies: anti-Rat Alexa 488 and anti-Mouse Alexa 594 (Thermo Fisher; Massachusetts, USA). Finally, they were abundantly rinsed with PBS Tween Triton and mounted using Fluoromount-G mounting medium (Invitrogen; Massachusetts, USA) (Natera de Benito et al., 2020, Torelli et al., 2021).

The percentage of the staining area was calculated for both merosin and dystrophin by measuring the average signal intensity in immunofluorescence microphotographs. Four images were randomly selected within the muscle tissue area using a DFC7000 fluorescence microscope (Leica Microsystems GmbH, Mannheim, Germany); image acquisition was performed using LAS X (Leica Microsystems GmbH, Mannheim, Germany). All analyses and measurements were performed in a blinded fashion by the same observer to avoid bias in the results. The percentage area of merosin and dystrophin within each section was calculated by applying an intensity threshold in ImageJ analysis software (National Institutes of Health, Bethesda, MD) (Natera de Benito et al., 2020, Torelli et al., 2021).

Statistics

The distribution of each variable was tested using the Shapiro–Wilk test. One- or two-way repeated-measures ANOVA or Friedman analysis followed by a paired *t* test with a Bonferroni correction were used when appropriate, depending on the distribution of variables, to identify significant differences from baseline values.

Effect sizes (ES) (Cohen's d) were calculated to assess the difference between means when statistically significant differences were found and/or at MD_{+3}. Thresholds for standardized difference statistics were trivial < 0.20, small 0.20 < 0.59, moderate 0.60 < 1.19, large 1.20 < 1.99, and very large > 2.0 (W. G. Hopkins et al., 2009). Pearson's correlation coefficient with 90% confidence intervals (CI) was used to evaluate the association between the variables of interest.

We evaluated our baseline test-retest reliability of dependent variables using the Intraclass Correlation Coefficient (ICC), along with a 95% CI, Typical Error (TE) and Coefficient of Variation (CV%): CV = (SD/mean)*100 (Weir, 2005). Reliability thresholds for ICC values were defined as poor (<0.50),

moderate (0.50–0.75), good (0.75–0.90), and excellent (>0.90) (Koo & Li, 2016).

Data are presented as the mean ± SD or median ± interquartile range (IQR) depending on the distribution of variables. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using Hopkins spreadsheet for analysis of validity and reliability (W. Hopkins, 2015) or SPSS v25.0.0.0 (IBM, Armonk, NY, USA).

Results

Match external and internal load

On average, players completed 10,405 ± 661 m DT, 458 ± 260 m HSR, 45.4 ± 20.2 ACC, and 59.5 ± 23.3 December during the football match and their declared RPE was (median and interquartile range, IQR) 9 [IQR = 7–9] arbitrary units (a.u.). No correlations were found between external or internal loads and sprint performance-related factors, HSI risk factors or BFlh fibre disruption.

General residual fatigue (muscle damage) and physical performance indicator

Significant changes from baseline were found for hamstring muscles' perceived soreness at MD_{+1} (dominant leg: from 0.5 [IQR = 0.00–2.00] to 3 [IQR = 1.25–3.75] a.u., $p = 0.001$, ES = 1.05; non-dominant leg: from 0.5 [IQR = 0.00–4.00] to 2.5 [IQR = 1.25–4.00] a.u., $p = 0.01$, ES = 1.54) and at MD_{+2} (non-dominant leg: 2 [IQR = 1.00–3.75] a.u., $p < 0.001$, ES = 1.14), and there were no differences between legs. Significant CMJ height decrements (measured through the CMJ test, which showed an "excellent" baseline test-retest reliability, ICC = 0.97–0.99; TE range = 0.64–1.00; CV% = 2.64 ± 1.90) were observed only at MD_{+1} (from 34.9 ± 6.2 cm at baseline to 32.6 ± 5.6 cm) (Figure 2(a)). CK serum levels were only significantly increased at MD_{+1} ([median and IQR] 1296 [IQR = 661–1669] $U{\cdot}L^{-1}$) and MD_{+2} (572 [IQR = 402–1135] $U{\cdot}L^{-1}$) (Figure 2(b)). All those variables trended back to baseline at MD_{+3}, where no significant changes were observed.

Sprint performance-related factors

The changes observed in all sprint performance-related variables at MD_{+3} were found to be significant. Specifically, sprint times demonstrated a *moderate* increase, with values changing from 2.24 ± 0.11 s at baseline to 2.37 ± 0.14 s, and from 4.76 ± 0.22 s at baseline to 5.03 ± 0.26 s for 10 and 30 m split times, respectively, at MD_{+3} (Figure 3(a,b)). Additionally, there was a decrease in F0 from 7.46 ± 0.71 N·kg-1 at baseline to 6.94 ± 0.69 $N{\cdot}kg^{-1}$, as well as a decrease in V0 from 8.74 ± 0.56 $m{\cdot}s^{-1}$ at baseline to 8.25 ± 0.68 m·s-1 (Figure 3(c,d)).

We obtained a "moderate-to-excellent" baseline test-retest reliability for sprint mechanical properties (F0: ICC = 0.66–0.94; TE range = 0.32–0.60; CV% = 6.41 ± 6.12, and V0: ICC = 0.56–0.91; TE range = 0.22–0.42; CV% = 2.57 ± 1.93).

Modifiable risk factors for hamstring strain injury

Non-significant *moderate* posterior chain muscles' strength decreases were detected until MD_{+3} in the 90:20 IPC test (from 218 ± 50 N at baseline to 189 ± 32 N and from 198 ± 43 N at baseline to 174 ± 29 N for dominant and non-dominant legs, respectively) (Figure 4(a)). Significant *moderate* hamstring muscles' strength decreases were observed until MD_{+3} in the ILC test (from 394 ± 80 N at baseline to 343 ± 73 N and from 378 ± 78 N at baseline to 308 ± 88 N for dominant and non-dominant legs, respectively) (Figure 4(b)). Both 90:20 IPC (dominant leg: ICC = 0.87–0.97; TE range = 11.35–17.59; CV% = 6.51 ± 4.59, and non-dominant leg: ICC = 0.87–0.97; TE range = 9.99–15.49; CV% = 6.39 ± 6.23) and ILC (dominant leg: ICC = 0.93–0.98; TE range = 13.12–20.23; CV% = 4.09 ± 2.19, and non-dominant leg: ICC = 0.96–0.99; TE range = 9.88–15.32; CV% = 3.18 ± 2.02) strength tests showed a "good-to-excellent" baseline test-retest reliability.

Moderate significant ROM reductions were observed until MD_{+3} for the dominant leg only (from 49.2 ± 13.7 degrees at baseline to 44.2 ± 12.8 degrees) (Figure 4(c)). The Jurdan ROM test showed an "excellent" baseline test-retest reliability (dominant leg: ICC = 0.97–1.00; TE range = 1.17–2.35; CV% = 2.83 ±

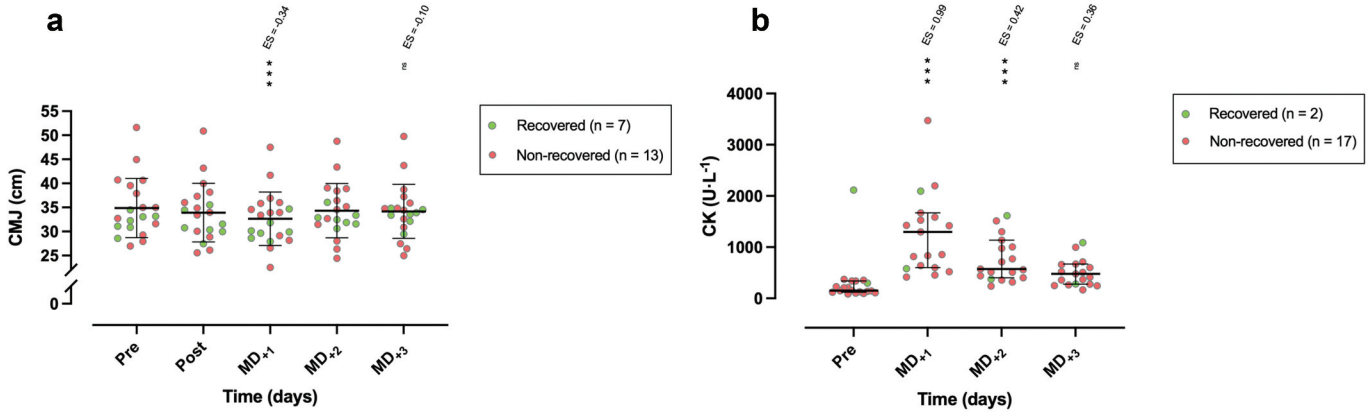


Figure 2. (a) individual, mean and SD values of counter movement jump height (CMJ). (b) Individual, median and interquartile range values of creatine kinase concentration (CK). Note that CK data from player 10 were lost. Player 15 was considered an outlier and his data from MD_{+1}, MD_{+2}, and MD_{+3} are not shown in the graph (8,589, 28288, and 35,126 $U{\cdot}L^{-1}$ at MD_{+1}, MD_{+2}, and MD_{+3}, respectively). Players who did not recovered their baseline values at MD_{+3} were considered non-recovered (red). Players who recovered their baseline values at MD_{+3} were considered recovered (green). ** and *** indicate a significant difference from baseline (pre) values at $p < 0.01$, and $p < 0.001$, respectively. ES: Cohen's d effect size. MD: match day.

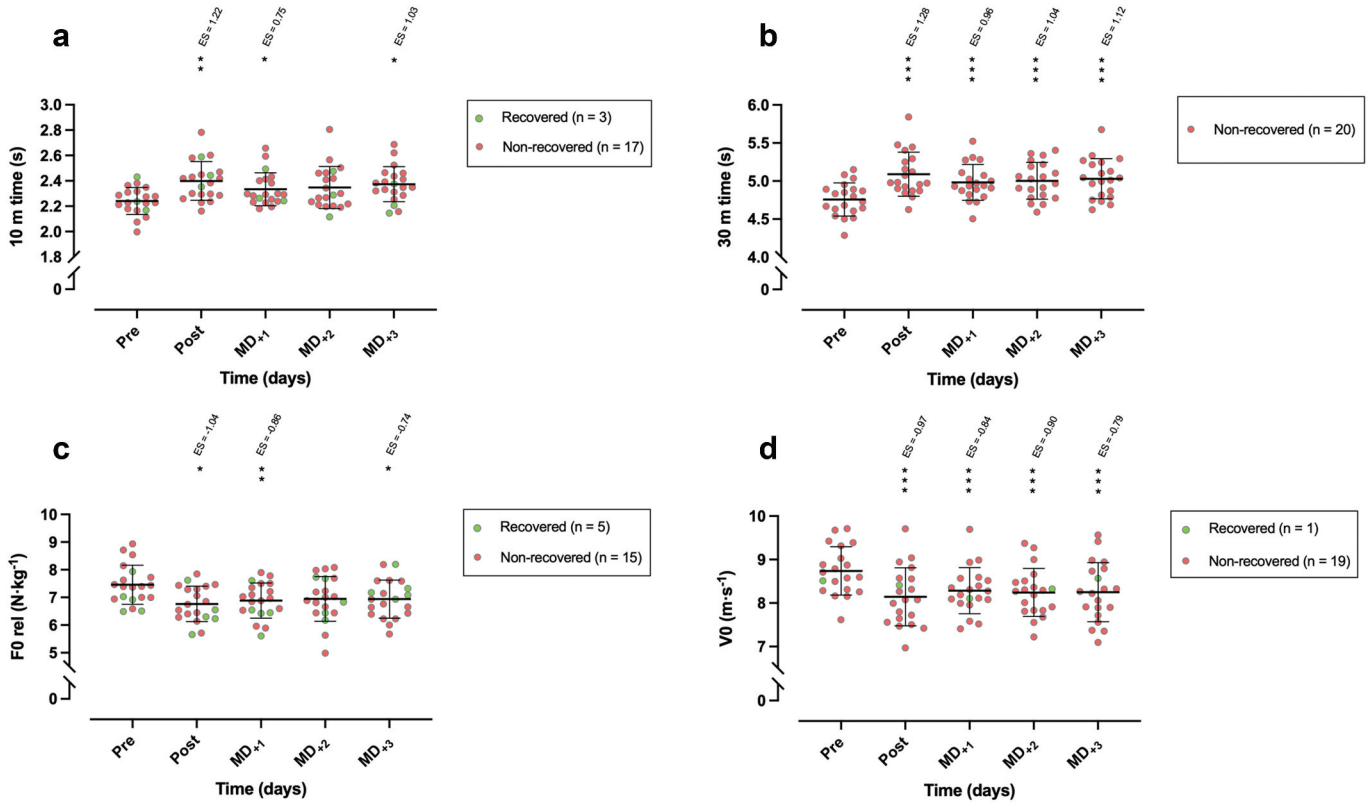


Figure 3. Individual, mean and SD values of 10 m times (a), 30 m times (b), theoretical relative (to body mass) horizontal maximal force (F0) (c), and theoretical maximal horizontal velocity (V0) (d). Players who did not recovered their baseline values at MD $_{+3}$ were considered non-recovered (red). Players who recovered their baseline values at MD $_{+3}$ were considered recovered (green). *, ** and *** indicate a significant difference from baseline (pre) values at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ES: Cohen's d effect size. MD: match day.

1.76, and non-dominant leg: ICC = 0.92–0.99; TE range = 1.73–3.47; CV% = 3.20 ± 2.59).

No differences between legs were observed for strength and ROM (Figure 4).

Dynamic LPC, measured as an increase in APT during maximal speed sprinting, showed no changes from baseline following the match (Figure 5). We obtained an "good-to-excellent" baseline test-retest reliability for dynamic LPC (ICC = 0.85–0.98; TE range = 1.49–2.90; CV% = 1.94 ± 2.04).

Basic histomorphologic study and fibre type distribution

BFlh fibre biopsy specimens appeared normal in the basic histomorphologic study (Supplementary material 2). BFlh exhibited an ATPase-based balanced fibre type distribution (type I 55%; type II 45%). No differences from baseline were observed at MD_{+3} (Figure 6(a,b)). There was a correlation between the percentage of type II fibres and sprint performance (Figure 6(c,d)).

Fibre disruption

Focal BFlh fibre disruptions (disruption occupying two sarcomeres and associated Z-lines) were significantly increased from baseline at MD_{+3} ($p = 0.025$) (Figure 7). Other minor structural alterations are shown in Figure 8. No correlations were found between BFlh fibre disruption and strength, ROM or CK.

Immunohistochemistry

BFlh fibre merosin (4H8) and dystrophin (Dys2) immunoreactivity were measured in five players, revealing a heterogeneous between-subjects response (Figure 9) (Supplementary material 3).

Individual total percentages of change at MD_{+3} for the main variables are summarized in Figure 9. Players who did not recover their baseline values at MD_{+3} were classified as "non-recovered", while those who did were classified as "recovered".

Discussion

The central question addressed in this study was whether a 72-h recovery period, a commonly observed timeframe between matches in elite football, was sufficient for hamstring muscles concerning both HSI risk factors and BFlh fibre structure following a football match. This is the first study to analyse the time course and magnitude of acute changes in sprint mechanism-related HSI risk factors following a football match using a football-contextualized multifactorial approach accompanied by an optical and ultrastructural microscopic analysis (histological) of BFlh fibre structure. Additionally, we analysed BFlh fibre composition. The main findings from the present study were as follows: (i) at MD $_{+3,}$ sprint performance-related and HSI risk factors were not recovered; (ii) there were significant moderate increases in focal BFlh fibre disruptions at MD $_{+3}$.

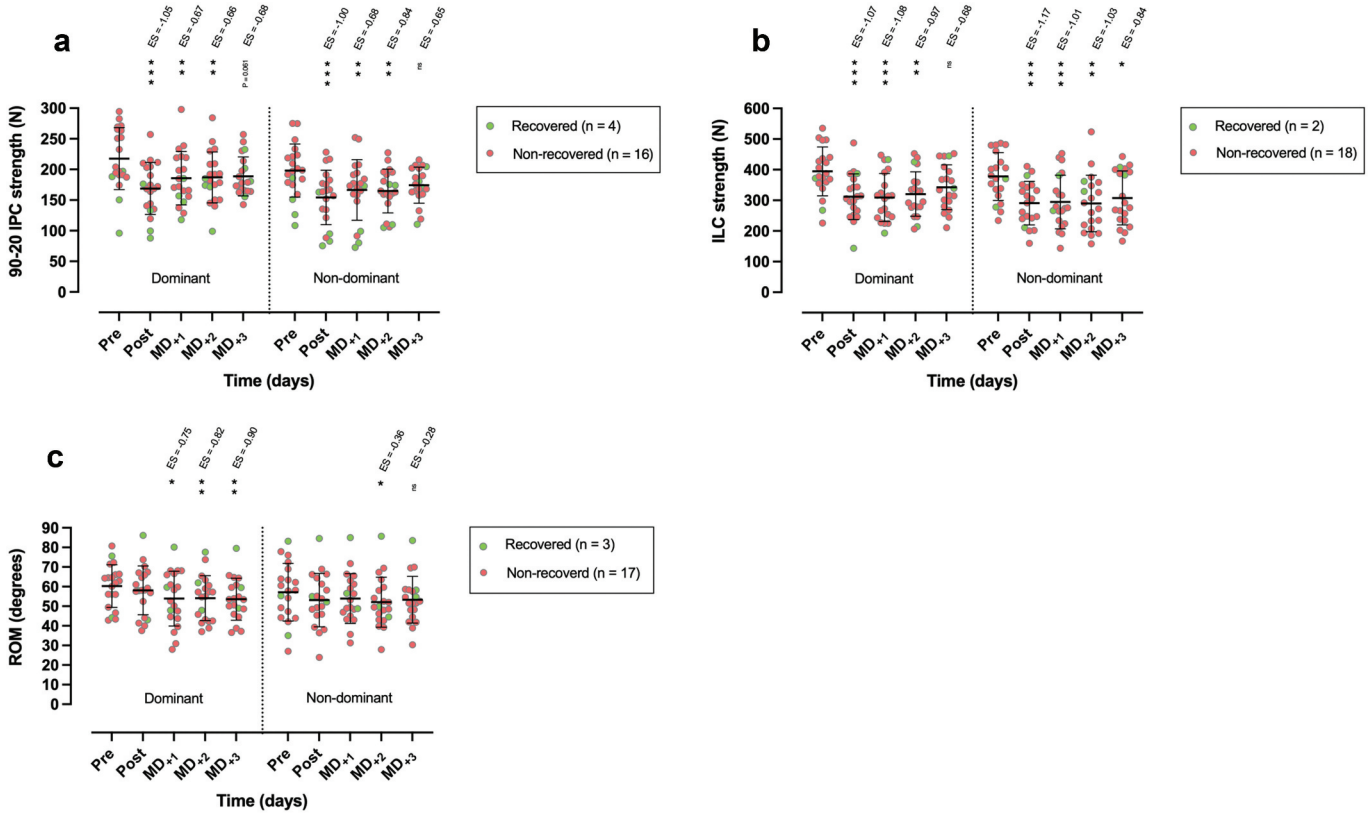


Figure 4. (A) individual, mean and SD values of posterior chain strength measured as the maximal voluntary contraction using the hands-on chest 90:20 isometric posterior chain (90:20 IPC) strength test from dominant and non-dominant leg; (B) hamstring muscles strength measured as the maximal voluntary contraction using the isometric leg curl (ILC) strength test from dominant and non-dominant leg; (C) range of motion (ROM) measured as shin angle from the dominant leg minus the thigh angle from the non-dominant leg (dominant), and shin angle from the non-dominant leg minus the thigh angle from the leg (non-dominant). Players who did not recover their baseline values at MD $_{+3}$ were considered non-recovered (red). Players who recovered their baseline values at MD $_{+3}$ were considered recovered (green). *, ** and *** indicate a significant difference from baseline (pre) values at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ES: Cohen's d effect size. MD: match day.

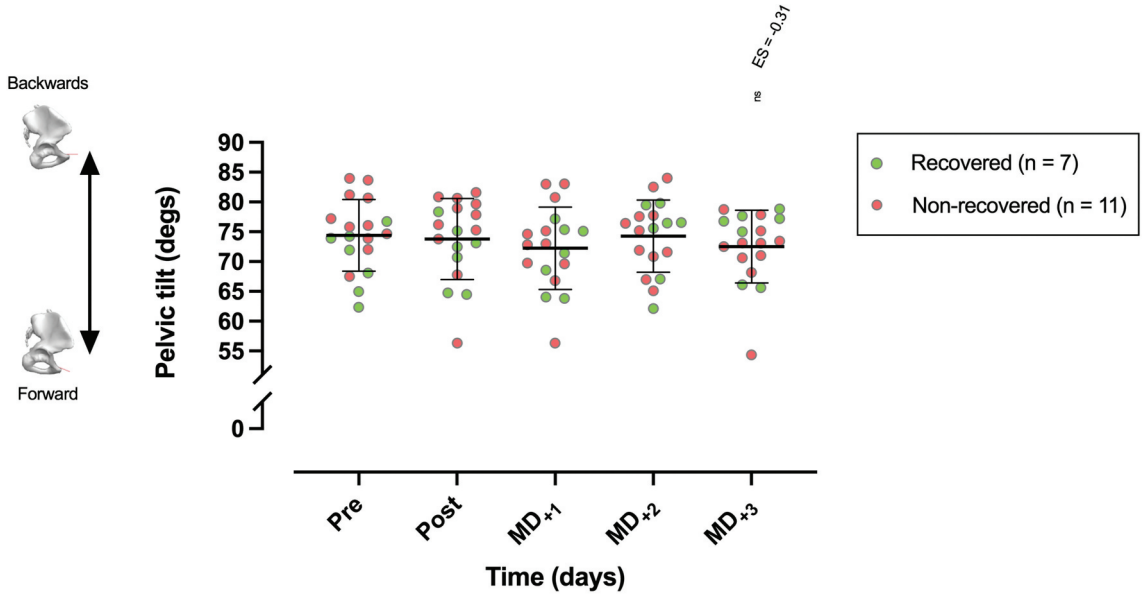


Figure 5. Individual, mean and SD values of dynamic lumbo-pelvic control (LPC) measured as mean peaks of pelvic tilt (degrees) during 20–30 m of sprint section. Players who did not recover their baseline values at MD $_{+3}$ were considered non-recovered (red). Players who recovered their baseline values at MD $_{+3}$ were considered recovered (green). MD: match day.

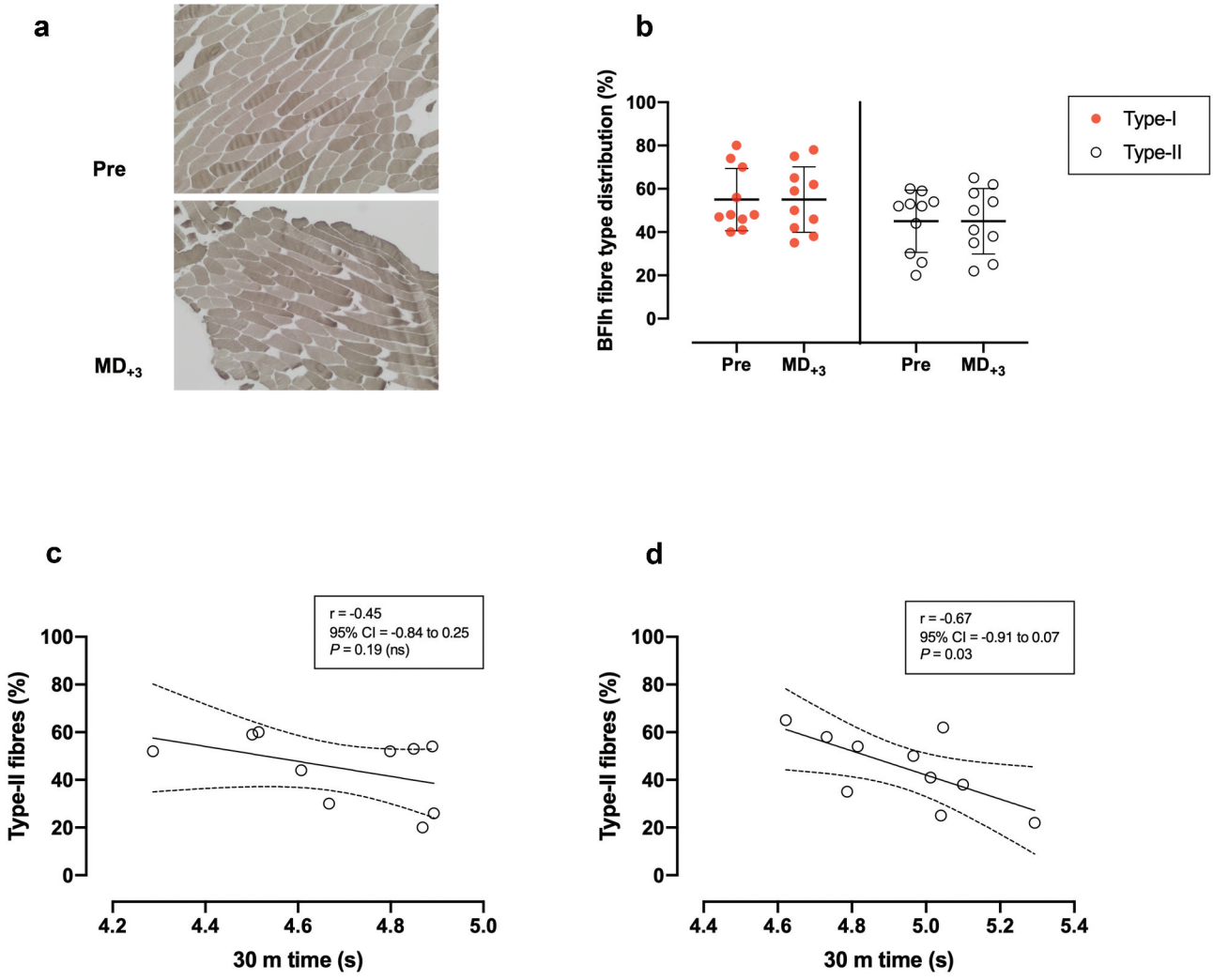


Figure 6. Non-specific esterase and adenosine triphosphatase at pH 9.4 showing chequerboard pattern with pale type I fibres and dark type II fibres before (upper panel) and three days following the match (MD $_{+3}$) (lower panel) (example from player 14). Images show: (A) (x100) microphotographs for both panels; (B) biceps femoris long head percentage of fibre type distribution (type I and type II) before and three days following the match (MD $_{+3}$); (C) correlation between percentage of type II fibres and sprint performance (30 m time) before and (D) three days following the match (MD $_{+3}$).

Additionally, (iii) match load (both external and internal [RPE]) did not show any associations with sprint performance-related factors, HSI risk factors, or BFlh fibre disruption; (iv) BFlh exhibited an ATPase-based balanced fibre type distribution, and the percentage of type II fibres was associated with sprint performance.

Before discussing the study's main findings, it should be noted that the external load during the match was similar to that previously reported in the literature for under-23 professional players (Reynolds et al., 2021). Additionally, the players' sprint performance, as measured by their 30-m time, was found to be comparable to that of professional players previously reported in the literature (Barrera et al., 2023).

Sprint performance and hamstring injury risk factors

At MD $_{+3}$, while no significant changes, or minor changes, were found in general residual muscle damage and physical performance markers (i.e., CK and hamstring perceived soreness, CMJ height), both sprint performance-related factors (i.e., 10 and 30 m split times, F0 and V0) and HSI risk factors (i.e., posterior chain and hamstring muscles' strength, and ROM) were still significantly (ES, moderate) decreased.

Sprinting is key both from a performance and injury prevention standpoint. Considering the main injury mechanism in football together with its frequency and relevance in decisive actions of the game (Danielsson et al., 2020; Faude et al., 2012), the sprint should be regarded as a focal point for any preventive measures to be implemented. The hamstrings are one of the muscle groups that mainly contribute to the development of sprint abilities, participating in both early acceleration and application of the rotation-extension strategy (producing force backwards). Additionally, in high-speed phases where vertical forces are decisive, the hamstrings play a crucial role in utilizing the impact-limb deceleration mechanism (active swing leg retraction) (Morin et al., 2015). However – and attending to the demands of football where the ability to accelerate

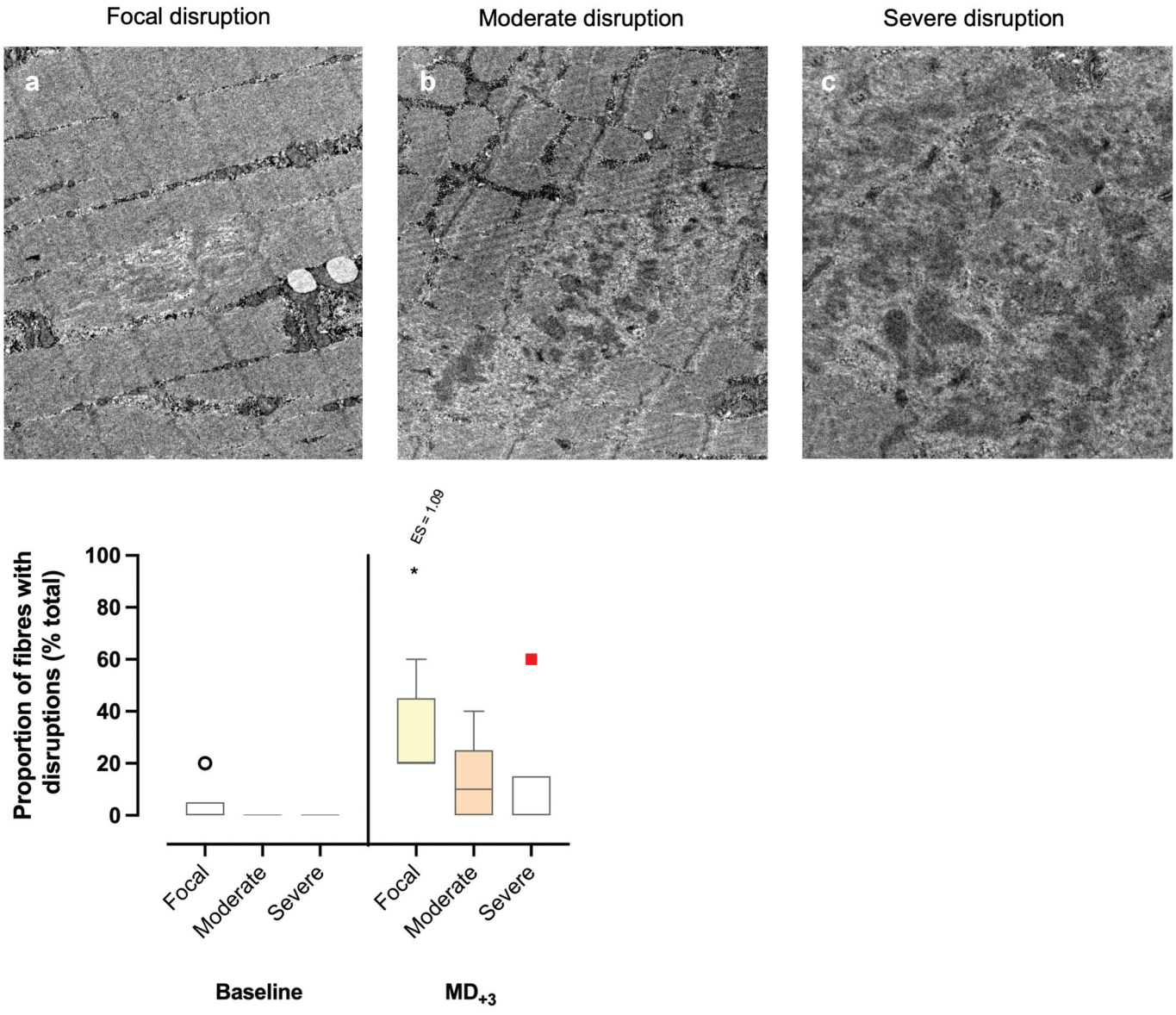


Figure 7. Upper panels: electron micrograph of longitudinally oriented skeletal muscle fibres exhibiting (A) a focal myofibrillar disruption occupying two sarcomeres and associated Z-disks, (B) a moderate myofibrillar disruption occupying several adjacent sarcomeres (3 to 9), and (C) a severe myofibrillar disruption occupying more than 10 adjacent sarcomeres. Original magnification x10,000 (A) and x8000 (B and C). Lower panel: Tukey box plots indicating the proportion of biceps femoris long head fibres showing focal, moderate and severe myofibrillar disruptions at baseline and three days following the match (MD $_{+3}$). Five fibres were investigated in each player ($n = 10$). Sarcomere morphological abnormalities were observed in five players in samples from MD $_{+3}$, where all samples exhibited focal disruptions, three presented moderate disruptions, and one showed severe disruptions. The dot indicates that only one player's sample exhibited focal disruptions at baseline (player 4). The red square indicates that only one player's sample showed severe disruptions at MD $_{+3}$ (player 5). * indicates a significant difference from baseline at $p > 0.05$. ES: Cohen's d effect size.

(decelerate) and rapidly change one's velocity and momentum to evade opponents is crucial – forward acceleration capabilities can be of far greater assistance to an athlete's on-field performance compared to the individual top speed that is occasionally reached (Hicks et al., 2020). Sprint acceleration performance has been shown to be associated with the ability of hip extensors (mainly biarticular hamstring muscles) to produce and apply high levels of force in the horizontal direction over the entire acceleration, which may explain the higher rate of hamstring injuries recently reported in football during acceleration (54%) compared to high speed (Gronwald et al., 2022; Morin et al., 2015). This ability is well described by a macroscopic linear relationship between horizontal force and velocity obtained during sprint acceleration (i.e., F-v relationship) (Morin et al., 2019). The F-v relationship is an integrative descriptor of an athlete's mechanical output capabilities during maximum sprinting accelerations, representing the maximum force an athlete can produce in the horizontal direction during sprinting at either slow (F0) or fast (V0) velocities (Morin et al., 2019). In line with the close relationships between sprinting mechanics (especially the F-v profile) and hamstring function, a clear decrease in F0, with no change in V0, has been prospectively associated with the risk of sustaining a new hamstring injury and to post-injury processes like

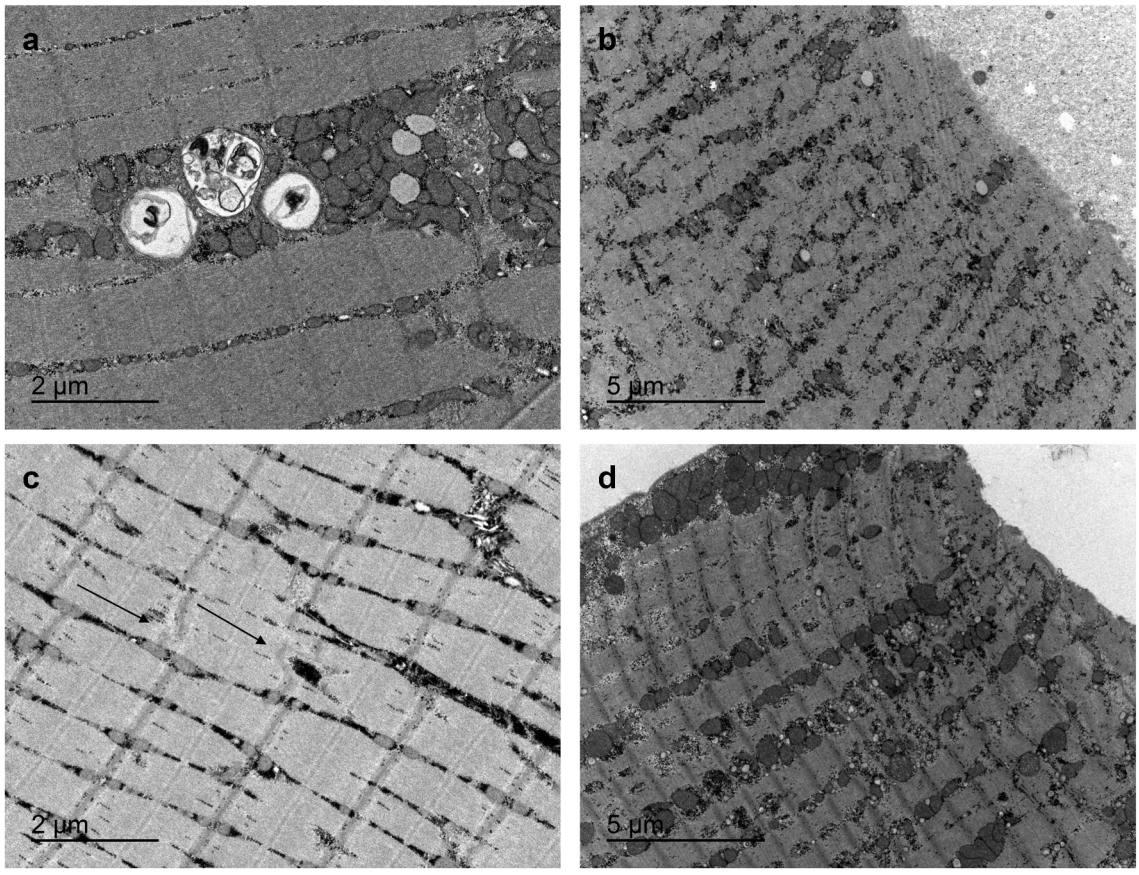


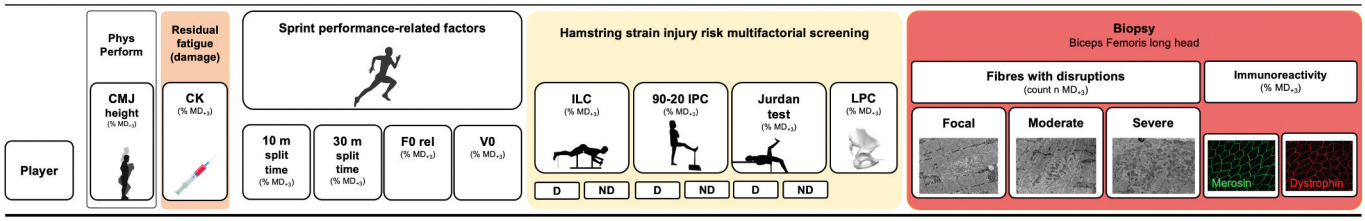
Figure 8. Transmission electron micrograph of skeletal muscle showing (A) autophagic vacuoles with residual bodies constituting dense membranous structures; (B) a fibre with initial necrotic changes with loss of myofibrils; (C) focal Z-line streaming affecting a few sarcomeres (arrows); (D) hypercontraction throughout one fibre.

returning to play after hamstring injury rehabilitation in footballers (Mendiguchia et al., 2014).

Based on the results of the present study, it seems justified to assume that 72 h after a match are not enough to recover the players 'mechanical ability to apply horizontal force and accelerate towards maximum speed, which explains a decrease in performance over time at 10 and 30 m. As horizontal force production during sprinting offers an integrative, macroscopic overview of lower limb function during the sprinting action, alterations in these capacities could potentially contribute to functional limitations in the lower limbs, particularly affecting the muscles in the posterior chain. However, this macroscopic evaluation used in the present study does not pinpoint the specific segment responsible for the decrease in function. Nevertheless, the consistent decrease in isolated hamstring strength until 72-h post-match, coupled with its greater susceptibility to fatigue compared to the gluteus maximus (Edouard et al., 2018), suggests a significant role for hamstring muscles in influencing macroscopic performance within the kinetic chain. Thus, a real match is likely not only to lead to long-lasting sprint performance impairments but also to persistent decrements in mechanical properties (particularly F0) that may be associated with an increased risk of HSI (Edouard et al., 2021).

To the abovementioned arguments, we can add that a certain degree of posterior chain muscle damage was observed (strength and ROM reductions at MD_{+3}) in most of the players. Although the role of decreased single joint strength as a risk factor for hamstring injury is still controversial (Ruddy et al., 2018), physiology studies have unanimously considered the inability to produce force as the best indirect marker of muscle damage and therefore of residual fatigue after exercise (Paulsen et al., 2012) or a football match (Nédélec et al., 2012), as in the present study. Accordingly, while the immediate hamstring strength loss following a sprint-based intervention is attributed to both central and peripheral fatigue, significantly prolonged (~72 h) strength (90:20 IPC and ILC) loss after a match, as shown in the present study and similar to the findings in other studies, is attributed to exercise-induced muscle damage – consisting of metabolic and mechanical alterations that impair the excitation-contraction coupling and the contractile process (Baumert et al., 2021).

This study's finding of a parallel and significant decrease in ROM as in the strength until MD_{+3} following a match could reflect the rise in muscle passive tension potentially originating from the development of a local contracture in fibre segments as a result of membrane damage produced in both iliopsoas and/or hamstring muscles assessed during the Jurdan test (Lahti et al., 2020) and that are subjected to continuous



Player	CMJ height (% MD+3)	CK (% MD+3)	10 m split time (% MD+3)	30 m split time (% MD+3)	F0 rel (% MD+3)	V0 (% MD+3)	ILC (% MD+3) D	ILC (% MD+3) ND	90-20 IPC (% MD+3) D	90-20 IPC (% MD+3) ND	Jurdan test (% MD+3) D	Jurdan test (% MD+3) ND	LPC (% MD+3)	Fibres with disruptions (count n MD+3) Focal	Fibres with disruptions (count n MD+3) Moderate	Fibres with disruptions (count n MD+3) Severe	Immunoreactivity (% MD+3) Merosin	Immunoreactivity (% MD+3) Dystrophin
1	-4,8	77,7	3,9	4,8	-6,0	-6,2	26,8	37,7	20,1	19,7	-27,0	-14,7	4,2	0	0	0		
2	-0,4	79,4	0,9	3,5	4,2	-8,0	-13,8	-7,3	-9,0	8,5	6,1	5,4	-4,9	0	0	0		
3	1,2	-48,6	1,1	4,0	3,2	-7,4	-10,7	-14,7	-14,7	-11,4	-9,6	12,2	-13,6	0	0	0		
4	7,5	71,9	7,5	4,3	-10,0	-0,6	-28,8	-59,8	-9,1	-9,6	-11,5	-10,9	-1,9	1	0	0	5.7	-16.9
5	-5,8	7,9	2,6	2,6	-6,7	-1,6	-17,5	13,8	2,3	10,1	-9,6	-17,0	3,9	1	1	3		
6	-0,4	48,7	14,1	7,8	-22,2	0,6	-11,8	-18,1	12,8	-24,8	-4,6	-9,6	5,3	-	-	-		
7	3,1	96,2	10,1	7,7	-13,9	-4,3	19,7	10,2	-21,7	-8,2	-2,1	2,1	0,7	-	-	-		
8	2,6	-6,0	11,3	7,9	-13,6	-4,6	-28,5	-19,9	61,8	1,7	7,2	-4,1	4,6	-	-	-		
9	-9,7	308	-2,3	3,2	10,6	-10,7	6,8	-40,2	23,7	35,1	10,9	56,3	-19,5	-	-	-		
10	8,0	-	-1,4	0,9	2,4	-4,7	21,3	-1,5	-16,0	-9,9	-4,5	-5,0	15,8	-	-	-		
11	0,2	216	9,6	7,8	-15,8	-4,7	-50,6	-40,8	-14,2	-8,6	-26,1	-7,6	-2,1	1	2	0	18.7	-19.6
12	-2,8	88,7	9,3	7,0	-14,4	-3,1	-17,6	-7,9	-39,2	-16,3	-15,0	-10,3	-4,1	0	0	0	-5.6	-4.0
13	4,1	213	2,0	4,4	-0,1	-8,3	-2,6	-15,4	-19,1	-27,0	-14,3	-4,8	-	3	0	0	-8.6	70.9
14	-3,6	397	15,8	7,8	-14,8	-1,1	-9,1	-4,6	-10,5	1,4	-16,4	-6,2	-12,6	1	1	0		
15	-2,4	32.424	9,0	8,2	-14,3	-6,9	-17,7	-36,4	-36,2	-43,2	-32,8	-22,9	1,8	2	0	0	112.6	-6.2
16	-0,6	191	-1,1	1,5	-0,4	-4,4	-11,4	-23,4	-5,6	2,4	-22,4	-3,1	-	-	-	-		
17	-1,7	723	14,4	10,2	-11,0	-6,9	-6,4	-12,5	-9,0	-5,8	5,2	0,4	-7,7	-	-	-		
18	-9,2	251	3,0	8,4	-3,0	-14,5	24,1	-9,0	-26,7	-25,8	-33,8	-16,5	-6,3	-	-	-		
19	-7,4	68,2	1,2	4,6	6,5	-9,8	-31,9	-44,8	-32,4	-38,9	-10,2	-24,1	-2,4	-	-	-		
20	-11,7	21,9	8,5	7,3	-12,5	-5,6	-49,9	-44,3	-38,7	-32,3	2,2	-8,6	-1,2	-	-	-		
Total	13 (20)	17 (19)	17 (20)	20 (20)	15 (20)	19 (20)	18 (20)		16 (20)		17 (20)		11 (18)	5 (10)	3 (10)	1 (10)		
	65%	89%	90%	100%	75%	95%	90%		80%		85%		61%	50%	30%	10%		

Figure 9. Individual and total percentage of change three days following the match (MD $_{+3}$) for main variables. Players who did not recover their baseline values at MD $_{+3}$ were grouped as non-recovered (red). Players who recovered their baseline values at MD $_{+3}$ were grouped as recovered (green). Dashed rectangles indicate players showing MD $_{+3}$ values below baseline (pre) in at least one leg or side in unilateral measurements (considered non-recovered). CK: creatine kinase; CMJ: counter movement jump; D: dominant; F0: theoretical relative (to body mass) horizontal maximal force; ILC: isometric leg curl; IPC: isometric posterior chain; LPC: lumbo-pelvic control; ND: non-dominant; V0: theoretical maximal horizontal velocity.

eccentric contractions during high-intensity sprinting actions (Proske & Morgan, 2001). In the context of sprint biomechanics, acknowledging the interaction between legs (commonly referred to as "switching") and the thigh separation is crucial, considering both performance and injury perspectives (Clark et al., 2017). The fact that the hamstring length was influenced more by the iliopsoas of the contralateral leg than by the hamstring itself during running (Thelen et al., 2006) and that peak elongation of the iliopsoas of the stance leg and the hamstring of the swing leg occur simultaneously justify the selection of the Jurdan test as a functional ROM measurement (Riley et al., 2010) to reflect the same leg interaction that occurs during sprinting. Continuing the examination of leg interaction as a critical indicator of injury and performance in sprinting, it is worth noting that the pelvis is the sole anatomical structure that connects both legs and facilitates the transfer of mechanical energy between them at a frequency of four or five times per second (Clark et al., 2017). From a hamstring injury perspective, during sprinting, APT has been found to be closely related to the phases (late swing/early stance) when the hamstring muscle – tendon tissues face the highest mechanical strain (main injury determinant) and potential hamstring strains occur (Danielsson et al., 2020). An increase in the APT through a posterior-superior translation of the ischial tuberosity would raise the eccentric load and elongation imposed on the hamstring musculature due to a greater moment arm derived from the increased hip flexion created (Mendiguchia et al., 2021). The aforementioned arguments may explain the association found between APT and hamstring injury risk in both prospective and retrospective studies (Daly et al., 2016; Schuermans et al., 2017). Although we did not find significant decreases in dynamic LPC, measured as an increase in APT during maximal speed sprinting, a heterogeneous response between players was observed (Figure 5), and 11 of them exhibited persistent dynamic LPC decrements at MD_{+3} (Figure 9). Therefore, it cannot be ruled out that football match play has the potential to anatomically alter the players' structure by increasing APT during the maximum speed phase of sprint. In this regard, Small et al. (2009), found a significant increase in APT under fatigue following a simulated football match. These authors employed the Soccer-Specific Aerobic Field Test (SAFT)^{90} protocol to replicate the physiological and mechanical demands of football match-play.

In summary, and according to the present results, from a sprint performance-related factors and HSI risk perspective, 72 h are not enough to recover from a real match, adding relevant hamstring-specific information to support the notion that football matches should be interspersed by more than 3 days to reduce individual injury risk, as previously suggested (Bengtsson et al., 2013; Silva et al., 2018). However, further research is needed to corroborate the present findings.

Biceps femoris long head fibre disruptions

For the first time, we report direct evidence of significant moderate increases in focal (myofibrillar disruption occupying two adjacent sarcomeres and associated Z-disks) BFlh fibre disruptions following a football match (MD_{+3}). Interestingly, the method quantifying myofibrillar disruptions (Gibala et al., 1995; Raastad et al., 2010) showed a heterogeneous response between players, ranging from no signs of BFlh myofibrillar disruption to one case of severe myofibrillar disruption (player 5). The mean proportion of fibres with myofibrillar disruptions was not correlated with muscle function (strength loss). In this regard, the large variation in the proportion of fibres with disruptions between biopsies from the same subject previously observed suggests that it may be difficult to detect the relationship between myofibrillar disruptions and changes in muscle function with single biopsies (Raastad et al., 2010). Moreover, it should be kept in mind that this association was previously found in a study that used a single joint exercise-induced muscle damage model (laboratory setting) that led to moderate-to-severe vastus lateralis myofibrillar disruptions in the majority of participants (Raastad et al., 2010). Therefore, in line with Warren et al. (Warren et al., 1999), who found that the histopathology of muscle fibres was poorly correlated with functional measurements, it is possible that this association only occurs when a large amount of damage is inflicted on a single muscle group and seen when at least two biopsies taken from different parts of the muscle are included (Raastad et al., 2010).

Anecdotally, in only some specimens at MD_{+3}, Z-disk streaming, autophagic vacuoles, hypercontracted fibre segments, and isolated necrotic fibres were observed (Figure 8). Moreover, sections stained with dystrophin (to label the sarcolemma) along with a basal lamina protein (merosin or laminin-2) (Shibuya et al., 2003) revealed unequal responses between players at MD_{+3} that might indicate remodelling processes such as basal membrane engrossment as a potential extracellular matrix (ECM) protective mechanism against strain (Hyldahl et al., 2015) (Supplementary material 2). Overall, and although anecdotal, these observations constitute the first evidence of BFlh structural alterations following a football match and reinforce the notion that this muscle, which is largely exposed to strain (the main determinant of tissue failure) during sprint acceleration (Thelen et al., 2006) suffers a certain degree of tissue damage in some players.

Therefore, although a heterogeneous response was observed between and within individuals, a real match has the potential to induce a certain degree of BFlh muscle tissue damage that is persistent at MD_{+3}. However, caution should be taken when universalizing these results since the present study used a single micro-biopsy sample that could exacerbate the inherent limitations of the biopsy technique itself (there remains the question of whether this small piece represents the entire muscle) (Paulsen et al., 2012).

Hamstring injury risk factors and match load

As stated before, at MD_{+3}, most players had not recovered their HSI risk factors, and histological evidence of BFlh tissue damage was reported at that time. Interestingly, none of these changes were associated with match load (both external [common metrics such as HSR, TD, ACC and DEC] and internal [RPE]). In this regard, according to a recent meta-analysis, there is a lack of evidence of an association between internal and external load metrics and injury risk in young football players (Verstappen et al., 2021). Only HSR (set at 19.7 $m \cdot s^{-1}$) has been linked to post-match (up to 24 h but not at 72 h) changes in general residual markers of muscle damage (CK) and physical performance (CMJ) (Hader et al., 2019). Accordingly, since GPS were introduced in football two decades ago to measure external load objectively, not only have hamstring injuries not decreased but they have actually increased (Ekstrand et al., 2023). The mechanical-psychological-physiological load-response pathways (Vanrenterghem et al., 2017) may be related to certain types of injury (Kalkhoven et al., 2021). In practice, however, load measures and metrics are limited in their ability to reflect either of these pathways, so a clear aetiology between sport injuries and load has yet to be established (Kalkhoven et al., 2021). Load monitoring may assist coaches in making informed decisions about player availability (especially regarding competition exposure time), but load responses such as HSI risk factor monitoring are of prime importance in this regard.

Since HSI risk factors can be altered at MD_{+3} independent of load levels, clubs should allocate their efforts to implementing robust football-contextualized, individualized multifactorial prevention strategies around the most prevalent injury in men's football, HSI (Ekstrand et al., 2023), in addition to (external [GPS metrics focused]) load monitoring.

Biceps femoris fibre composition and sprint performance: Implications for hamstring injury prevention?

In line with Evangelidis et al. (2017), who analysed BFlh fibre composition by measuring myosin heavy chain distribution in young active men, we found that amateur football players' BFlh exhibited an ATPase-based balanced fibre type distribution. While caution should be exercised due to the relatively small sample size analysed, this study presents novel direct evidence suggesting that BFlh muscle fibre composition in football players may not be predominantly "fast". Therefore, it appears that muscle fibre composition does not explain the high prevalence of HSI. However, the BFlh type II fibre percentage was correlated with sprint performance at baseline and at MD_{+3} (Figure 6(c,d)). From a practical perspective, this means that it is reasonable to expect that faster players will have fast BFlh. In comparison to type I fibres, type II fibres are more prone to damage (from eccentric contractions) because of their morphological and biomechanical characteristics – less elastic titin isoform, lower desmin content, narrower Z-disks, and short optimum length – a combination that results in lower sarcomeric integrity and a higher vulnerability to strain (Schiaffino & Reggiani, 2011). Moreover, type II fibres are more fatigable, and

their energy failure implies a great decrease in muscle power output, especially at fast movement rates (Schiaffino & Reggiani, 2011). The abovementioned characteristics could indicate why a high BFlh type II fibre percentage might constitute an HSI risk factor. However, a retrospective study recently suggested that amateur footballers with a prevalence of fast fibre typology (estimated by proton magnetic resonance spectroscopy for quantification of the semitendinosus muscle carnosine content) were not associated with HSI history (Schuermans et al., 2023). Currently, there is no conclusive evidence linking fibre composition to hamstring injury risk. Nonetheless, the mechanistic explanations provided above may suggest the potential for a high type II fibre percentage to be a contributing factor to HSI risk. However, if this was the case, a high percentage of type II fibres in the BFlh is essentially an unmodifiable risk factor, as what coach would willingly choose to make their players slower?

From our perspective, this reinforces the notion that prevention strategies should be focused on individual modifiable risk factors. As an example, player 14, who was the fastest player at baseline and showed the highest type II fibre percentage in his BFlh (65%) at MD_{+3}, also presented impaired ROM and strength at both posterior chain and knee joint levels on the dominant leg, impairments at F0 (but not V0) and a tilted pelvis at that time (Figure 9). Therefore, instead of a one-size-fits-all prevention training programme or a general load-based individualized approach (such as reducing player exposure time or individual HSR distance in specific tasks), an individualized multifactorial prevention approach centred on those specific altered risk factors would be more beneficial.

Conclusions

After analysing the time course and magnitude of acute changes in HSI risk factors following a football match in amateur football players by using a football-contextualized multifactorial approach accompanied by an optical and ultrastructural microscopic analysis (histological) of BFlh fibre structure, the question of whether 72 h is enough time to recover from a football match in terms of hamstring muscles perspective was investigated. Based on the main findings of the study, the answer appears to be negative. At MD_{+3}, most players had not recovered their sprint performance-related and HSI risk factors, and histological evidence of BFlh tissue damage was reported at that time. Additionally, none of these changes were associated with match load measurements. Lastly, the BFlh fibre composition in the investigated cohort of football players was not predominantly fast, suggesting that this factor may not contribute significantly to the high prevalence of injuries. However, the percentage of type II fibres was associated with sprint performance, and because these fibres are more prone to damage, it suggests that a high percentage of type II fibres could be a potential contributing factor to HSI.

Limitations

We acknowledge that professional players, who have higher physical fitness and are used to congested fixture periods, may not experience the magnitude of change in sprint performance-related and HSI risk factors reported in this study. However, the interindividual variability that can be induced by a real match strengthens the notion that understanding individual variability is fundamental to understanding individuals (Figure 9 illustrates how multiple combinations of pieces can represent the individual football match response puzzle). It is our opinion that, instead of directly generalizing the present results to professional football players, what should be highlighted from this study is the need to implement a football-contextualized multifactorial approach. This approach enables the classification of players based on their unique sprint mechanism-related HSI risk factor(s) profile. This understanding of individual responses, particularly from the perspective of hamstring muscles, is crucial, given that this muscle group is responsible for 24% of total injuries in modern football (Ekstrand et al., 2023). Future research analysing the time course and magnitude of acute changes in sprint performance-related factors and HSI risk factors following scenarios where football players' hamstrings are compromised using a football-contextualized multifactorial approach is warranted.

Finally, although the device employed for assessing sprint performance and mechanical properties utilizes friction encoder-based technology, ensuring high distance and temporal accuracy, future studies should be specifically designed to evaluate the validity and reliability of the device.

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Data availability of statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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