DOCTORAL THESIS

UCAM

UNIVERSIDAD CATÓLICA
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Doctoral Programme in Sport Science

FATIGUE MONITORING AND MECHANICAL PROPERTIES OF THE HAMSTRING MUSCLES IN FOOTBALL PLAYERS

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THESIS SUPERVISORS' AUTHORISATION FOR THESIS SUBMISSION

Prof. Pedro E. Alcaraz and Prof. Tomás T. Freitas, as Supervisors(1) of the Doctoral Thesis 'FATIGUE MONITORING AND MECHANICAL PROPERTIES OF THE HAMSTRING MUSCLES IN FOOTBALL PLAYERS' by Mr. Antonio Martínez Serrano in the Doctorate Programme in Sport Science, **authorise(s) its submission**, given that it meets the required conditions for its defence.

Which I hereby sign in compliance with Spanish Royal Decree 99/2011, of 28 January, in Murcia, on 8th July, 2024.

(1) If the Thesis is supervised by more than one Supervisor, both must be mentioned and both must sign.

ABSTRACT

The high prevalence and significant performance and economic impact of hamstring injuries in football present considerable challenges to teams and their players. Understanding the factors contributing to these injuries, including neuromuscular fatigue and its effects on muscle mechanical properties, is crucial for developing effective prevention and rehabilitation strategies. This thesis aimed to analyse neuromuscular fatigue in the hamstring muscles and its influence on maximal force production capacity, mechanical properties, and load-sharing strategies in healthy footballers compared to previously hamstring-injured players. Three studies were designed to address the objectives. In study 1, neuromuscular fatigue in the posterior chain muscles of young football players was assessed using the 90:20 isometric test after an in-season training session to evaluate the effects of different high-speed running demands. Whereas in Studies 2 and 3, shear-wave elastography was used to indirectly assess the stiffness of the biceps femoris long head and semitendinosus muscles. Specifically, in Study 2 the effects of different contraction intensities during a knee flexors submaximal isometric task until exhaustion on muscles' active stiffness and load-sharing were tested. Meanwhile, in Study 3, muscles' active stiffness and load-sharing strategies were compared between healthy football players and previously hamstring-injured players. The findings of this thesis revealed that the volume of high-speed running demands during a training session was the most effective global positioning system-derived external load variable when discriminating neuromuscular fatigue in the posterior chain muscles of young football players. Additionally, it was found that submaximal isometric knee flexor contractions to exhaustion at 20% and 40% of the maximal voluntary isometric contraction differently impacted the active stiffness of biceps femoris long head and semitendinosus muscles, highlighting different load-sharing strategies at different contraction intensities. Furthermore, in highly-trained footballers, the active stiffness responses of the biceps femoris long head and semitendinosus were similar during submaximal isometric contractions at 40% of the maximal voluntary isometric contraction until exhaustion. Consequently, load-sharing patterns between previously hamstring-injured and non-injured limbs were similar, indicating that a hamstring injury history may not influence muscle stiffness and intermuscular coordination under fatigue.

Keywords: Biceps femoris long head; neuromuscular; semitendinosus; shear-wave elastography; ultrasound; soccer; stiffness.

TÉRMINOS TESAURO: Fisiología muscular; Fisiología del ejercicio.

RESUMEN

La alta prevalencia e impacto negativo en el rendimiento y economía de las lesiones de la musculatura isquiosural en el fútbol presentan un desafío considerable para los equipos y sus jugadores. Comprender los factores que contribuyen a estas lesiones, incluida la fatiga neuromuscular y sus efectos en las propiedades mecánicas del músculo, es crucial para desarrollar estrategias efectivas de prevención y rehabilitación. El objetivo de esta tesis fue analizar la fatiga neuromuscular en la musculatura isquiosural y su influencia en la capacidad de producción de fuerza máxima, las propiedades mecánicas y la coordinación intermuscular en futbolistas sanos en comparación con jugadores previamente lesionados en la musculatura isquiosural. Se diseñaron tres estudios para abordar los objetivos. En el estudio 1, se evaluó la fatiga neuromuscular en los músculos de la cadena posterior de jóvenes futbolistas mediante la prueba isométrica 90:20 después de una sesión de entrenamiento durante la temporada para evaluar los efectos de diferentes demandas de carrera a alta velocidad. Mientras que en los Estudios 2 y 3, se utilizó la técnica de elastografía a través de ultrasonido para evaluar indirectamente la rigidez de los músculos cabeza larga del bíceps femoral y semitendinoso. Concretamente, en el estudio 2 se comprobaron los efectos de diferentes intensidades de contracción durante una tarea isométrica submáxima de los flexores de la rodilla hasta el agotamiento sobre la rigidez activa de los músculos y la coordinación intermuscular. Mientras tanto, en el Estudio 3, se compararon la rigidez activa de los músculos y las estrategias de coordinación intermuscular entre futbolistas sanos y jugadores previamente lesionados en la musculatura isquiosural. Los resultados de esta tesis revelaron que el volumen de carreras a alta velocidad durante una sesión de entrenamiento fue la variable de carga externa derivada del sistema de posicionamiento global más efectiva para discriminar la fatiga neuromuscular en los músculos de la cadena posterior de los jóvenes futbolistas. Además, se observó que las contracciones isométricas submáximas de los flexores de la rodilla hasta el agotamiento al 20% y 40% de la contracción isométrica voluntaria máxima impactaron de manera diferente en la rigidez activa de la cabeza larga del bíceps femoral y semitendinoso, destacando diversas estrategias de coordinación a diferentes intensidades de contracción. Asimismo, en futbolistas entrenados, las respuestas de rigidez activa de la cabeza larga del bíceps femoral y del semitendinoso fueron similares durante las contracciones isométricas submáximas al 40% de la contracción isométrica voluntaria máxima hasta el agotamiento. Por lo tanto, las estrategias de coordinación intermuscular entre los miembros previamente lesionados en la musculatura isquiosural y los no lesionados fueron similares, lo que indica que un historial de lesiones previas en la musculatura isquiosural podría no influir en la rigidez muscular y la coordinación intermuscular en condiciones de fatiga.

Palabras clave: Cabeza larga biceps femoral; neuromuscular; semitendinoso; elastografía; ultrasonido; fútbol; rigidez.

TÉRMINOS TESAURO: Fisiología muscular; Fisiología del ejercicio.

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A mi familia, amigos y a todas las personas que me han brindado su apoyo emocional y académico. Gracias de corazón.

‘Me enervan los que no tienen dudas y aquellos que se aferran a sus ideales sobre los de cualquiera.’ **Ideario, Francisco M. Ortega Palomares.**

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ACRONYMS AND ABBREVIATIONS

ACC, Accelerations

ACL, Anterior cruciate ligament

ACT, Activation

BAMIC, British athletics muscle injury classification

BF, Biceps femoris

BFlh, Biceps femoris long head

BFsh, Biceps femoris short head

BM, Body mass

CV, Coefficient of variation

DEC, Decelerations

ES, Effect size

GPS, Global positioning system

HSIs, Hamstring strain injuries

HSR, High-speed running

ICC, Intraclass correlation coefficient

KE, Knee extensors

MD, Match-day

MRI, Magnetic resonance imaging

MTU, Muscle-tendon unit

MVIC, Maximal voluntary isometric contraction

NMF, Neuromuscular fatigue

PCSA, Physiological cross-sectional area

PREV, Preventive

ROI, region of interest

RPE, Rating of perceived exertion

SM, Semimembranosus

SPM, Statistical parametric mapping

sRPE, Session rating of perceived exertion

SSG, Small-sided games

ST, Semitendinosus

SWE, Shear-wave elastography

TD, Total distance

TR, Training session

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I – INTRODUCTION

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1.1. FOOTBALL DEMANDS

Football, also known as soccer in some regions, is a team sport characterised by its dynamic and multifaceted nature that demands significant physical, physiological, and psychological aspects on its players. During the competition, players typically cover an average distance of 10-13 km, involving high-intensity running, sprints, changes of direction, and prolonged periods of low to moderate-intensity activity (1,2). Therefore, physiologically, players must develop their aerobic capacity to sustain prolonged activity and anaerobic capacity to perform high-intensity efforts intermittently (3). Additionally, players must develop neuromuscular performance, particularly in the lower limbs, to execute powerful movements such as sprinting, kicking, jumping, or tackling (2,4).

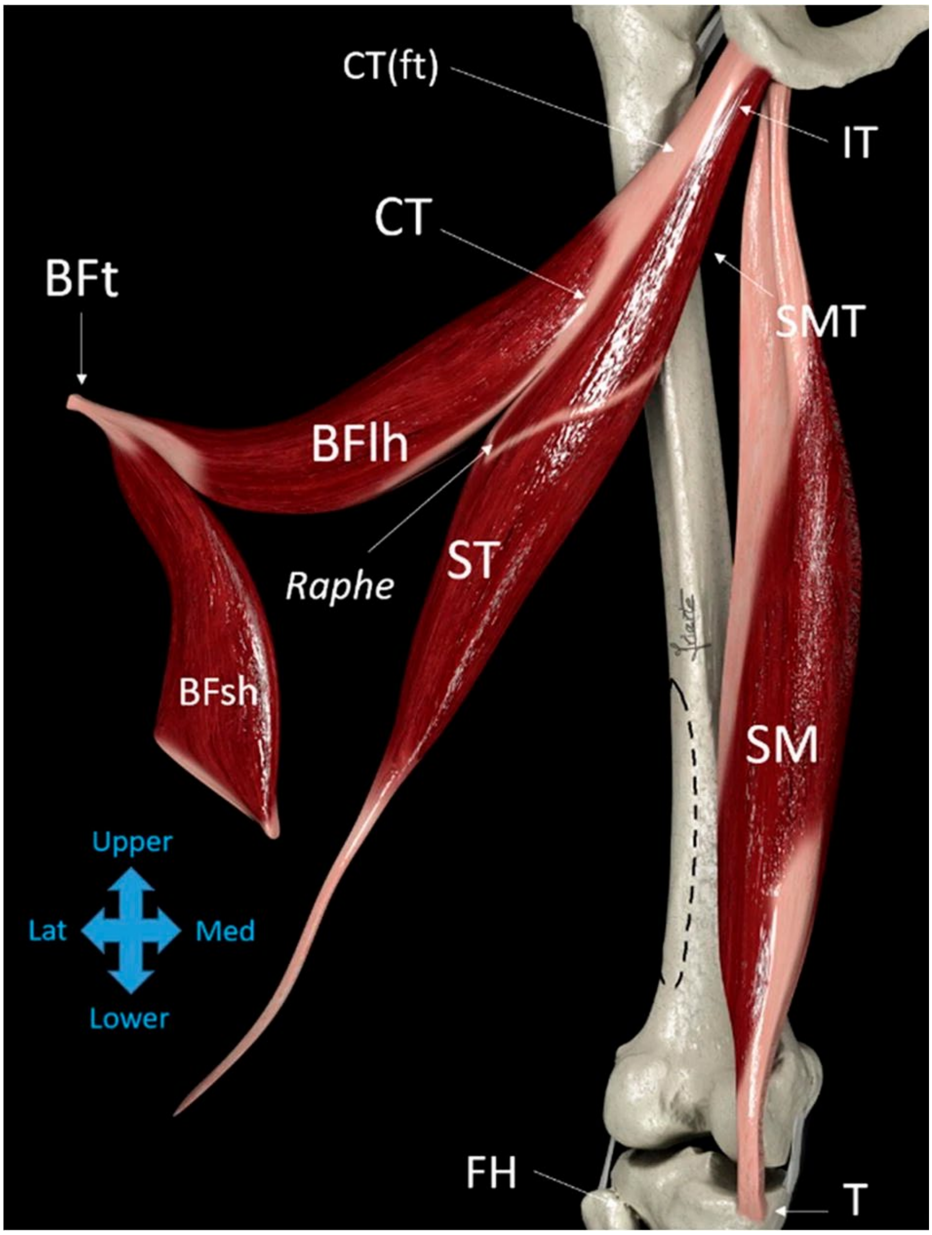
Over the past decades, the demands of football have evolved significantly due to advancements in sports science, changes in playing styles, and increased competition levels. These changes have led to an increase in the frequency and intensity of sprints, as well as a higher total distance (TD) covered during matches in modern football (5,6). Technological advancements in performance monitoring and analysis have further highlighted the increased physical demands on players, necessitating comprehensive conditioning and recovery strategies to prevent the occurrence of injuries (6,7).

Therefore, the performance and health of the lower limbs are crucial in football due to their role in nearly all fundamental actions of the sport. Consequently, players should develop and maintain an optimal lower limb function for both performance enhancement and injury prevention issues (8–10). Among the various lower limb injuries commonly seen in football, hamstring injuries are particularly prevalent and present significant challenges for football players and medical teams due to the considerable time loss and recurrence risks they imply (11,12).

1.2. HAMSTRING MUSCLES

The hamstrings are a complex and challenging muscle group, commonly injured in athletic populations (13,14). The muscles that comprise this group are the biceps femoris long head (BFlh) and short head (BFsh) on the lateral side, and the semitendinosus (ST) and semimembranosus (SM) on the medial side of the posterior thigh (15–18). Excluding the BFsh, the hamstring muscles cross the hip joint originating in the ischial tuberosity (19). Therefore, the primary functions of the hamstring muscles are knee flexion and hip extension, except for the monoarticular BFsh, which primarily functions as a knee flexor. Additionally, the BFlh and BFsh contribute to the external rotation of the hip, whereas the SM and ST assist in the internal rotation of the hip when the knee is partially flexed (20). The complexity of the hamstring muscles arises from the present significant variability in their distal attachments, tendon size, and muscle-to-tendinous tissue ratio (21), influencing their functional capabilities and architecture (22,23). The BFlh originates at the medial superior half of the ischial tuberosity and inserts, together with the distal tendon of the BFsh, to the head of the fibula and the lateral condyle of the tibia (19). Proximally, it shares a common tendon with the ST, the only hamstring muscle whose a portion of its fibres directly attaches to the posteromedial part of the ischial tuberosity (15). The ST and SM muscles run along the medial side of the posterior thigh to their respective attachment sites, with the SM inserting at the medial tibial condyle and the ST joining the sartorius and gracilis tendons to form the per anserinus attachment on the medial border of the proximal tibial shift (19) (Figure 1).

Figure 1. *Diagram of expanded hamstring muscles. BFlh biceps femoris long head, BFsh biceps femoris short head, ST semitendinosus, SM semimembranosus, SMT semimembranosus tendon, CT common tendon, CT(ft) free-tendon of the common tendon. The dotted line on the femoral diaphysis marks the linea aspera, the origin of the BFsh. IT ischial tuberosity, T medial side of the tibia, BFt distal BF tendon. From Balius et al. (18)*



Regarding their muscle architecture, the hamstring muscles are generally designed for high contractile and stretching velocity with low force production (24). This is contrary to its antagonist, the quadriceps musculature, due to their higher muscle mass and physiological cross-sectional area (PCSA) (25). Nevertheless, it is important to consider that not all hamstring muscles have the same properties and that architectural variability between muscles has been found (21,22,26). For example, the BFlh and SM display higher PCSA, pennation angle, and shorter fascicles whereas the ST and BFsh show longer fascicles and almost double the normalized fascicle length to muscle length ratio compared to BFlh and SM (21,22,25) (Table 1).

Table 1. *Descriptive architectural values of the hamstring muscles. BFlh biceps femoris long head, BFsh biceps femoris short head, SM semimembranosus, ST semitendinosus. Data retrieved from Kellis et al. (21, 281).*

	BFlh	BFsh	ST	SM
Pennation angle (°)	7.80	13.86	7.80	15.60
Fascicle length (cm)	7.99	11.72	13.59	6.22
Muscle length (cm)	29.56	21.17	27.71	25.83
Muscle length/Fascicle length ratio	0.27	0.55	0.49	0.24
Physiological cross-sectional area (cm^2)	14.73	5.81	8.51	22.82

These characteristics translate into ST and BFsh providing a superior excursion capacity, while BFlh and SM have greater force production (27–29). Tendon mechanical properties, regulated by the stress-strain relationship, also play an important role in how forces are transmitted from the muscle to the bone. Stiffer tendons, characterized by a steeper slope on the stress-strain curve, enhance force transfer efficiency (30), crucial for rapid muscle contractions (31) and high-impact movements (32). Conversely, a compliant tendon, which allows more stretch, necessitates greater shortening of muscle fascicles to compensate for this elasticity (33). While this can absorb excessive forces by dissipating energy (34,35), it potentially shifts muscle fibres to less optimal positions on the force-length curve (36–39), thereby decreasing muscle efficiency (39) and potentially increasing injury risk (40). Recently, Nakao et al. (41) explored the stress-strain relationship in complete hamstring muscles (i.e., tendon and muscle tissue) of human cadavers and found that the stress in each hamstring muscle subjected to the same strain was greater in the BFlh and SM than in the ST. The authors suggested that the considerably lower fascicle length, sarcomere length, and fascicle length/muscle length ratio of the BFlh and SM compared to ST could explain why these muscles are more prone to strain.

On the other hand, hamstring muscles also present intra-musculotendinous variations such as the tendinous inscription or "raphe" that divides the ST muscle into two compartments (16,42,43), regional variations in terms of muscle architecture (44), and aponeuroses morphology and function (45). The ST inscription has been hypothesized to improve force transmission from the fibres to the tendons (46,47), increase resistance to stretch (48,49), and therefore, it may protect the muscle against injuries. Although this "protective role" hypothesis still needs to be further proven, it is important to note that most of the injuries occur in the proximal site of the BFlh and SM (50), located near the ST inscription. Considering proximo-distal variability, the BFlh presents longer fibres with greater pennation angle proximally (44), implying greater force production and excursion capacity compared to its distal fibres (51), therefore increasing tissue strain in this region (27). Although similar observations have been detected for the ST, simulations have shown that the proximo-distal variability in parallel fibered muscles such as the ST presents little practical influence (27). Lastly, Rehorn et al. (52) showed that muscles that present a narrow proximal aponeurosis and wide distally such as the BFlh could increase strain at the musculotendinous junction area, making it more injury-prone compared to SM and ST with wider aponeuroses. However, heterogeneous inter-individual aponeurosis size has been found at the proximal region of the BFlh (53), hindering the understanding of this muscle-specific injury susceptibility.

These intra- and inter-musculotendinous variations could partly explain the high incidence of injuries in this complex muscle group and confirm that muscles within synergistic groups exhibit architectural variations that allow them to generate forces across a wider spectrum of magnitude, range, and velocity (21).

1.3. EPIDEMIOLOGY OF HAMSTRING INJURIES

Hamstring injuries are among the most common and limiting injuries in sports that involve high-speed running and sudden, explosive changes in speed or direction, making them particularly prevalent in different team sports (14). For example, from 2015 to 2019 in the United States National Football League, injuries located in the hamstrings represented 54.7% of the total lower extremity strains (54); in the Australian Football League, the most prevalent injuries were hamstring strains (19.1 missed games/club/season in 2015), even higher than anterior cruciate ligament (ACL) injuries (16.7 missed games/club/season in 2015) (55). Moreover, alongside concussion injuries, hamstring strains had the highest injury incidence (4.6 injuries/1000h) during three years in the rugby European Super League (56). Recently, Ekstrand et al. (12) presented relevant findings regarding the trend of hamstring injury rates during 21 seasons in men's professional football. The authors indicated a notable increase in the prevalence of hamstring injuries, which doubled from 12% to 24% of all injuries, and the proportion of total injury-related absences due to hamstring injuries also doubled from 10% to 20% (12). Notably, it was found that structural injuries prevailed over functional injuries during the most recent 11 seasons, suggesting an increase in injury severity, partially explaining the change in absence days (12). Consistent with prior research, the authors highlighted that: a) hamstring injuries typically occurred during running or sprinting activities (57); b) were more likely to occur in the final 15 min of either half of a match (58); c) predominantly affected the biceps femoris (BF) muscle compared to the SM or ST (59,60); and d) had a high recurrence rate within two months at the same location (61). This high injury incidence and prevalence are also supported by different professional football leagues (62–66), professional football clubs (67,68), and in youth players (69,70). Interestingly, hamstring strain injuries now represent 12% of all injuries in women's elite clubs, becoming the most frequent over anterior cruciate ligament injuries (71), suggesting a shift from ligament to muscle injuries as in men's football likely due to the increased high-intensity demands of match-play.

Moreover, the impact of hamstring injuries extends to team performance and economic aspects as well. For instance, teams with a high injury burden and low match availability are associated with lower league rankings (72), which could impact financially due to the underachievement of goals, salaries paid to the injured players, loss of players' worth, transfer fees, or loss of audiovisual rights. Notably, the financial cost of hamstring injuries has been established at 40,021A$ for a single hamstring strain injury in the Australian Football League (73), whereas, in professional European football, the financial burden is even higher at 90,367€/1000 h (74). For instance, the mean financial cost per year due to hamstring injuries in Spanish clubs participating in LaLiga® ranged from 719,480€ to 7M€ (75). As a result, professional football associations are increasing their dedication to implementing injury prevention methods and optimizing protocols to increase player availability and profitability (76).

1.4. HAMSTRING INJURIES CLASSIFICATION

Hamstring injury classification systems are essential tools for clinicians and researchers to diagnose, treat, and study hamstring injuries effectively. Various systems exist, differing in their use of imaging techniques, grading scales, identification of the specific anatomical tissues involved, and the mechanisms of injury they address. Despite the diversity of injury classification systems available, there is a consensus among experts that the British athletics muscle injury classification (BAMIC) (77), Munich (78), and (M)echanism (L)ocation (G)rading of severity – (R)e-injuries (79) methods are the most preferred (80). All the abovementioned systems use magnetic resonance imaging (MRI) as an imaging tool to diagnose and have a grading system based on the severity of the injury; however, they present different characteristics in terms of the specific criteria and measurement parameters used for the assessment of the injury. For example, the Munich system performs a differentiation between direct/indirect as well as functional/structural injuries, but it does not include the location (i.e., proximal, medial, or distal) contrary to the BAMIC and MLG-R systems. Despite having somewhat similar sub-grading systems based on the site and extent of the injury, the MLG-R is the only system to consider the injury mechanism and number of re-injuries. Regardless of the system used, it seems crucial for experts to consider: a) MRI as the main imaging tool for diagnosis; b) history and physical examination of the player; c) unified grading severity and anatomical description terminology; and d) individual muscles affected, mechanism of injury data, athlete susceptibility (i.e., age or previous injuries), and player demands (80). This information could improve the prognosis, treatment, and decision-making during the return-to-play process.

1.5. HAMSTRING INJURY MECHANISMS

Hamstring injury mechanisms are broadly categorized into two primary types: sprint-type and stretch-type (81). The sprint-type, predominantly observed in the BFlh, typically impacts the muscle-tendon junction (82). This injury is primarily attributed to increased strain on the muscle-tendon unit (MTU) due to the eccentric loading of the hamstrings throughout various phases of high-speed running (i.e., acceleration, deceleration, and maximal velocity phase) (83,84). Conversely, stretch-type injuries occur under conditions where the hamstrings are excessively elongated, such as during activities involving kicking or lunging. These movements often combine excessive hip joint flexion with an extended knee, primarily affecting the SM tendon tissue (85). The specific locations of the injury and tissue affected, while occasionally deviating from the aforementioned types, normally characterize stretch-type injuries with a prolonged rehabilitation and return to competition time compared to sprint-type injuries (86).

However, it is important to consider that this binary classification might oversimplify the diverse mechanisms leading to hamstring injuries. Such injuries may manifest combined biomechanical characteristics inherent to both sprinting and stretching actions. Recent studies employing in-depth video analysis have aimed to clarify the mechanisms and patterns underlying hamstring injuries in football players (57,87–89). These systematic analyses across various football leagues indicate that hamstring injuries generally occur in non-contact scenarios during rapid forceful actions. Moreover, the categorization of stretch-type injuries has been refined to distinguish between close and open-chain actions. Close-chain actions, characterized by intense decelerations, braking, stopping, or lunging, place high eccentric forces on the hamstring muscles at extended muscle lengths. In contrast, open-chain actions, typically involving kicking, result in overstretching of the hamstrings at long muscle lengths. Furthermore, Jokela et al. (2023) introduced the concept of a mixed-type injury mechanism, indicating an overlap between sprint and stretch-type mechanisms. Understanding these detailed injury mechanisms could allow the design of specialized prevention and readaptation programs that focus on replicating specific injury actions or movements and not solely implementing global resistance exercises.

Regarding the sprint-type mechanism, contrasting theories can be found in the literature, differing in terms of injury timing and contraction type. The timing of hamstring injuries during sprinting has been debated extensively, with prevailing theories focusing on either the late swing phase (90,91) or the early stance phase (92) of the gait cycle. The late swing phase is widely regarded as the most critical for hamstring injuries, particularly involving the BFlh (82). During this phase, the hamstring muscles, especially the BFlh, undergo large eccentric contractions to decelerate the leg in preparation for the foot strike (83,84). This action results in peak musculotendon strain and significant negative work, making the muscle fibres highly susceptible to overstretching. Musculoskeletal modelling studies support this, indicating that peak musculotendon stretch and force occur during the late swing, aligning with the highest observed risk of injury (93). Large force loads have been also found during the early stance; however, Chumanov et al. (94) showed that, only in the late swing phase, peak musculotendon forces of the BFlh increased significantly when increasing running velocity. These findings, together with the large muscle activation of the BFlh, support the hypothesis that the late swing phase is a high-risk phase for hamstring injury occurrence.

Conversely, some researchers argue that the early stance phase, when the foot first contacts the ground, also presents a significant risk for hamstring injuries (92,95). This phase involves a rapid shift from muscle lengthening during the late swing to muscle shortening as the body absorbs the peak loads produced by the high knee flexion and hip extension moments. The theory suggests that these abrupt changes in force and muscle activity, along with the strong counteracting ground reaction forces, create an injury-prone situation for hamstring injuries. However, this theory is less supported in the literature, lacking extensive biomechanical evidence to verify an increased injury risk relative to the late swing phase.

Considering both lines of thought, Liu et al. (96) proposed a more integrative approach suggesting that the transition between late swing and early stance should be considered as a continuous risk phase. This theory explains that the muscle is vulnerable not only due to the peak strain at the end of the swing but also due to the immediate loading impacts at the beginning of the stance. This perspective encourages for a broader understanding of the dynamic interactions and suggests that interventions should address the muscles' behaviour throughout these critical phases to effectively reduce injury risks.

Lastly, Van Hooren and Bosch (97) challenged the conventional interpretation of hamstring muscles functioning eccentrically during the swing phase of running. The authors explained that the hamstrings might predominantly exhibit isometric behaviour during this phase, challenging the assumption that an increase in the distance between the attachment points of the hamstring MTU necessarily indicates fascicular lengthening. Furthermore, Van Hooren and Bosch (97) argued that studies performing computational modelling could be oversimplifying complex aspects of muscle function by underestimating the effects of muscle slack (98) and muscle gearing (99). It is important to note that these authors do not exclude eccentric muscle action as the cause of hamstring injuries in favour of isometric action, but rather suggest that the contractile elements remain isometric until exceeding high levels of force during the late swing may cause an eccentric muscle action, probably causing the injury.

1.6. HAMSTRING INJURY RISK FACTORS

Although the nature of hamstring injuries has been suggested to be multifactorial, age and injury history have been identified as the most consistent risk factors for these injuries across different studies (100–103). The fact that players with a history of hamstring injuries would be at a higher risk of re-injury could be partially explained by tissue and neurological maladaptations during the rehabilitation process (104). The formation of non-functional scar tissue could predispose adjacent muscle fibres to undergo a higher strain during eccentric contractions, altering tissue lengthening mechanics (105), and reducing flexibility and eccentric strength capabilities (106–108). Considering neurological maladaptations, it has been proposed that neuromuscular inhibition could play a major role by decreasing the maximal voluntary activation of the muscles (102,104), as experienced in traumatic knee injuries requiring surgery (e.g., ACL injuries) (109,110). It is thought that the neuromuscular inhibition of the knee flexors is mainly found in eccentric actions performed at longer muscle lengths (111), probably due to the traditional rehabilitation process of limiting stretching and range of motion during the early and middle stages of the treatment. The fact that eccentric actions at longer muscle lengths are introduced at the last stages of the treatment could imply that players may already display persistent muscle atrophy (105,112,113), eccentric weakness (106,111,114–117), coordination issues (118–122), and a shift in the peak of the knee flexor torque-joint angle curve to much shorter muscle lengths due to reduced in-series sarcomeres (123,124). Notably, it has been found that individuals with a history of hamstring injuries presented lower BFlh fascicle length (125), larger BFlh proximal aponeuroses and smaller muscle-to-aponeurosis volume ratios (126) compared to healthy individuals. Therefore, the early introduction of high-intensity lengthening exercises in the rehabilitation process has been recently proposed as a safe and effective method to overcome these negative aspects (127,128). Nevertheless, further research should prospectively analyse if neuromuscular inhibition could be a causative factor for recurrent hamstring injuries.

Another aspect that has been shown to increase injury susceptibility is neuromuscular fatigue (NMF). NMF is a complex, multifaceted phenomenon that involves both central and peripheral components, affecting the ability of muscles to generate force or power (129–131). Fatigue-induced changes include a reduction in the hamstrings' ability to generate maximum voluntary force (132–136), decreased muscle stiffness (121,137,138), or modified running biomechanics (132,136,139–143). These changes can lead to a higher strain on the hamstring muscles during eccentric contractions, which are critical during the late swing phase of sprinting (144). Furthermore, fatigued muscles may exhibit delayed neuromuscular responses (145), which can compromise the stability of the knee joint and increase the risk of strain. During a football match, players perform repeated high-intensity activities such as sprints, jumps, and changes of direction (146,147), imposing significant neuromuscular demands and substantial fatigue on the hamstring muscles (148,149). Notably, studies have shown that hamstring injuries frequently occur in the latter stages of the first and second halves of matches (58). This timing seems to coincide with high NMF levels, characterized by decreased muscle force output and impaired neuromuscular function.

Besides the acute effects of NMF in match-play, it is important to consider the effective management of NMF within the microcycle for minimizing injury risk and optimizing performance. Typically, a competitive microcycle in football includes several types of training sessions and stimuli such as technical and tactical drills, high-intensity interval training, or strength and conditioning and recovery sessions. The interaction between the different external load demands and tasks within the microcycle (150) can increase neuromuscular and perceptual fatigue (151,152). Similarly, congested competition periods with insufficient recovery could lead to a cumulative fatigue effect, impairing neuromuscular function (152,153) and possibly increasing the likelihood of injuries (154). In this context, implementing strategies such as reducing training loads, including active recovery techniques, or monitoring NMF could help mitigate these detrimental effects. For example, different isometric tests have been applied, specifically in the hamstring muscles, to assess posterior lower-limb NMF in a simple, fast, and reliable way (149,155–157). The information provided by these tests might facilitate an early intervention that could decrease the likelihood of sustaining an injury (158). Moreover, it is becoming increasingly common for football clubs to incorporate external load monitoring through global positioning system (GPS) devices, both at professional level and in their youth academies. This allows strength and conditioning coaches to quantify the different patterns of external loads in their training sessions and possibly estimate the incidence of muscle injuries (159). However, these devices sometimes provide a large number of variables, requiring a specific selection of the ones that are closely associated with hamstring neuromuscular performance, if the objective is to monitor fatigue within the microcycle in this muscle group. In this case, the variable high-speed running (HSR) has been widely used due to the relation of increased running speeds with activation and kinematic demands of the hamstrings (160).

On the other hand, recent research has indicated that the force distribution among synergistic muscles in the hamstring muscle group, known as load-sharing or load-bearing, could potentially contribute to the incidence of hamstring injuries. Schuermans et al. (118) utilized MRI and T2 relaxation quantification to investigate the metabolic response of the hamstrings in amateur footballers with previous hamstring injuries. The researchers discovered that these individuals exhibited a more balanced metabolic response between the BF, ST, and SM muscles after performing an eccentric prone leg curl exercise until exhaustion. This response differed from the non-injured control group, suggesting that the injured individuals experienced a modified activity pattern, with the BF and SM compensating for the reduced activity in the ST. These findings were further contrasted in a subsequent prospective study, which demonstrated a significant association between elevated levels of metabolic activity in the BF after eccentric exercise and a marked decrease in ST assistance with the risk of experiencing an initial hamstring injury (119). Interestingly, Avrillon et al. (120) aimed to investigate if the intermuscular coordination of the hamstring muscles of injured and non-injured elite athletes was different. They found that the contribution of BF to total knee flexion torque was reduced in injured athletes, caused by decreased force-generation capacity of BF or increased contribution of the SM. Considering the modified force-sharing strategies of injured limbs found in these studies, it would be possible that this mechanism could constitute an intrinsic risk factor for hamstring injuries. However, the fact that these studies measured different muscle properties (i.e., metabolic activity, muscle activation) makes comparisons challenging. Moreover, limited evidence has been found considering the effects of hamstring injuries on the muscles' mechanical properties. Lastly, it is important to consider that the current determination of risk factors mainly follows a reductionist approach and, therefore, does not address the complex interactions between intrinsic and extrinsic factors, which limits the understanding of injury occurrence by simplifying the phenomenon.

1.7. STIFFNESS AND SHEAR-WAVE ELASTOGRAPHY

Understanding the mechanical properties of muscle tissue is essential for evaluating muscle function, performance, and adaptation under various conditions, including exercise, injury, and rehabilitation. Among the different passive (e.g., elasticity, viscoelasticity, stress, stress relaxation, creep, or hysteresis) and active (e.g., force-length-velocity relationships) mechanical properties of the skeletal muscle, stiffness is crucial in sports performance contexts. Stiffness or modulus, based on Hooke's law (161), is defined as a measure of a structure's resistance to deformation under an applied force (162) and is necessary for efficient force production and energy storage (163), particularly in activities requiring explosive movements or high-impact landings (164). In terms of injury prevention, inappropriate leg stiffness can predispose athletes to a higher risk of musculoskeletal injuries (165). In this regard, insufficient stiffness may lead to altered joint kinematics and increased strain on soft tissues (166), whereas excessive levels may result in increased peak forces and loading rates, thus increasing the risk of stress injuries (167). However, it is important to note that the leg stiffness would not necessarily reflect the stiffness of the individual muscles as results from an arrangement of contractile (i.e., synergists and antagonists) and non-contractile (i.e., tendon, skin, ligament, nerve, and articular structure) tissues in the hip, knee, and ankle joints. Therefore, investigating the specific stiffness of individual muscles and their impact on athletic performance and injury risk presents a compelling area of study. For instance, Miyamoto et al. (168) demonstrated that the passive stiffness of the vastus lateralis was negatively correlated with 100-m race times in sprinters but positively correlated with 5000-m race times in long-distance runners. This suggests that the role of muscle stiffness varies significantly with different athletic demands and distances.

In this regard, different methods have been used to assess skeletal muscle stiffness such as palpation, myotonometry, or magnetic resonance elastography (169–171). However, some of these methods do not provide objective measurements, have limited penetration depth, or are limited to measurements in passive conditions. Therefore, ultrasound-based shear-wave elastography (SWE) has been recently used to indirectly assess skeletal muscle stiffness by providing a measurement of muscle elasticity (172,173). Unlike conventional B-mode ultrasound, which visualizes tissues based on sound wave reflections, SWE measures the speed of shear wave propagation through tissues. The velocity of these waves is directly proportional to tissue stiffness, with stiffer tissues exhibiting faster propagation speeds. Initially, an acoustic radiation force impulse is generated by the ultrasound transducer, creating a localized displacement within the tissue. This displacement produces shear waves that propagate laterally from the point of generation. Then, ultrafast ultrasound imaging captures the displacement of tissue particles over time as the shear waves move through the tissue. The speed of these waves is calculated by tracking the time it takes for the waves to travel a known distance. The speed of shear wave propagation (shear wave speed) is then used to derive the shear modulus (μ) of the tissue, a measure of stiffness, using the equation:

$$\mu = \rho \cdot v_s^2$$

where ρ is the density of the tissue (in kg/m^3) and v_s the shear wave speed (in m/s). However, the stiffness of the tissue is often expressed in terms of Young's modulus (E) (174) in kPa, which is calculated using the equation:

$$E \approx 3\mu$$

SWE has been demonstrated to be a reliable tool for assessing muscle stiffness across passive (175–179) and active (121,137,138,180,181) conditions and in different muscle groups, even in pennate muscles (182) such as the BF (183). Notably, SWE can provide a valid in vivo estimation of individual muscle force, based on the linear force-stiffness relationship (184), being even more accurate than surface electromyography (185). This estimation has been further demonstrated in fatiguing isometric contractions by following changes in torque (186). Considering this, SWE could be used to note changes in the load-sharing between synergistic muscles, such as the hamstrings, during fatiguing constant-load isometric contractions. This could provide information on the mechanical properties of previously injured hamstring muscles and verify, as previously mentioned, if distinct load-sharing patterns could be considered a potential risk factor for hamstring injuries.

For instance, Freitas et al. (121) measured the BFlh and ST of previously injured limbs of elite footballers with SWE and found a lower absolute BFlh active stiffness and, consequently, a lower BFlh/ST ratio only at the beginning of a submaximal isometric contraction compared to healthy limbs. However, this study used a low contraction intensity, at 20% of the maximal voluntary isometric contraction (MVIC). Bearing in mind that at this intensity the active stiffness of the ST is much greater than that of the BFlh (i.e., a BFlh/ST ratio=0.42-0.50), it would be interesting to determine whether at a higher intensity level, the contribution of the BFlh, the most injured muscle, would be increased and therefore, greater differences in load-sharing between limbs would be observed throughout the contraction and not only at the beginning. Although a higher contribution of the BFlh at increased intensities (ranging from 10 to 70% of MVIC) has been observed in healthy individuals and under non-fatigued conditions by Mendes et al. (183)

and Evangelidis et al. (187), more information is needed to verify if the mechanical properties and load-sharing strategies of experienced footballers with a previous hamstring injury are affected under higher contraction intensities and fatigue conditions.

To conclude, based on the current literature, NMF, post-injury alterations in the mechanical properties of the muscle, and load-sharing among synergists could be identified as potential risk factors for hamstring injuries. However, there remains a need for a deeper understanding of the acute and residual effects of the multiple tasks and demands faced by football players during a competitive microcycle. Furthermore, identifying which GPS variables are most relevant for strength and conditioning coaches to monitor is crucial for effectively managing NMF in the posterior chain muscles, such as the hamstrings. Moreover, given the limited information on the impact of injury on muscles' mechanical properties and its potential inhibitory effects, it would be valuable to investigate whether muscle stiffness and coordination patterns change between BFlh and ST during submaximal contractions under fatigue conditions. Notably, these two synergistic muscles exhibit completely different injury rates, with BFlh being the most frequently injured and ST the least. Understanding these dynamics could provide critical insights into injury prevention and rehabilitation strategies that may help reduce the impact of these injuries.

II – OBJECTIVES

II - OBJECTIVES

2.1. GENERAL OBJECTIVE

The general objective of this compendium of articles is to analyse NMF on the hamstring muscles and its influence on maximal force production capacity, mechanical properties, and load-sharing in healthy footballers compared to previously hamstring-injured players.

2.2. SPECIFIC OBJECTIVES

The specific objectives for each study included in this thesis are detailed below:

Study 1:

- Determine which external load variable given by GPS better discriminates NMF on the knee extensors (KE) and posterior chain muscles in young soccer players.
- Analyse the acute and residual effects of different HSR demands during a training session in young soccer players on localised NMF of the posterior chain muscles.
- Examine if players with different HSR demands in the training session perceived the intensity differently.

Study 2:

- Compare the effects of knee flexors' isometric contraction until exhaustion performed at 20% vs 40% of MVIC on the active stiffness responses of BFlh and ST.
- Examine if the load-sharing strategies of BFlh and ST are different at 20% vs 40% of MVIC during a knee flexors' isometric contraction until exhaustion.

Study 3:

- Analyse BFlh and ST active stiffness during a submaximal contraction at 40% of MVIC until exhaustion between previously injured and non-injured limbs of highly-trained national-level male footballers.
- Determine if previously injured and non-injured limbs present different load-sharing patterns of BFlh and ST during a submaximal contraction at 40% of MVIC until exhaustion.
- Explore whether passive stiffness, knee flexors’ MVIC, and endurance capacity would differ between previously hamstring-injured and non-injured limbs.

III – HYPOTHESES

III - HYPOTHESES

3.1. GENERAL HYPOTHESIS

It is hypothesised that the NMF will decrease the maximal force production capacity of the hamstrings and football players with a history of hamstring injuries will exhibit altered mechanical properties and load-sharing strategies in their hamstrings compared to healthy players, potentially indicating a predisposition to re-injury.

3.2. SPECIFIC HYPOTHESES

The specific hypotheses for each study included in this thesis are detailed below:

Study 1:

- HSR will better discriminate the NMF of the posterior chain muscles while variables related to the number of accelerations (ACC) and decelerations (DEC) will be linked to the NMF of the KE.
- Players with higher HSR demands will present an acute decrease in the neuromuscular performance of the posterior chain muscles. This decreased performance will be maintained after 24h of the training sessions.
- Players with high HSR demands will present higher rating of perceived exertion (RPE) values compared to the players who performed lower HSR demands.

Study 2:

- During the 40% of MVIC contraction intensity, the BFlh would have a greater active stiffness increase with a lower ST active stiffness decrease.
- At 40% of MVIC, the BFlh/ST active stiffness ratio would be higher than at 20% of MVIC.

Study 3:

- The BFlh active stiffness levels of previously hamstring-injured limbs would be decreased in non-fatigued states.
- Due to the lower BFlh active stiffness at the beginning of the contraction, previously hamstring-injured limbs would present a lower BFlh/ST ratio and it will have a steeper increase in the presence of fatigue.
- No differences would be observed for muscles' passive stiffness, knee flexors' MVIC, and endurance capacity between the previously hamstring-injured and non-injured limbs.

IV – GENERAL OVERVIEW OF THE STUDIES

IV - GENERAL OVERVIEW OF THE STUDIES

4.1. STUDY 1: DOES EXTERNAL LOAD REFLECT ACUTE NEUROMUSCULAR FATIGUE AND RATING OF PERCEIVED EXERTION IN ELITE YOUNG SOCCER PLAYERS?

Abstract

This study aimed to analyse the acute and residual effects of increased HSR demands during an in-season training microcycle in young elite soccer players on localized NMF of the KE, posterior chain muscles, and RPE. Thirty-four elite young soccer players (age = 17.1 ± 0.8 years) were assessed on 2 consecutive days at different time points (baseline, POST-activation gym-based session [ACT], POST-small-sided game [SSG], POST-training 1 [TR1], POST-6H, POST-24H, POST-preventive gym-based session [PREV], and POST-training 2 [TR2]). NMF of the KE and posterior chain muscles was measured with a MVIC. External (TD, number of ACC or DEC, and HSR distance) and internal (RPE) loads were assessed during the SSG, TR1, and TR2 sessions. Players were divided through a median split, into "HIGH" or "LOW" groups according to the training demands. The alpha level was set at $p \leq 0.05$. A 2-way mixed effects model ANOVA showed a significant decrease in 90:20 MVIC after TR1 in the "HIGH" HSR group ($p = 0.037$; effect size [ES] = 0.45). No significant differences in RPE were found after TR1 ($p = 0.637$; ES = 0.58) and TR2 ($p = 0.109$; ES = 0.62) when comparing the "HIGH" HSR group with the "LOW" HSR group. Assessing players' force production capabilities can be an effective strategy to detect NMF when HSR demands are acutely increased. Special caution should be taken when prescribing the training load of the training session based solely on RPE, as NMF might be present.

4.2. STUDY 2: CHANGES IN HAMSTRINGS' ACTIVE STIFFNESS DURING FATIGUE TASKS ARE MODULATED BY CONTRACTION DURATION RATHER THAN INTENSITY

Abstract

The mechanical characteristics of muscles have been studied using ultrasound-based SWE, allowing the determination of muscle stiffness and load distribution. However, despite NMF impairing hamstrings' function, the active stiffness of BFlh and ST muscles under fatigue conditions at various contraction intensities has not been explored. This study aimed to compare the effects of knee flexors' isometric contraction until exhaustion performed at 20% vs. 40% of MVIC, on the active stiffness responses of BFlh and ST. Eighteen recreationally active males performed two experimental sessions. The knee flexors' MVIC was assessed before the fatiguing task, which involved a submaximal isometric contraction until failure at 20% or 40% of MVIC. Active muscle stiffness of the BFlh and ST was assessed using SWE. BFlh active stiffness remained relatively unaltered at 20% of MVIC, while ST active stiffness decreased from ≅91% contraction time (55.79 to 44.52 kPa; $p<0.001$). No intramuscular stiffness changes were noted in BFlh (36.02 to 41.36 kPa; $p>0.05$) or ST (63.62 to 53.54 kPa; $p>0.05$) at 40% of MVIC session. Intermuscular active stiffness at 20% of MVIC differed until 64% contraction time ($p<0.05$) whereas, at 40% of MVIC, differences were observed until 33% contraction time ($p<0.05$). BFlh/ST ratios were not different between intensities (20%=0.75±0.24 ratio vs. 40%=0.72±0.32 ratio; $p>0.05$), but a steeper increase in BFlh/ST ratio was found for 20% (0.004±0.003 ratio/%) compared to 40% (0.001±0.003 ratio/%) of MVIC ($p=0.003$). These results suggest that contraction duration could play a major role in inducing changes in hamstrings' mechanical properties during fatigue tasks compared to contraction intensity.

4.3. STUDY 3: HIGHLY-TRAINED FOOTBALLERS WITH AND WITHOUT PREVIOUS HAMSTRING INJURIES HAVE SIMILAR BICEPS FEMORIS LONG HEAD AND SEMITENDINOSUS STIFFNESS RESPONSES DURING A KNEE FLEXORS' SUBMAXIMAL ISOMETRIC CONTRACTION UNTIL EXHAUSTION

Abstract

Despite various proposed prevention strategies, the incidence of hamstring injuries in modern soccer is still elevated. Recent research has focused on exploring how muscle tissue stiffness behaves under fatigue conditions as a potential risk factor. This study aimed to examine the active stiffness of BFlh and ST muscles using ultrasound-based SWE during a knee flexors' submaximal contraction until exhaustion in highly-trained national-level male footballers, comparing previously injured and non-injured limbs. A cross-sectional study was performed including 94 highly-trained male footballers. Using SWE, we assessed the passive and active stiffness of the BFlh and ST at rest and during a knee flexors' submaximal isometric contraction at 40% of MVIC until exhaustion. Differences in stiffness patterns between previously hamstring-injured and non-injured limbs were analysed, along with passive muscle stiffness, knee flexors' MVIC, and endurance capacity. No significant differences in the active stiffness of BFlh and ST between previously hamstring-injured and non-injured limbs throughout the contraction task were found ($p>0.05$; 0-100% contraction time). Similarly, there were no differences in BFlh (mean diff.=0.2 kPa; $p>0.05$) and ST (mean diff.=0.9 kPa; $p>0.05$) passive stiffness, knee flexors' MVIC (mean diff.= -8.5 Nm; $p>0.05$), or time to exhaustion (mean diff.= 6.95 s; $p>0.05$). Load-sharing between the BFlh and ST did not change significantly throughout the contraction ($p>0.05$; 0-100% contraction time). These results suggest that players with a history of hamstring injuries may retain similar mechanical properties and coordination strategies as non-injured players.

V – STUDY I

V - STUDY I

DOES EXTERNAL LOAD REFLECT ACUTE NEUROMUSCULAR FATIGUE AND RATING OF PERCEIVED EXERTION IN ELITE YOUNG SOCCER PLAYERS?

5.1. INTRODUCTION

Throughout the years, muscle injuries have consistently constituted a substantial concern for players and clubs in modern soccer, both at professional and amateur levels (188). The hamstring muscles are particularly susceptible to injury, with an incidence rate in soccer players ranging from 0.3 to 1.9 injuries per 1,000 h exposure and a high recurrence rate (4–68%) (11) that negatively affects team performance. Despite the fact that numerous exercise-based prevention protocols have been proposed to reduce the risk of muscle injuries (189–191), the overall muscle injury rate has not decreased in the past 18 years, neither in training nor in matches (192).

This phenomenon might be attributed to the greater number of high-intensity actions (e.g., ACC, DEC, HSR distance, changes of directions, jumps, kicks, or tackles) performed in match-play (6), as well as because of the congested competitive calendar that players usually face during the season, where the recovery times are limited, or even incomplete (193), thus potentially increasing the risk of injury. Therefore, to reduce the likelihood of players suffering an injury, sports practitioners conduct sessions with different objectives and stimuli (e.g., resistance training sessions, SSG, tactical sessions, or real match-play situations) to prepare the athlete to cope with the increased match demands. Those capable of adapting to the training load will be better protected in real-match situations (3), thus reducing the risk of injury. However, if the implementation of these injury prevention sessions is performed inadequately (i.e., prolonging the absence of training in the off-season, performing high-eccentric load exercises 48 h before the competition (194), or planning excessive tactical training that underestimates critical attributes such as HSR (195), it might have opposite effects to those initially intended (196).

Hence, it becomes important to assess the effects that the training sessions performed throughout the season have on player's physical performance as a result of NMF (197). Although adequate and sufficient training stimuli are necessary to achieve neuromuscular adaptations, significant increases in acute fatigue during the competitive microcycle should be avoided due to the limited recovery time and the wide diversity of training contents, which could negatively affect muscle activation and coactivation, biomechanical properties, leg stiffness, and reactive strength (198). Consequently, focusing specifically on the hamstring muscles, different isometric tests have been developed to assess posterior lower-limb NMF in a simple, fast, and reliable way (149,155,157) and to identify neuromuscular imbalances between dominant and non-dominant legs. The implementation of these tests might be a useful strategy to evaluate the NMF of specific muscle groups after the match, facilitating an early intervention that could decrease the likelihood of sustaining an injury (158).

Another key aspect to optimize performance and increase player availability is to monitor training and competition loads, specifically in soccer, in which cycles of training, tapering, competition, and recovery are repeated systematically during the competitive microcycle. As such, most teams have integrated GPS with built-in accelerometers (199) and measurements of the players' RPE to analyse external and internal load, respectively. The first instrument (i.e., GPS) allows sport scientists and strength and conditioning coaches to assess players' performance, physical stress, physiological demands, and several external load metrics (200,201) during the training session. The second (i.e., RPE), aimed at assessing internal load, allows the understanding of players' global perception of effort after practice, enabling the development of training load planning strategies based on these values (202). Of note, the sensitivity of RPE to detect specific alterations at the muscular level (i.e., localized), in response to different external load demands in young soccer players, is not clear (203). Indeed, it is possible for local NMF to be produced despite players perceiving the session (as a whole) as low or moderate intensity. As such, if the training load distribution is performed exclusively considering RPE values without assessing local NMF, the risk of suffering a muscle injury because of an inadequate load programming could be increased (204).

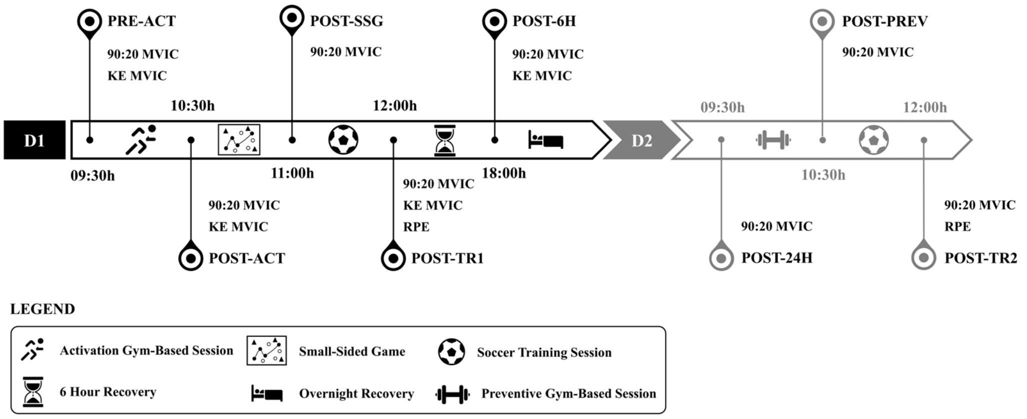
Therefore, this research seeks to analyse the acute and residual effects of different HSR demands (high and low, as divided through a median split) during a training session in young soccer players on localized NMF of the KE, posterior chain muscles, and RPE. We hypothesize that increasing HSR demands would cause a significant impairment in the neuromuscular performance of posterior chain muscles, while the KE force production capabilities would remain relatively unaffected. Moreover, players with increased HSR demands would report higher perceived exertion values.

5.2. METHODS

5.2.1. Experimental approach to the problem

A cross-sectional, repeated-measures study design was used. Players were assessed on 2 consecutive days at different time points (Figure 2). On day 1 (match day [MD-4]), players completed 2 isometric strength tests (i.e., KE and posterior chain muscles): (a) at rest, before an ACT gym-based session; (b) immediately after the ACT gym-based session and before a SSG (4v4 + 3) activity where 4 attackers with the assistance of 3 floaters had to maintain ball possession against 4 defenders; (c) immediately after the SSG task (only posterior chain muscles were assessed at this time point); (d) immediately after the soccer TR1; and (e) 6 h after the end of the morning soccer training. On day 2 (MD-3), the posterior chain muscles assessment was performed: (a) at rest, before a PREV gym-based session (coinciding with ~24 h after the MD-4 session); (b) immediately after the PREV gym-based session; and (c) after the soccer TR2. Players were divided into groups of 4 to ensure a constant recovery time (1–2 min) between tasks and isometric strength tests. Owing to time constraints, frequent in applied research settings, KE force was not assessed on day 2. Players' external load during the SSG, TR1, and TR2 were assessed using a WIMU PRO Local Positioning System (RealTrack Systems, Almeria, Spain). The training session's content was based on a typical in-season microcycle and defined by the team's coaching staff. On TR1, players performed a 6v6 + 2 goalkeepers match on a 40 x 40 m space and a 10v8 offensive-defensive transition in 3/4 of the soccer field. On TR2, they performed a 7v7+3 game situation on a 90 x 60m space and a 10v8 offensive-defensive transition in 3/4 of the soccer field. Both sessions had an approximate duration of 90 min.

Figure 2. *Timeline of neuromuscular fatigue assessment during 2 days of the in-season competitive microcycle. 90:20 = isometric posterior chain test; ACT = activation gym-based session; KE = knee extensors; MVIC = maximum voluntary isometric contraction; PREV = preventive gym-based session; RPE = rating of perceived exertion; SSG = small-sided game; TR1 = training session 1; TR2 = training session 2.*



5.2.2. Subjects

The sample consisted of 34 postpeak height velocity (from 2 to 5 years postpeak) elite male young soccer players (age ranging from 16 to 18 years, mean = 17.1 ± 0.8 years; body mass [BM] = 62.9 ± 7.9 kg; height = 168.0 ± 32.2 cm). Players were excluded from the study if they were taking any nutritional supplementation that could influence test results or if they had suffered a lower-limb musculoskeletal injury 6 months before the beginning of the study. They were already familiarized with the gym-based sessions, SSG task, and training sessions. Each player provided individual consent to participate in the study as part of the team requirements. Moreover, after reading the information sheet, a parental or guardian informed consent form was obtained. The Ethics committee of the Catholic University of Murcia approved the study's procedure (CE022106), conforming to the Declaration of Helsinki.

5.2.3. Procedures

5.2.3.1. *Knee extensors maximal voluntary isometric contraction*

To assess KE MVIC, players sat upright in a leg extension machine (Technogym, Cesena, Italy), with the dominant leg flexed at 90° and the ankle strapped directly to a load cell (Model SML500; Interface Scottsdale, AZ) that was fixed to a customized apparatus. Subjects performed 3 trials of MVIC, each lasting for 3 s (intraclass correlation coefficient [ICC] = 0.978; coefficient of variation [CV] = 6.20%). 2 min of rest were given between contractions. To ensure maximal effort, subjects were encouraged verbally and at least 2 MVICs had to be within 10% of one another. The highest peak force trial was used for analysis.

5.2.3.2. *Posterior chain maximal voluntary isometric contraction*

The posterior chain MVIC was assessed with the 90:20 test, previously shown to be a reliable method (157). Players stood with their glutes, upper back, head, and non-tested leg heel against a wall; arms crossed on chest; and hands touching the opposite acromion. The dominant leg's heel was positioned on the centre of a force platform, sampling at 1,000 Hz (Kistler 9286BA, Kistler Group, Winterthur, Switzerland), and placed on a customized wooden box. The height of the box and its distance to the wall were individually adjusted so that each player was tested in a 90° and 20° of hip and knee flexion, respectively (measured using a goniometer). Subjects were instructed to "exert maximal force vertically into the force plate" for 3 s. To ensure that the player's initial whole-body position was kept as described above during the test, a researcher applied the necessary amount of force to prevent the non-test leg from flexing. The time between trials was 2 min. The dominant leg was tested twice (ICC = 0.981; CV = 13.35%), and the highest peak force value, obtained instantly from ForceDecks software (Vald Performance, Brisbane, Australia), was retained for analysis.

5.2.3.3. *Activation and preventive gym-based sessions*

The ACT session was focused on displacements, ACC and DEC, and jumps and included the following exercises: (a) 2 x 10 barbell glute bridge from the bench (in a loading scheme in which players left 3 repetitions in reserve); (b) 2 x 10 ab wheel crunches; (c) 2 x 2 15-m resisted sprint (10–20% BM); (d) 2 x 10 unilateral backwards displacement with flywheel; (e) 2 x 8 unilateral oblique turns with flywheel; (f) 2 x 2 10-m ACC and DEC sprints; (g) 2 x 10 yo-yo squats; (h) 2 x 10 isometric ab crunch on BOSU; and (i) 2 x 2 3 hurdle jumps and 2 headers. Exercises a, c, d, f, g and i were performed at maximum speed, whereas exercises b, e and h were executed in a controlled manner. On the other hand, the PREV session specially targeted the hamstrings and groin muscles. This session included (a) 2 x 10 unilateral lying flywheel hamstring kicks, (b) 2 x 10 unilateral lying quadriceps eccentric contraction with elastic bands, (c) 2 x 10 eccentric Nordic hamstring, (d) 2 x 10 ab wheel crunches, (e) 2 x 10 unilateral fitball glute bridge, (f) 2 x 10 Copenhagen hip adduction, (g) TRX plank, and (h) 2 x 10 side lying clams with an elastic band. In this session, players were asked to control the exercise's speed execution. The ACT and PREV sessions lasted approximately 21–22 min.

5.2.3.4. *Soccer-specific activity profile*

Players wore a local portable positioning system WIMU PRO (RealTrack Systems) equipped with 4 3D accelerometers (full-scale output ranges ±16 g, ±16 g, ±32 g, ±400 g at 100 Hz sample frequency), 3 gyroscopes ($8000°\cdot s^{-1}$ full-scale output range at 100 Hz sample frequency), a 3D magnetometer (100 Hz), a GPS unit (10 Hz), and a UWB (18 Hz). The system used has been previously shown to be valid and reliable for time-motion analysis in soccer (205). Devices were placed inside an adjustable vest located at the upper back of each player. The data collected in the SSG task, TR1, and TR2 were analyzed using the proprietary software (WIMU Software; Realtrack Systems SL). The following external load variables were collected: TD covered, number of total ACC and DEC, and HSR distance (>19.8 $km\cdot h^{-1}$) covered.

5.2.3.5. *Rating of perceived exertion*

Borg's CR10 scale was used to measure the perception of players' exertion after TR1 and TR2. This scale is comprised of 11 points ranging from 0 to 10, 0 being "no exertion at all" and 10 "maximal exertion" (206).

5.2.4. Statistical analyses

The statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA). The Shapiro-Wilk test was used to assess the normality of the distribution of the variables. The external load metrics (i.e., TD, ACC, DEC, and HSR) were not modified by the research team at any time. They were only retrospectively used to divide the players (i.e., after the end of the experimental period, when analysing the data) into "HIGH" or "LOW" groups, through a median split based on the summated external load for each variable after TR1. This was performed to investigate whether a specific metric could be used to discriminate NMF levels of the KE and the posterior chain. A 2-way repeated measure ANOVA with Tukey's multiple comparisons test was used to analyse whether any change in the KE MVIC values after the TR1 were the result of the interaction between the "external load demands" (i.e., HIGH and LOW demands) and "time point" (i.e., PRE-ACT, POST-ACT, POST-TR1, and POST-6H). A 2-way mixed effects model ANOVA (because of the presence of some missing values: 5 and 6 in the "HIGH" and "LOW" groups, respectively, across all 8 time points) with Tukey's multiple comparisons test was performed to analyse whether any change in the 90:20 MVIC values after the SSG task, TR1, and TR2 was the result of the interaction between the "external load demands" (i.e., HIGH and LOW) and "time point" (i.e., PRE-ACT, POST-ACT, POST-SSG, POST-TR1, POST-6H, POST-24H, POST-PREV, and POST-TR2). Finally, a 2-way ANOVA with Tukey's multiple comparisons test was used to assess whether significant differences were detected between the "HIGH" and "LOW" HSR group in RPE after TR1 and TR2. If sphericity was not met, the Greenhouse-Geisser correction was applied. Cohen's d ES for each comparison were calculated manually in Microsoft Excel 16.36 (Microsoft, Redmond, Washington) and were classified as trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2), very large (2–4), and near perfect (>4) (207). Data are presented as mean ± SD. The alpha level was set at $p \leq 0.05$.

5.3. RESULTS

Table 2 provides the descriptive data of the external and internal load during the SSG task, TR1, and TR2.

Table 2. *Descriptive data (mean ± SD) of the summated external load variables and RPE after TR1 (time point when the median split was performed).**

	Group	TR1
TD (m)	**High**	7139.321 ± 895.04
	Low	4151.167 ± 702.57
HSR (m)	**High**	290.703 ± 199.63
	Low	10.610 ± 9.49
ACC (no.)	**High**	54.235 ± 15.20
	Low	29.235 ± 4.98
DEC (no.)	**High**	53.167 ± 14.02
	Low	27.688 ± 6.39
RPE (1-10)	**High**	6.824 ± 1.29
	Low	6.000 ± 1.46

ACC = accelerations; DEC = decelerations; HSR = high-speed running; m = meters; no. = number; RPE = rating of perceived exertion; TD = total distance; TR1 = training session 1.

No significant effects were found in KE MVIC peak force after TR1 and POST-6H between the players in the "HIGH" or "LOW" group in TD (F = 1.155; p = 0.332), ACC (F = 0.450; p = 0.718), DEC (F = 0.185; p = 0.906), and HSR (F = 0.153; p = 0.928) (Table 3).

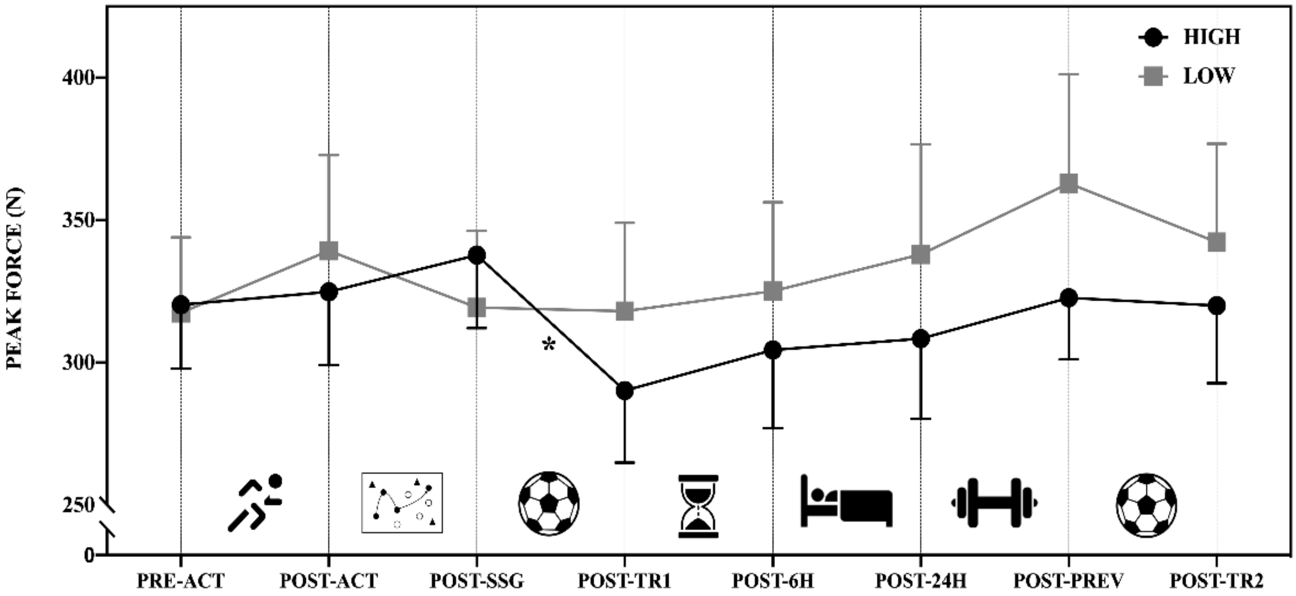
Table 3. *Knee extensors maximum voluntary isometric contraction after TR1 and POST-6H according to external load demands.**

	Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P value	ES
TD	**HIGH**				
	POST-ACT vs. POST-TR1	0.231	-28.33 to 28.80	>0.999	0.00
	POST-TR1 vs. POST-6H	-11.350	-48.62 to 25.93	0.816	0.09
	LOW				
	POST-ACT vs. POST-TR1	3.465	-23.69 to 30.62	0.982	0.03
	POST-TR1 vs. POST-6H	-2.406	-29.36 to 24.54	0.994	0.02
HSR	**HIGH**				
	POST-ACT vs. POST-TR1	2.758	-27.06 to 32.57	0.993	0.02
	POST-TR1 vs. POST-6H	-11.310	-48.16 to 25.55	0.813	0.09
	LOW				
	POST-ACT vs. POST-TR1	0.770	-24.82 to 26.36	0.999	0.01
	POST-TR1 vs. POST-6H	-2.451	-30.07 to 25.17	0.994	0.02
ACC	**HIGH**				
	POST-ACT vs. POST-TR1	0.342	-31.27 to 31.95	>0.999	0.00
	POST-TR1 vs. POST-6H	0.947	-30.71 to 32.60	0.999	0.01
	LOW				
	POST-ACT vs. POST-TR1	3.347	-19.57 to 26.26	0.973	0.03
	POST-TR1 vs. POST-6H	-15.520	-48.94 to 17.90	0.549	0.13
DEC	**HIGH**				
	POST-ACT vs. POST-TR1	-1.747	-37.28 to 33.79	0.999	0.01
	POST-TR1 vs. POST-6H	-5.362	-40.90 to 30.17	0.979	0.04
	LOW				
	POST-ACT vs. POST-TR1	5.575	-31.13 to 42.28	0.979	0.05
	POST-TR1 vs. POST-6H	-8.791	-45.49 to 27.91	0.923	0.08

ACC = accelerations; ACT = activation gym-based session; CI = confidence interval; DEC = decelerations; ES = effect size; HSR = high-speed running; TD = total distance; TR1 = training session 1.

After performing a median split analysis for all external load variables, no significant differences were found in 90:20 MVIC between "HIGH" and "LOW" groups based on TD (F = 1.324; p = 0.240), ACC (F = 0.518; p = 0.821), and DEC (F = 0.485; p = 0.845). Only HSR was found to discriminate posterior chain NMF when comparing "HIGH" and "LOW" groups. As such, when considering the latter variable, 90:20 MVIC peak force decreased significantly after TR1 in the "HIGH" HSR group (p = 0.037; ES = 0.45 [-0.24 to 1.12]) (Figure 3). Descriptive data are reported in Table 4.

Figure 3. *Development of posterior chain neuromuscular performance according to HSR distance demands performed in training session 1. Error bars indicate the standard error of the mean. ACT = activation gym-based session; PREV = preventive gym-based session; SSG = small-sided game; TR1 = training session 1; TR2 = training session 2.*



No significant differences were found after the SSG task and TR2 between those in the "HIGH" or "LOW" groups in TD, HSR, ACC, or DEC (Appendix 1).

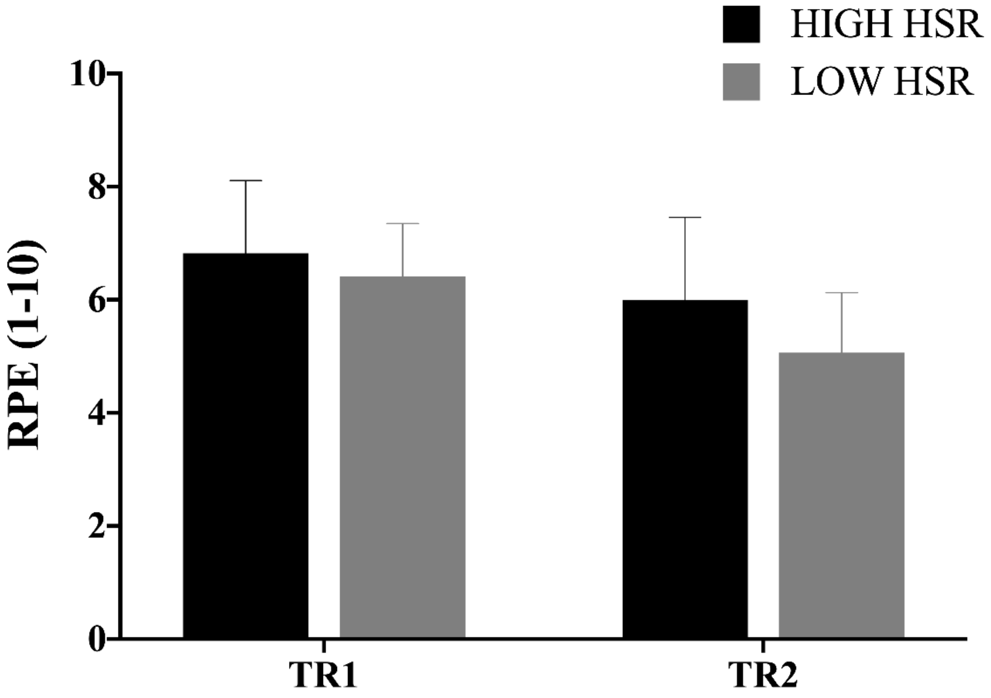
Table 4. *Descriptive data of 90:20 maximum voluntary isometric contraction according to HSR demands performed in TR1 shown as mean ± SD.**

	"HIGH" HSR	**"LOW" HSR**
PRE-ACT	320.30 ± 92.7	317.44 ± 109.4
POST-ACT	324.85 ± 102.7	339.26 ± 129.8
POST-SSG	337.73 ± 105.8	319.30 ± 110.8
POST-TR1	**290.16 ± 104.2†**	317.99 ± 128.1
POST-6H	304.38 ± 106.4	325.05 ± 128.5
POST-24H	308.40 ± 116.0	337.92 ± 149.9
POST-PREV	322.75 ± 86.8	362.93 ± 148.0
POST-TR2	319.94 ± 108.7	342.21 ± 138.3

*HSR = high-speed running; TR1 = training session 1; ACT = activation gym-based session; PREV = preventive gym-based session; SSG = small-sided game; TR2 = training session 2. †Significant differences regarding POST-SSG (highlighted in bold). All values represent maximal isometric force and are presented in N.

Finally, there was no statistically significant effect of HSR demands on RPE (F = 1.115; p = 0.308) after TR1 and TR2. Moreover, no significant differences were found after TR1 (p = 0.637; ES = 0.58 [-0.18 to 1.44]) and TR2 (p = 0.109; ES = 0.62 [-0.04 to 1.21]) when comparing the "HIGH" HSR group with the "LOW" HSR group (Figure 4).

Figure 4. Comparison of player's rate of perceived exertion after training session 1 and 2 according to HSR distance demands. HSR = high-speed running; RPE = rating of perceived exertion; TR1 = training session 1; TR2 = training session 2.



5.4. DISCUSSION

The present findings indicated that the increase in HSR demands in a typical in-season training session (i.e., POST-TR1) in young elite soccer players acutely affected the neuromuscular performance of the posterior chain muscles. Although both groups subjectively perceived the overall session in a similar way, only those with higher HSR demands suffered from acute NMF. Finally, no acute or residual changes in KE MVIC with different external load demands were found suggesting that the hamstring muscles were more taxed during the training sessions.

To our understanding, few studies have analyzed the acute and residual effects of training load on neuromuscular performance during the competitive microcycle in young soccer players (208). Despite TD being one of the most frequently used variables in elite soccer to monitor training load, our findings indicated that HSR was the most sensitive GPS metric to detect acute changes on posterior chain MVIC after TR1 (-14.08%; from 337.7 ± 105.8 to 290.2 ± 104.2 N). These results are in line with Hader et al. (209) as they showed that very high-intensity running (18–19.8 km·h^{-1}) was highly correlated with acute fatigue (postmatch) of the hamstring muscles, explaining up to ~50% of the neuromuscular state postmatch. Interestingly, although no significant differences between groups were found, the "HIGH" HSR group had a delayed recovery dynamic throughout the experimental period (-9.4%, -5.0%, and -3.7% at POST-TR1, POST-6H, and POST-24H, respectively) compared with baseline values. By contrast, the "LOW" HSR group did not exhibit any decreases in posterior chain MVIC (0.2, 2.4, and 6.5% at POST-TR1, POST-6H, and POST-24H, respectively). These findings might suggest that limiting HSR distance during the training session might be an effective strategy to avoid fatigue accumulation in congested microcycles where recovery time is reduced.

Notably, KE MVIC force was not acutely or residually (POST-6H) affected by none of the GPS metrics. This might be explained by the fact that hamstring muscles, such as BF, are activated for a greater amount of time relative to stride duration at higher running velocities (i.e., greater work-to-rest ratio) in comparison with vastus lateralis that tends to decrease (210). Similar results were found by Camic et al. (211) during an incremental treadmill running test. According to the authors, the hamstring-to-quadriceps ratio increases significantly when the exercise intensity is higher (from 0.841 ± 0.142 at 40% Vo_2peak to 0.999 ± 0.071 at 100% $V\dot{o}_2$peak). Furthermore, running at high speed causes the knee to be in an almost fully extended position during the late swing phase, resulting in greater eccentric force in the hamstring in comparison with the quadriceps, because of its function of decelerating the limb (212). The continuous repetition of these eccentric actions has been suggested to lead to greater muscle damage (213), which, together with alterations at the central nervous system level (i.e., decreased recruitment and discharge rates of motor units) (129), could explain the higher NMF found post-TR1 in this muscle complex.

Contrary to expected, the RPE values of the "HIGH" and "LOW" HSR groups after the training session were similar (p = 0.087; ES = 0.60). These results are not in line with those recently published by Marynowicz et al. (214) who found that RPE and session-RPE (sRPE) increased when HSR demands during a youth soccer training session were higher, being sRPE the highest correlated variable ($p < 0.001$; $r = 0.52$). Nonetheless, it has been suggested that RPE may underestimate the stress caused by very short bouts of HSR because these actions performed during the training session are interspersed with extensive recovery time (215), thus reducing player's perception of effort. This could explain why our results did not show significant differences in RPE between groups. Notwithstanding these findings, it is worth noting that although both groups reported similar RPE (Figure 3), only the group with higher HSR demands had acute neuromuscular impairments. From an applied perspective, these findings suggest that subjective information must be complemented with muscle-specific neuromuscular assessments to accurately discriminate players' level of fatigue at the muscular level.

One of this study's main limitations was that, because of players' time unavailability, KE MVIC was not assessed at all time points, so the neuromuscular recovery pattern between sessions was incomplete. Moreover, only the dominant limb was assessed, not allowing to identify possible changes between limbs. According to our findings and the current body of knowledge, future studies should focus on how drills with different high-intensity actions (e.g., ACC, DEC, HSR, and COD) in the training session could alter the neuromuscular performance of the posterior chain and KE muscles.

In conclusion, young soccer players who performed higher HSR demands throughout an in-season training session acutely decreased posterior chain neuromuscular performance of the dominant limb. By contrast, KE neuromuscular performance was not impaired. Contrary to our hypothesis, there were no significant differences in RPE when comparing players with higher and lower HSR demands during the training session.

5.5. PRACTICAL APPLICATIONS

Considering the growing tendency to increase the number of tasks with different training demands and stimuli during the competitive microcycle in soccer, our data suggest that the implementation of fatigue monitoring practices could help strength and conditioning coaches and practitioners to have a better knowledge of players' neuromuscular state. Moreover, it would allow to optimally adjust the training load of the next sessions, thus avoiding the development of injury risk factors related to NMF. Based on our findings, HSR demands had the most influence on posterior chain neuromuscular performance. Therefore, caution should be taken when prescribing exercises that involve high volumes of HSR on consecutive days of the microcycle because greater HSR distances might induce acute NMF in these muscles. Finally, although RPE may be a good indicator of how intense the session was perceived by the players, practitioners should consider that it may not necessarily discriminate between athletes with higher and lower HSR distances and NMF. As such, this metric should ideally be used in combination with external load tracking and muscle-specific assessments for a more thorough monitoring of localized fatigue.

VI – STUDY II

VI -STUDY II

CHANGES IN HAMSTRINGS’ ACTIVE STIFFNESS DURING FATIGUE TASKS ARE MODULATED BY CONTRACTION DURATION RATHER THAN INTENSITY

6.1. INTRODUCTION

Skeletal muscle stiffness during contraction has been investigated in recent years through the use of ultrasound-based SWE in both fatigue and non-fatigue conditions (121,137,183,216). Based on the known force-stiffness relationship (184,185), and considering that it can be affected by different methodological aspects (e.g., probe orientation and compression), tissue properties (e.g., tissue density, viscosity, temperature, anisotropy), and participants' characteristics (e.g., tissue length, physical activity, history, or injury state) (173,217–220), SWE can be used to infer the load distribution among agonist muscles crossing a given joint (187,221,222). Thus, several fundamental and applied physiological questions have been investigated through the quantification of localized muscle stiffness assessments during contraction (i.e., active stiffness) (223,224).

When it comes to the lower-body musculature, previous studies have focused on the hamstring muscles (225–229), due to their relevance in human locomotion (230,231) and the high injury incidence (especially for BFlh) in team sports where sprinting is key (12,14). Understanding the mechanical properties of the hamstrings could provide valuable information in developing effective injury prevention and rehabilitation protocols by addressing the hamstrings’ biomechanical load, strain, and coordination in different actions and joint positions (93,232). Although the distribution of load-sharing among hamstrings appears to be individual-specific (233), different coordination patterns have been observed in hamstring-injured limbs compared to healthy (120), especially in fatigue conditions (118,121). In this regard, the load-sharing between ST and BFlh has been shown to be dependent on the knee flexor's isometric contraction intensity (183,187). For instance, Mendes et al. (183) reported that the ST displays greater active stiffness compared to BFlh during knee flexion isometric contractions at lower intensities, and the BFlh/ST active stiffness ratio increases with the contraction intensity. The same research group also found that the BFlh/ST active stiffness ratio of healthy individuals is altered during a knee flexors' isometric contraction at 20% of MVIC until exhaustion; whereas the BFlh active stiffness tends to remain unaltered along the task, indicating a change in muscle coordination patterns which could potentially predispose athletes to injury. Moreover, the ST active stiffness starts to significantly decrease from 40% of the time task until reaching values ≆20% below baseline at the end of the task (137). Similar responses have also been observed with elite footballers (121) but not with healthy males in a study from another research group that used a different fatiguing protocol (i.e., multiple short-duration isometric contractions) (138). Nevertheless, it is important to note that at 20% of MVIC of knee flexors' isometric contraction, but not at 40% of MVIC, the active stiffness of ST is much higher than BFlh (i.e., a BFlh/ST ratio=0.42-0.50 and 0.96-1.03, for the 20% and 40% conditions, respectively) (183). Therefore, it is conceivable that the BFlh-ST active stiffness responses during a knee flexors' isometric contraction until exhaustion would be different between 20% and 40% of MVIC protocols, due to the distinct load distribution between BFlh and ST, even though research addressing this issue in-depth is scarce.

Thus, this study aimed to further explore previous findings by comparing the effects of knee flexors' isometric contraction until exhaustion performed at 20% vs. 40% of MVIC, on the active stiffness responses of BFlh and ST. We hypothesized that during higher knee flexor contraction intensity the BFlh would have a greater active stiffness increase with a lower ST active stiffness decrease, since, at 40% of MVIC, the BFlh/ST active stiffness ratio is higher than at 20% of MVIC.

6.2. METHODS

6.2.1. Participants

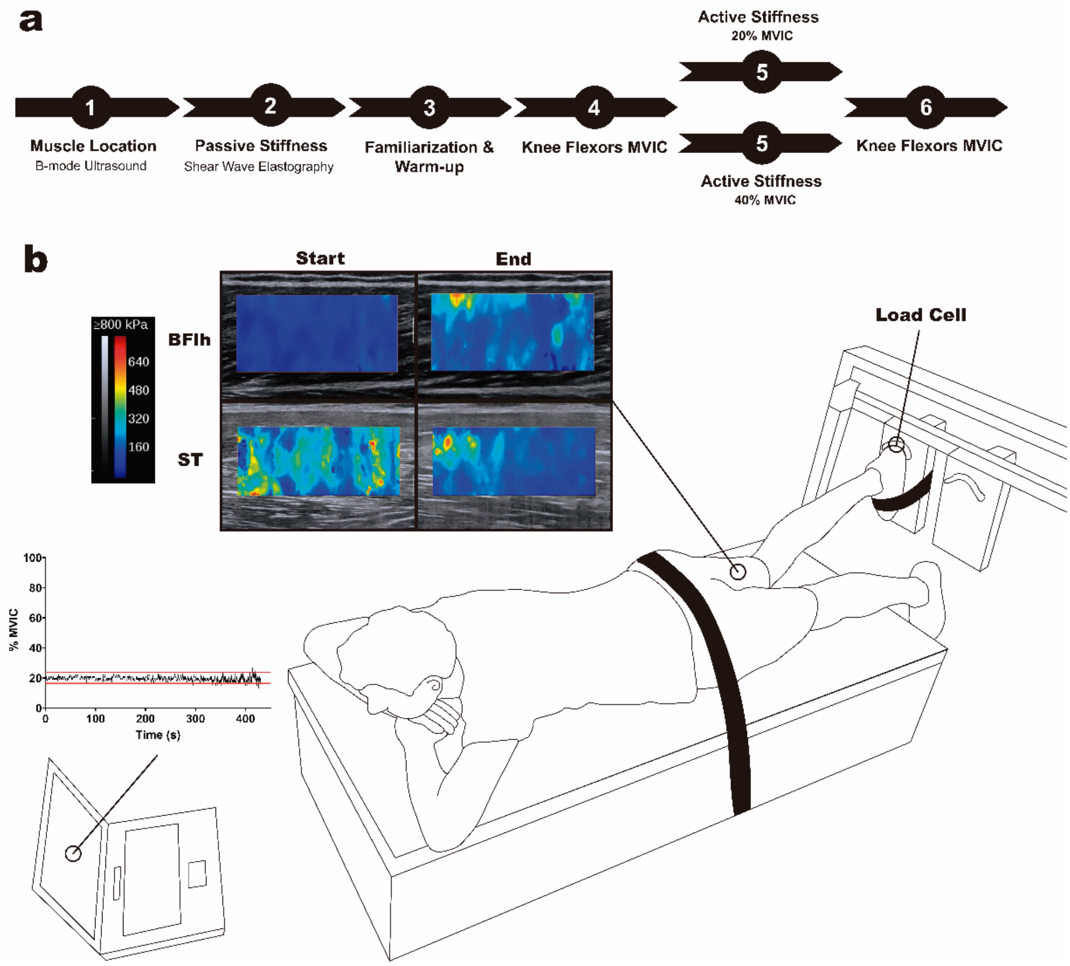
Based on a priori sample size calculation using G*Power software (version 3.1.9.3, Universität Düsseldorf), sixteen participants were required assuming a statistical power of 80%, effect size of 0.25, and an alpha error of 0.05. Eighteen recreationally active males (age [mean±SD]: 27.1±6.8 years; height [mean±SD]:

176.4±6.2 cm; BM [mean±SD]: 73.6±8.3 kg) participated in this study. Participants were free from any lower limb musculoskeletal injury in the past 2 years and were asked to cease resistance or flexibility training at least 72 h before the experimental sessions. Before data collection, informed consent was obtained from participants. The study was approved by the local ethics committee (#11/2022) and was conducted under the principles outlined in the Declaration of Helsinki.

6.2.2. Experimental design

The following information provides a concise overview of the methods employed in the study. Participants took part in a randomized crossover study design in which they had to attend two experimental sessions (Figure 5a). Sessions were separated by 3-7 days and conducted at a similar time of the day to minimize diurnal variations. Two evaluators identified the BFlh and ST muscles in the dominant limb using ultrasound and determined the muscles' passive stiffness. Participants were then familiarized with the equipment and performed a specific warm-up of 10 submaximal contractions at 50% perceived maximal intensity. Then, the knee flexors' MVIC of the dominant leg was assessed before the fatiguing task that involved the evaluation of the active stiffness behaviour of BFlh and ST during a submaximal isometric contraction until failure at 20 or 40% of MVIC. Afterwards, knee flexors MVIC was re-assessed to determine the presence of NMF. The order of the experimental sessions was randomly selected.

Figure 5. a) Timeline of the experimental design of the study. b) Experimental setup used in the study to assess passive and active stiffness of biceps femoris long head (BFlh) and semitendinosus (ST) during a knee flexion isometric contraction at 20 or 40% of maximal voluntary isometric contraction (MVIC). Visual feedback of knee flexion torque production is also shown, with the red lines showing the upper and lower torque limits. Participants' representative sonograms at the start and the end of the 20% of MVIC knee flexion submaximal isometric contraction from BFlh and ST and the elastogram scale are depicted to improve interpretation.



6.2.3. Procedures

6.2.3.1. *Knee flexor maximal voluntary isometric contraction*

Participants were positioned prone, with the hips in a neutral position (secured with a strap), and the knees flexed at ~30° in a custom-built equipment as shown in Figure 5b. The foot was positioned in a foot holder at 90° ankle joint angle in a neutral position to avoid the internal/external rotation of the tibia. The foot holder contained a force transducer (Model STC, Vishay Precision, Malvern, PA) measuring at 1 kHz to collect the linear force perpendicular to the leg orientation. Force was collected (Model UA73.202, Sensor Techniques, Cowbridge, UK), amplified (gain: 1000), digitally converted (USB-230 Series, Measurement Computing Corp., Norton, MA), and recorded using the DAQami software (v.4.1, Measurement Computing Corp., Norton, MA). Knee torque was estimated by multiplying the perpendicular distance between the force transducer centre and the femoral lateral condyle. Individuals performed two 3-5 s duration knee flexors' MVICs with the dominant limb separated by a 60 s rest period. Verbal encouragement was given by the evaluators to ensure maximal effort during the test. If the second MVIC differed 5% from the preceding contraction, more repetitions were performed. The highest peak force value within the 5% difference range was used for analysis.

6.2.3.2. *Fatigue task*

After a 5 min rest, participants performed a sustained submaximal isometric knee flexion at 20 or 40% MVIC until failure. The test was stopped when the participant was unable to produce force or when the force produced was below 5% of the established intensity and after encouragement, the participant could not increase the force to the determined level. During the execution of the test, participants had visual feedback of the torque production as well as a visual delimited line that corresponded to their 20% or 40% knee flexors' MVIC. The evaluators gave verbal encouragement throughout the test to maintain torque at the determined intensity.

6.2.3.3. *Muscle stiffness*

Two identical ultrasound systems (Aixplorer, v10; Supersonic Imagine, Aix-en-Provence, France) in B-mode, connected to a linear transducer array (SL10-2, 2-

10 MHz, Vermon, Tours, France) were used to identify the location of the largest cross-sectional areas of the BFlh, and ST muscles. To find these regions, the probe was placed, first transversely and then longitudinally, following fascicle orientation at ~55% of the distal-to-proximal femur length and during submaximal contraction in each muscle. Once the region of interest (ROI) was identified, and to ensure a stable and precise recording of muscles' passive and active stiffness, a 3D printed plastic cast with the form of the probe was attached to the skin using bi-adhesive tape. The cast was oriented according to the fascicle direction. Then, a 60 s recording in SWE mode (musculoskeletal preset, penetrate mode, smoothing level 5, persistence off; scale: 0 – 800 kPa) at a frequency of approximately 1 Hz (range: 0.7-1.1 Hz, depending on the size of the selected ROI) of both muscles was taken to assess passive muscle stiffness. During the passive recording, participants were instructed to remain completely relaxed. Although muscle electromyography was not measured, researchers ensured participants' relaxation by considering the elastogram map and knee flexor's torque production values during the assessment. A pedal switch was used to simultaneously start data acquisition from both ultrasound systems. Active stiffness of BFlh and ST was continuously assessed during the fatigue task with the same settings used in the passive measurement. As the maximum recording time for SWE videos was 60 s, clips were taken continuously until the end of the fatigue task (less than 5 s intervals between clips due to ultrasound processing time).

6.2.4. Data analysis

Torque signal was filtered using a 4th order Butterworth low-pass filter with a cutoff frequency of 10Hz. The highest peak torque obtained in pre- and post-fatigue knee flexors' MVIC trials was considered for analysis and used to calculate the fatigue index (i.e., the percentage of MVIC torque loss after the fatigue task relative to baseline knee flexors' MVIC). Endurance time was calculated as the total time duration from the onset of the submaximal contraction in the fatigue task until exhaustion. Torque data in the fatigue task at 20% and 40% of MVIC was normalized to the respective MVIC of the session and from 0 to 100% contraction time. Lastly, it was interpolated to match the same contraction duration (i.e., from 0 to 100% contraction time, a total of 100 data points).

Shear modulus data from the passive and active assessments were analyzed using customized Matlab® (version R2022a, The Mathworks, Inc., Natick, MA, USA) routines (script code files can be found at https://cimt.uchile.cl/mcerda/). Each video clip was extracted from the ultrasound software and converted into ".avi" format. The Matlab® routine allowed users to manually select the largest rectangular ROI in the elastogram window of the video and convert the pixels containing elastogram measurements into elastic moduli values based on the recorded scale (800 kPa). These values were then averaged to obtain a representative muscle value and exported to individual Excel files. The values displayed in the elastogram windows of the Aixplorer's ultrasound scanner represent Young's modulus (E) of the medium measured as: $E \approx 3\mu$, being (μ) the shear modulus. Therefore, to quantify the shear moduli of the muscles, the values were divided by 3 following the equation: $\mu = E/3$ (174). The muscle active stiffness during the initial 5 s after the onset of muscle contraction was discarded from analysis, in order to exclude the period in which the individual adapted to reach the target contraction intensity with a stable elastogram. Customized Matlab® (version R2022a, The Mathworks, Inc., Natick, MA, USA) routines were then used to: 1) detect and remove outliers using the interquartile method; 2) replace missing values (caused by ultrasounds' processing time between clips) by fitting a 4th order polynomial function; 3) smooth the signal applying a Savitzky-Golay filter; 4) normalize the time series from 0 to 100% contraction time; and 5) interpolate the individuals' data to match the same contraction duration (i.e., from 0 to 100% contraction time, a total of 100 data points). BFlh/ST ratio values for each data point during the fatigue task were determined, as well as the individuals' slope of the linear contraction duration-BFlh/ST and ratio relationship, was considered for analysis. The BFlh/ST ratio was calculated by dividing the BFlh active stiffness values at each percentage of the contraction duration with the corresponding ST stiffness values. These variables were selected as indicative of potential load-sharing alterations. Ultimately, to examine the magnitude of inter-individual responses in each muscle and testing condition, the CV of the active stiffness responses of BFlh and ST in the different conditions was calculated.

6.2.5. Statistical analysis

Descriptive data are presented as mean±SD. Normal distribution of the data was confirmed using the Shapiro-Wilk test. A two-way repeated measures ANOVA [muscle (BFlh, ST) x session (20%, 40%)] was used to analyse muscles' passive shear modulus and slopes, and knee flexors MVIC [time (PRE, POST) x session (20%, 40%)]. If significant effects were found, a post hoc multiple comparisons test with Holm's correction was performed. To verify if torque was maintained at the determined intensity levels (i.e., 20% and 40% of MVIC) during the fatigue task, statistical parametric mapping (SPM) one-sample two-tailed t-tests were performed, with 20 and 40% of MVIC as criterion values, respectively.

A two-way repeated measures SPM ANOVA [muscle (BFlh, ST) x session (20%, 40%)] was performed to analyse active stiffness throughout the submaximal contraction. When a significant effect was found for muscle, session, or session x muscle interaction, post hoc SPM t-tests were performed to identify the direction of the differences. SPM paired samples two-tailed t-tests were used to assess between muscle active stiffness differences. Statistical significance was set to $p \leq 0.025$ to correct for multiple comparisons accordingly. Then, to analyse within muscle active stiffness changes throughout the contraction at the different sessions, SPM one-sample two-tailed t-tests were performed, with the starting average stiffness values used as baseline comparisons. Furthermore, a one-way repeated measures SPM ANOVA was used to analyse BFlh/ST ratio differences at each contraction intensity.

Lastly, a paired sample t-test was used to analyse the differences in fatigue index, and the BFlh/ST ratio slopes between sessions. Wilcoxon signed-rank test was used to analyse endurance time (as data was non-normal distributed). Eta squared was calculated from the repeated measures ANOVA and categorized as small (0.01-0.06), moderate (0.06-0.14), and large (>0.14) (234). The alpha level was set at $p \leq 0.05$. Statistical analysis of discrete variables was performed using JASP (version 0.17.1.0) whereas the analysis of continuous variables was performed in Matlab® (version R2022a, The Mathworks, Inc., Natick, MA, USA) by using the open source SPM code (version M.0.4.10, SPM1D open-source package, spm1d.org) (235).

6.3. RESULTS

A total of 17 participants were included in the study. Data from one subject was removed from analysis due to failure to perform tests properly.

6.3.1. BFlh and ST passive stiffness, MVIC, fatigue index, and endurance time

Table 5 shows the MVIC and fatigue task performance and muscles' passive stiffness outcomes. At the start of both testing sessions, BFlh and ST showed similar passive stiffness, with no muscle x session interaction ($p=0.507$; $\eta^2=0.009$), or effects for muscle ($p=0.133$; $\eta^2=0.056$) and session ($p=0.374$; $\eta^2=0.013$) factors. For the knee flexors MVIC, an effect for time was noted ($p<0.001$; $\eta^2=0.644$), but not for session ($p=0.708$; $\eta^2=0.001$) or time x session interaction ($p=0.067$; $\eta^2=0.017$). The fatigue protocols decreased MVIC to a similar extent [average MVIC loss: -18.9% (mean diff.= -30.4 Nm); $p<0.001$; ES= 1.11 (0.61 to 1.60)]. However, the fatigue index was significantly higher at 20% of MVIC (mean diff.= -8.7%; $p=0.033$; ES= -0.57 [-1.07 to -0.05]). As expected, endurance time at 20% of MVIC was significantly higher than at 40% of MVIC (mean diff.= 382.5 s; $p<0.001$; ES= 1.43 [0.73 to 2.10]). Participants maintained torque at the established intensity, both at 20% ($p>0.05$) and 40% of MVIC ($p>0.05$) sessions (Appendix 2).

Table 5. *Descriptive data for individuals' fatigue task performance, muscles' passive stiffness, and individual muscles and ratios slopes in both testing sessions.*

	20% of MVIC	**40% of MVIC**
BFlh passive stiffness (kPa)	7.1±1.5	7.8±2.0
ST passive stiffness (kPa)	8.2±3.2	8.3±2.2
MVIC (Nm) - PRE	130.5±23.2	126.7±29.6
MVIC (Nm) - POST	95.1±32.1	101.3±24.3
Fatigue index (%)	28.3±17.3*	19.6±11.9
Endurance time (s)	623.1±350.8*	153.1±40.0
BFlh slope (kPa/%)	0.032±0.1	0.005±0.1
ST slope (kPa/%)	-0.171±0.1**	-0.066±0.2
BFlh/ST ratio slope (ratio/%)	0.004±0.003*	0.001±0.003

Legend: BFlh, biceps femoris long head; MVIC, maximum voluntary isometric contraction; POST, after the fatigue task; PRE, before the fatigue task; ST, semitendinosus. Data are presented as mean±SD. *Significantly different than 40% of MVIC ($p<0.05$). **Significantly different than BFlh slope at 20% of MVIC ($p<0.05$).

6.3.2. BFlh and ST active stiffness

SPM analysis showed significant muscle ($p<0.001$), session ($p<0.001$), and muscle x session ($p=0.041$) interaction effects (Appendix 3). Overall, ST showed greater active stiffness than BFlh between 0 and 90% of contraction time ($p<0.001$), while muscles had greater stiffness at 40% than 20% of MVIC in most of the contraction time ($p<0.05$; Appendix 3).

Between muscles analyses showed that ST active stiffness was higher than BFlh from the start until ≅ 64% of contraction time at 20% of MVIC (Figure 6a and b); whereas at 40% of MVIC, this difference was just noted until ≅33% of contraction time (Figure 6e and f). Within muscles analyses revealed that at 20% of MVIC the ST active stiffness decreased from ≅91% until the end of contraction time (Figure 7d), whereas the BFlh active stiffness remained largely unchanged for most of the contraction duration (Figure 7c). At 40% of MVIC, no changes in both BFlh and ST active stiffness were observed throughout the full contraction time (Figure 7g and h).

Regarding individual muscle slopes, significant effects were found for muscle x session interaction ($p=0.009$; $\eta^2=0.063$) and muscle ($p=0.001$; $\eta^2=0.268$), but not for session ($p=0.261$; $\eta^2=0.022$). The negative ST slope at 20% of MVIC was significantly steeper compared to BFlh at the same contraction intensity (mean diff.=0.203 kPa/%; $p<0.001$; ES=1.61 [0.57 to 2.64], but similar slopes between muscles were found at 40% of MVIC (mean diff.=0.071 kPa/%; $p=0.192$; ES=0.56 [-0.36 to 1.48]). Descriptive data of BFlh and ST slopes can be found in Table 5.

Notably, the coefficient of variation of BFlh active stiffness under the 20% (CV=26.0±3.4%) and 40% (CV=27.4±2.9%) of MVIC sessions was lower than for the ST (20% of MVIC: CV=28.8±3.0%; 40% of MVIC: CV=37.0±2.7%).

Figure 6. *Mean active stiffness of biceps femoris long head (BFlh) and semitendinosus (ST) during the knee flexors submaximal contraction until exhaustion at (a) 20% and (e) 40% of MVIC. Panels b and f show the statistical parametric mapping (SPM) analysis, i.e., t-statistics (SPM{t}), of the differences in active stiffness levels between muscles at 20 and 40% of MVIC, respectively. Legend: i) Data in panels a and e, are depicted as mean (solid line) and upper and lower 95% confidence intervals (shaded areas). ii) In panels b and f, the pink dashed line represents the critical threshold. The darker and lighter grey shaded areas in panels b and f represent the portion of the contraction in which muscles had different active stiffness values. Alpha level was set at p≤0.025 to correct for multiple comparisons.*

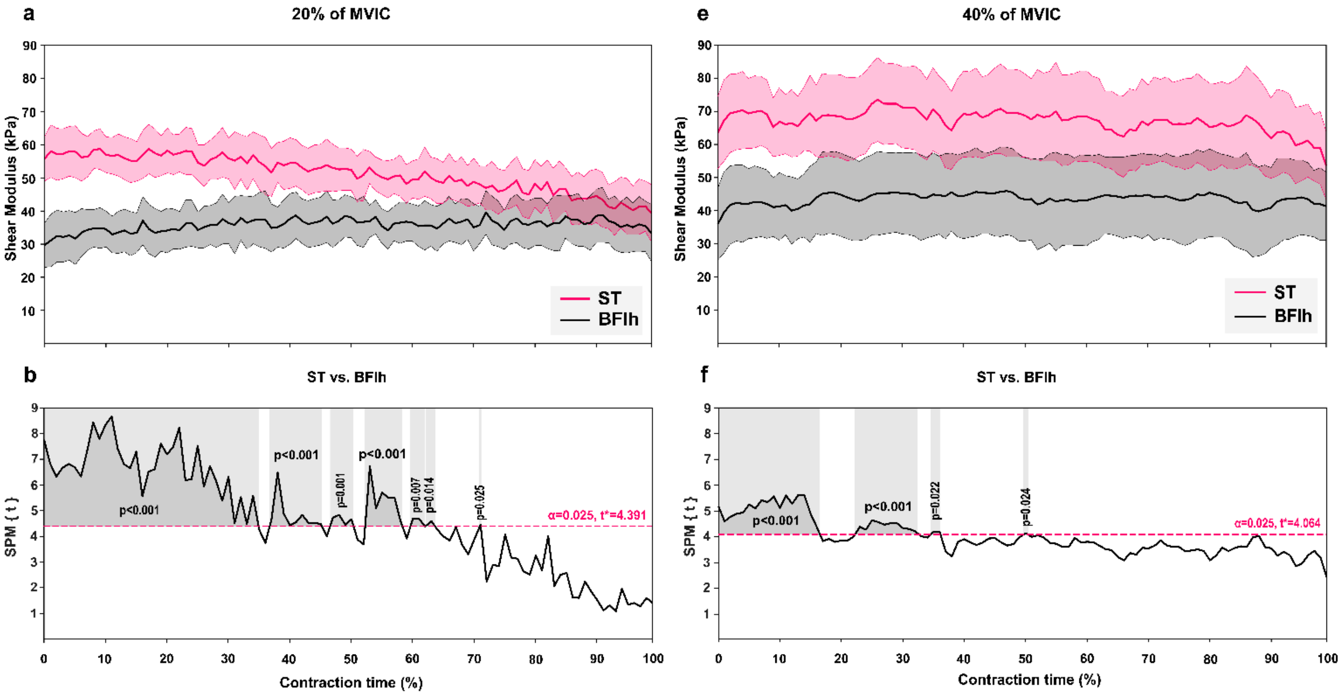
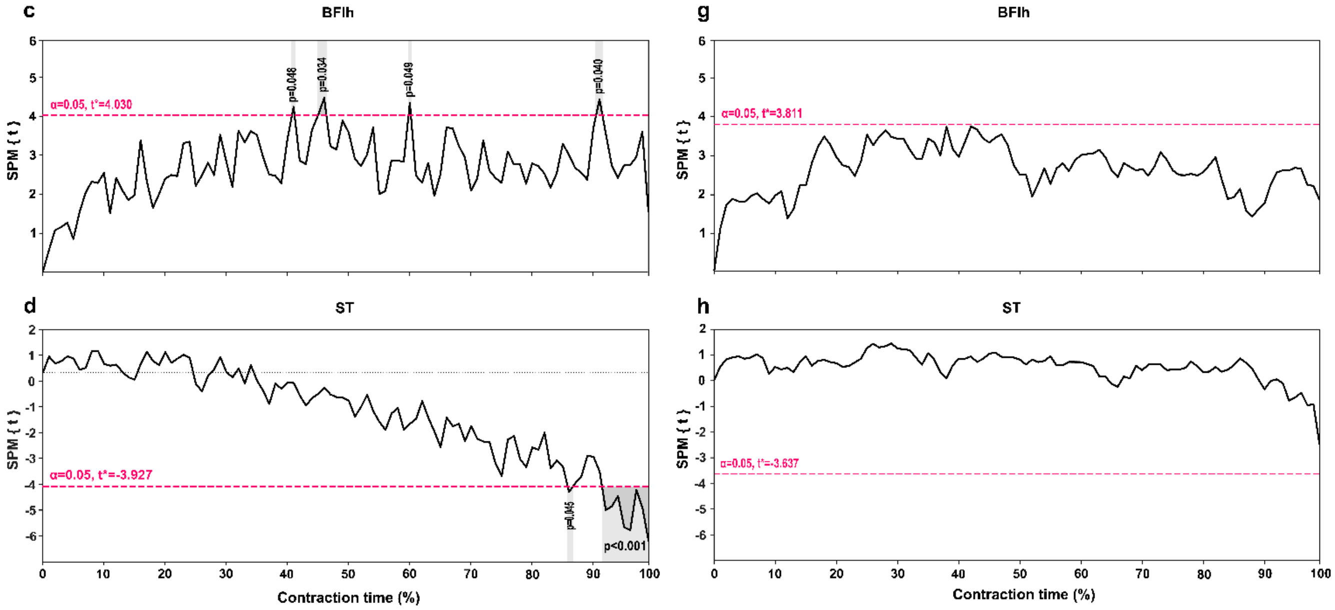


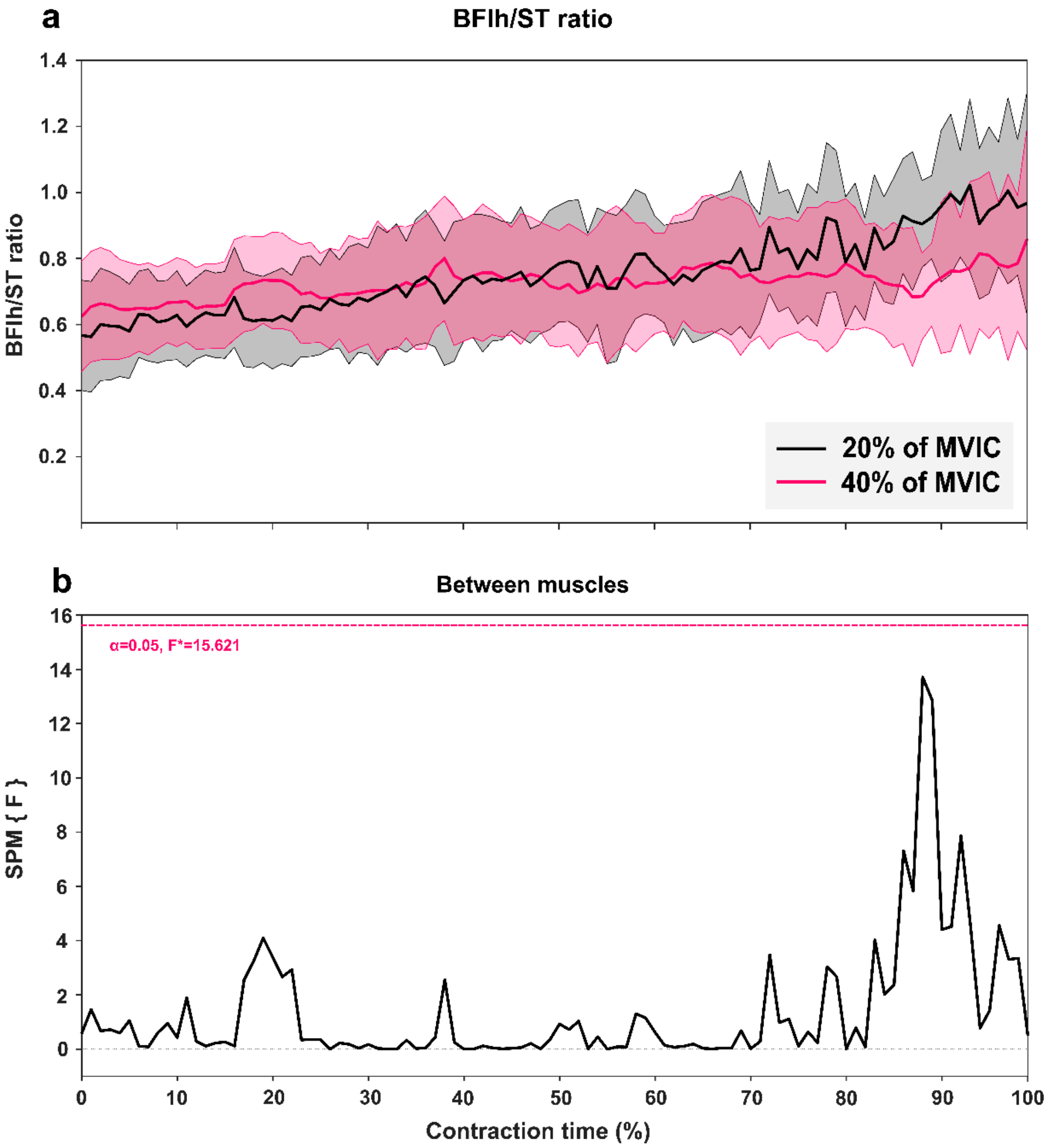
Figure 7. SPM analysis of the within muscles differences in active stiffness (BFlh and ST) at 20 (panels c and d) and 40% of MVIC (panels g and h) are also shown. Legend: i) In panels c, d, g, and h, the pink dashed line represents the critical threshold. ii) In panels c, d, g, and h, the darker and lighter grey shaded areas represent the portion of the contraction where significant differences in active stiffness compared to baseline values (i.e., the start of the contraction) were observed. Alpha level was set at p≤0.05.



6.3.3. BFlh/ST ratio

Regarding the BFlh/ST ratio, no significant session effects were found (Figure 8a and b). However, a steeper BFlh/ST ratio slope was found for 20% than 40% of MVIC (mean diff.= 0.003 ratio/%; p=0.003; ES=0.86 [0.29 to 1.41]) (Table 5).

Figure 8. *Mean biceps femoris long head/semitendinosus (BFlh/ST) ratio during the knee flexors submaximal contraction until exhaustion at 20% and 40% of MVIC (panel a). Panel b shows the statistical parametric mapping (SPM) analysis, i.e., F-statistics (SPM{F}), of the session effects on BFlh/ST ratios. Legend: Data in panel a is depicted as mean (solid lines) and upper and lower 95% confidence intervals (shaded areas). The pink dashed line represents the critical threshold. Alpha level was set at p≤0.05.*



6.4. DISCUSSION

This study examined the effects of a localized knee flexors' fatigue task performed at two intensities (i.e., at 20 and 40% of MVIC) on knee flexors' maximal strength and on the BFlh and ST active stiffness in recreationally active individuals. The main results of the current study indicated that, although a lower fatigue task time was observed in the 40% MVIC session (as expected), the ST active stiffness only decreased with fatigue task time in the 20% MVIC session, while BFlh active stiffness remained unchanged in both testing sessions. This led to a greater BFlh/ST ratio increase with fatigue task time at 20% compared to 40% of MVIC. Notably, muscles started with different active stiffness values (i.e., greater for ST) in both conditions but showed similar active stiffness from 64% and 33% contraction time in the 20% and 40% of MVIC testing sessions, respectively.

The findings of the present research also denote that both knee flexors' fatiguing tasks resulted in reduced MVIC; however, the 20% MVIC session evoked greater knee flexors fatigue index together with load-sharing alteration between BFlh and ST, compared to the 40% MVIC condition. This suggests that contraction duration could be more relevant than intensity to induce changes in load-sharing within hamstring muscles. In this regard, significant decreases in ST active stiffness occurred from ~90% of contraction time during the 20% MVIC session (without changes in the 40% MVIC session). Taking into consideration the average task duration, it appears that active stiffness changes would only be expected to occur from contraction durations of 561 s onwards, which could explain why no active stiffness changes occurred in the 40% MVIC condition as its maximum duration was, on average, 153 s. This observation supports previous literature that reported no alteration of the hamstring muscles' active stiffness following a maximal repeated sprinting task in trained footballers (i.e., 10 trials of 30 m interspersed with 30 s rest), although minimal changes were observed in non-athletic individuals (236). Interestingly, Evangelidis et al. (138) recently explored the effects of 99 knee flexors isometric contractions with 5 s duration (interspersed with 5 s rest, for a total contraction duration of 494 s) at 50% of MVIC in healthy individuals on the hamstring muscles' active stiffness. The authors found a knee flexors' MVIC loss of 18.4% with a tendency for ST active stiffness to decrease and BFlh to increase over the fatigue task time. Thus, it would be interesting for future studies to explore the effects of a protocol with intermittent contractions at 40% of the MVIC for the same contraction time of the 20% MVIC condition but using a fatigue protocol. In this sense, it can be hypothesized that, for the same contraction duration, an equal active stiffness ST decrease response might be obtained and the BFlh increase may become evident.

Nevertheless, the reason why contraction duration significantly affects ST stiffness during the fatigue task, while BFlh stiffness appears relatively unaltered is still unknown. We speculate that a combination of morphological, histological, and neural factors could explain, at least in part, these results. For instance, BFlh has been reported to have a greater PCSA (24) and a potentially higher proportion of oxidative fibres (237) than ST which may predispose BFlh to resist fatigue (contrary to ST) and to produce higher forces with the increase in contraction intensity. Consequently, the central nervous system may increase the neural drive to other knee flexor muscles (including BFlh) to compensate for the decreased ST force with fatigue. Nonetheless, since knee-dominant exercises are thought to preferentially recruit ST over the BFlh (118,238,239), it might be interesting to examine, in future research, whether the stiffness responses of ST and BFlh could be different with a combined knee flexion/hip extension fatigue task as previous research has shown higher recruitment of BFlh (240).

The active stiffness responses of the ST at 20% of MVIC were found to be consistent with previous studies using a similar methodology (121,137). These studies also observed a ~20% decrease in ST active stiffness by the end of the task, along with a tendency for BFlh active stiffness to increase. Notably, Freitas et al. (121) examined a sample of professional footballers and only noted changes from the percentile 80th of the task, while Mendes et al. (137) tested non-athletic individuals (as in the present study) and observed ST changes from the percentile 40th. It should be noted that in Freitas et al. (121), individuals had higher knee flexors' MVIC (i.e., 154-158 Nm) compared to individuals in Mendes et al. (137) study (i.e., 123-128 Nm), as well as the individuals of the present study (i.e., 126-131 Nm). This might suggest that the training status may influence the timing where the ST active stiffness alteration occurs. Interestingly, hamstring active stiffness alterations were observed following maximal repeated sprints in non-athletic individuals but not in trained footballers (236), which reinforces the importance of training status. However, individuals from our study maintained ST active stiffness levels even further than the sample of professional footballers of

Freitas et al. (121) (i.e., ~90% contraction time vs percentile 80th of the task, respectively), indicating higher fatigue resistance. These findings, however, should be taken with caution due to the different statistical analysis approaches employed between studies. In this regard, Pataky et al. (241) found discrepancies in the statistical outcome when comparing zero- vs. one-dimensional hypothesis testing as zero-dimensional methods inadequately represent the continuous variance observed in one-dimensional trajectories, a feature commonly found in one-dimensional biomechanical datasets. Therefore, future comparisons using both statistical approaches should be made, as conclusions could be different (Appendix 4).

Another interesting finding was the loss of heterogeneity of the intermuscular stiffness throughout the contraction in both the 20% and 40% of MVIC conditions, which means that at some point in the fatigue task, the active stiffness between ST and BFlh became similar. We are unaware of the relevance of this mechanical response. However, we speculate that it may affect the mechanical interaction between the BFlh and ST (i.e., two neighbouring muscles) by increasing the shear stress in the connective tissue interface, which is commonly prone to injury in athletes who perform repeated sprint efforts. In this regard, interestingly, a previous study by Schuermans et al. (118) reported that athletes with previous hamstring injuries had a more homogeneous pattern of T2 relaxation response (assessed using functional magnetic resonance) among the hamstring muscles after a knee flexors' fatigue protocol, compared to no previously injured individuals. Whether this aspect alters muscle mechanics during contraction and, potentially, increases the susceptibility to injury in this region warrants further investigation.

It is important to note the study's limitations. Firstly, the contraction intensities used in this study produced different mechanical and performance responses; however, they did not produce a statistically different BFlh/ST ratio at the beginning of the tasks (20%: BFlh/ST=0.57±0.22 ratio vs. 40%: BFlh/ST=0.62±0.25 ratio), which was in contrast with our initial expectation (183). The high ST active stiffness variability between the healthy individuals under the 40% of MVIC condition (20%: CV=28.8±3.0% vs. 40%: CV=37.0±2.7%) may explain why this difference did not reach statistical significance as similar BFlh active stiffness variability was found between the 20% (CV=26.0±3.4%) and 40% (CV=27.4±2.9%)

MVIC conditions. This means that, when increasing the hamstring contraction intensity, healthy individuals may use different load-sharing strategies, which denotes high individual heterogeneity as seen in Appendix 5. Nevertheless, the experimental condition provided evidence that different BFlh/ST active stiffness responses are obtained with different contraction intensities. Secondly, the present findings may not be generalized to other experimental conditions (e.g., muscle-tendon lengths, and contraction types used to induce fatigue), and we do not exclude different regional responses along the muscle length (i.e., proximal vs. distal regions) as testing was only performed in the mid-region of both muscles. Thirdly, a stress-relaxation effect could also have occurred in the ST connective tissue, particularly its distal tendon (whose length is considerably high), thus partly explaining the decrease of the muscle belly active stiffness. Thus, the physiological mechanism underlying the ST active stiffness response with long duration knee flexor isometric contraction deserves to be explored in future research. Moreover, it should be noted that, although local monitoring was conducted to ensure individuals executed the protocol correctly, we noted (after data processing) that some individuals, in some brief instants, had torque deviations below the 5% threshold. Despite this type of behaviour, it is implicit to this type of protocol, future research should make efforts to minimize this type of situation. Finally, for a complete understanding of ST and BFlh active stiffness characterization during the fatigue tasks, the full agonist (i.e., SM, BFsh, sartorius, gracilis, gastrocnemius, popliteus) and antagonist (i.e., quadriceps heads) crossing the knee joint would need to be assessed, as the torque production depends on the interaction between all muscle actuators acting on the joint (242). For instance, it has been suggested that the force production among hamstring heads varies (e.g., with SM producing ~80% of the hamstrings' total force at intermediate lengths (22), and the interplay between these synergistic muscles could explain the high inter-individual variability found in the present study. However, this full muscle assessment is very demanding and methodologically challenging, and thus it was not possible to be performed in the present study. Therefore, we consider that these results should be interpreted with caution and future studies should further characterize the mechanical interaction of this complex muscle group, as well as the potential influence of co-activation of the antagonist muscles (i.e., greater activation of the antagonists with increased contraction intensity) (243).

In conclusion, we found evidence showing that muscle contraction duration seems to have greater relevance than contraction intensity in altering the active stiffness of the hamstring muscles during fatiguing tasks, particularly in the ST. On this matter, during a submaximal isometric contraction until exhaustion, changes in stiffness appear to occur after a certain time threshold (i.e., ~90% of the contraction duration) in this muscle, while the BFlh remains relatively unchanged. Consequently, a steeper increase of the BFlh to ST active stiffness ratio during the fatigue task with the lower contraction intensity (i.e., 20% MVIC) was observed, which was not the case in the higher contraction intensity (i.e., 40% MVIC). Furthermore, the loss of intermuscular stiffness heterogeneity found at the last (20% of MVIC) and initial (40% of MVIC) stages of the contraction suggests a potential impact on the mechanical interaction between these hamstring muscles, which could alter muscle mechanics and increase susceptibility to injury in (non-)athletes exposing to rapid actions involving the hamstring activation.

VII – STUDY III

VII - STUDY III

HIGHLY-TRAINED FOOTBALLERS WITH AND WITHOUT PREVIOUS HAMSTRING INJURIES HAVE SIMILAR BICEPS FEMORIS LONG HEAD AND SEMITENDINOSUS STIFFNESS RESPONSES DURING A KNEE FLEXORS' SUBMAXIMAL ISOMETRIC CONTRACTION UNTIL EXHAUSTION

7.1. INTRODUCTION

Skeletal muscle injuries are currently under the spotlight of football medicine due to the financial burden and loss of individual/collective performance they imply (72,244). Among the different locations of muscle injuries, the hamstrings remain one of the most frequently injured muscle groups, with hamstring strain injuries (HSIs) accounting for 4-13% of all football-related injuries and presenting a recurrence rate of 4-68% (11). Despite different prevention strategies proposed in the scientific literature (245), the incidence of hamstring injuries has not decreased over 18 seasons in UEFA Champions League teams (192). Specifically, the BFlh seems to be the most affected muscle in footballers with the ST being less affected (67,88). Although the nature of HSIs has been suggested to be multifactorial (246), injury history has been identified as the most consistent risk factor for hamstring strain injuries across studies, while NMF has been shown to potentially increase injury susceptibility (101). Thus, research efforts should be conducted to identify modifiable risk factors that could allow reducing HSIs incidence.

Recent studies have suggested that the distribution of force amongst synergistic hamstring muscles (i.e., load-sharing) may play a role in hamstring injury occurrence (118,120,121). It is important to note that different methodological approaches could be used to assess and infer load-sharing (186,216,221,247). Through the use of T2 relaxation quantification with MRI, prior research found that previously injured amateur footballers had a more symmetrical metabolic response between BF, ST, and SM after a prone leg curl exercise performed until exhaustion, compared to the non-injured controls; suggesting that part of the ST activity was replaced by more synergistic activity of BF, and SM (118). However, the assessment of the metabolic response via functional MRI is limited to pre-post measurements taken at rest and, consequently, does not consider the effects of the fatigue developed during the active contraction. Also, T2 relaxation reflects metabolic activity, and not a mechanical parameter, which may be more relevant for injury mechanism understanding.

In this regard, ultrasound-based SWE is a technique that allows to assess the skeletal muscle mechanical properties (e.g., muscle stiffness) in both passive (120,229,248) and active conditions (137,183,227,249), and to infer the load-sharing strategy between the hamstring muscles (137,183). Interestingly, Freitas et al. (121) analysed the differences in the BFlh/ST active stiffness pattern of non-injured and hamstring-injured limbs of elite football players during a knee flexion submaximal isometric contraction until exhaustion at 20% of MVIC. The authors found that, at the start of the contraction, previously injured limbs had a lower absolute BFlh active stiffness and, consequently, a lower BFlh/ST ratio. Moreover, a tendency (although without reaching statistical significance at $p<0.05$) was found for a steeper increase in the BFlh/ST ratio in injured limbs which suggests a greater relative activity of the BFlh (compared to ST) throughout the development of fatigue. Similar conclusions (through the use of surface electromyography, MRI, and dynamometry) were reported by Avrillon et al. (120), where the contribution of BF torque at 20% of MVIC in a non-fatigued state was found to be lower in the hamstring-injured limb of elite male sprinters and long jumpers. These findings may corroborate the muscle inhibition that has been suggested to occur after the injury occurrence (102,104,250), and could play a major role in modifying the load-sharing strategies among hamstring muscles. Notably, both studies identified muscle imbalances in non-fatigued states at low-intensity isometric contractions; thus, the observation at higher contraction intensity remains to be explored. At 20% of knee flexors MVIC, the BFlh/ST active stiffness ratio is known to be lower than 40% of MVIC in healthy individuals, together with a higher strength endurance time for 20% (183). However, this has not been explored in experienced footballers, as well as the behaviour during an isometric contraction until exhaustion in those with previous injuries. As non-traumatic hamstring injury in footballers often involves fast movements that require rapid muscle contractions with high levels of muscle activation (160), it is important to examine if a knee flexion isometric task performed at a higher contraction intensity could provide better outcomes that could differentiate injured from non-injured players.

This study aimed to analyse BFlh, and ST active stiffness patterns assessed with SWE during a submaximal contraction at 40% of knee flexion MVIC until exhaustion between previously injured and non-injured limbs of highly-trained national-level male footballers. We also explored whether passive stiffness, knee flexors' MVIC, and endurance capacity would differ between limbs. We hypothesised twofold: i) the BFlh active stiffness levels of previously hamstring-injured limbs would be decreased in non-fatigued states (i.e., lower BFlh/ST ratio), and the BFlh/ST ratio would have a higher increase in the presence of fatigue; and ii) no differences would be observed for muscles' passive stiffness, knee flexors' MVIC, and endurance capacity between injured and non-injured limbs.

7.2. MATERIALS AND METHODS

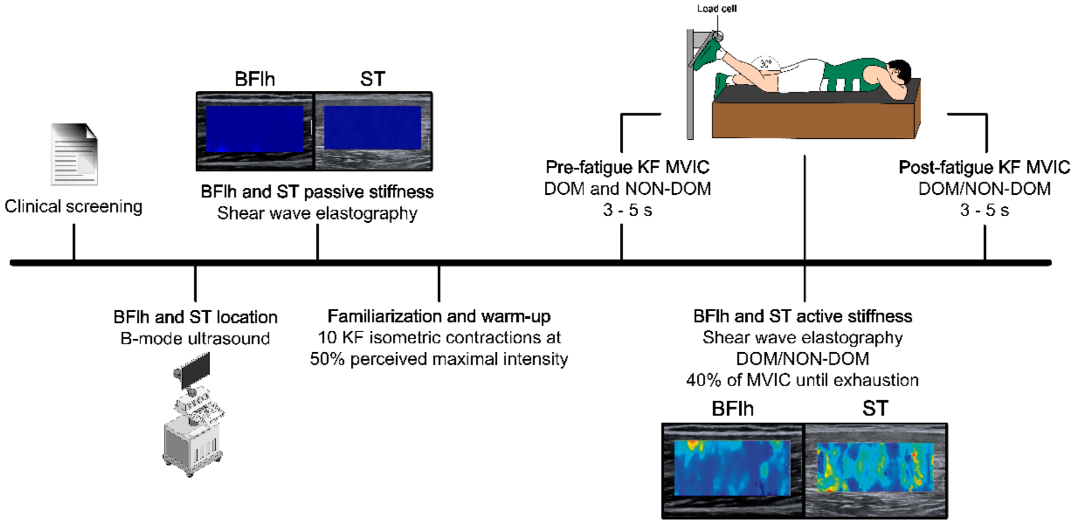
7.2.1. Sample

Participants' recruitment was performed through the researchers' personal contacts which were directed to the health and performance departments of teams participating in the main professional Portuguese football leagues. The sample size required to compare the shear moduli of BFlh and ST between injured and non-injured limbs was calculated on G*Power software (Version 3.1, Universität Düsseldorf) with an estimation of a minimum of 18 participants for each group, assuming a statistical power of 0.95, an effect size of 0.13, and alpha error probability of 0.05. Following previously published data (12) that reported that around 20-30% of the study sample would be found to have suffered a hamstring injury in the past 2 years, efforts were made to recruit a minimum of 90 male footballers. Players were asked to avoid any physical exercise 72 h prior to the testing session, and they were excluded from testing if they had a clinical injury at the time. HSIs were defined according to the Munich Consensus Statement (78). The injured group included players' limbs that sustained HSIs within 2 years of the testing session and were free from any major surgeries in the lower limb. On the other hand, the non-injured group included players' limbs that were free from HSIs or any major surgeries in the lower limbs. The contralateral non-injured limbs of the injured group were also excluded from the latter. For data analysis, only field players were included. Informed consent was given by the players before data collection. The study was conducted under the principles outlined in the Declaration of Helsinki and approved by the local ethics committee (#11/2022).

7.2.2. Study design and general procedures

A quasi-experimental and cross-sectional study design was conducted. Players performed one experimental testing session at the start of the 2022/2023 pre-season. The study's general procedures are schematized in Figure 9. Briefly, general demographic, anthropometric, and clinical lower-limb history information of the players was collected at the arrival to the laboratory. Two researchers identified both limbs' BFlh and ST localization via ultrasound, determined their muscles' ROI, and measured their passive stiffness using SWE. Then, players were familiarised with the equipment and performed a brief specific warm-up (i.e., ~10 knee flexion submaximal unilateral contractions at 50% perceived maximal intensity), followed by knee flexors' MVIC testing of both dominant and non-dominant limbs (assessed with random order). The fatigue task consisting of a knee flexion submaximal isometric contraction at 40% of MVIC until failure was then performed. Both limbs were tested in a random order and the active stiffness of BFlh, and ST muscles was recorded throughout the contraction. After the active stiffness measurements of each limb, knee flexors MVIC was re-assessed to identify the level of NMF.

Figure 9. *Study general procedures taken by the footballers. Representative sonograms of a participant's BFlh and ST are shown both in passive and active conditions (elastogram scale: 0-800 kPa). Legend: BFlh, biceps femoris long head; DOM, dominant limb; KF, knee flexion; MVIC, maximal voluntary isometric contraction; NON-DOM, non-dominant limb; ST, semitendinosus.*



7.2.3. Equipment and variables

7.2.3.1. *Clinical screening*

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

7.2.3.2. *Knee flexor maximal voluntary isometric contraction*

Players were positioned prone, with the hips in a neutral position (secured with a strap), and the knees flexed at ~30° in a custom-built equipment as shown in Figure 7. The foot was positioned in a foot holder at a 90° ankle angle and strapped to avoid the internal/external rotation of the tibia. The foot holder contained a force transducer (Model STC, Vishay Precision, Malvern, PA) measuring at 1kHz to collect the linear force perpendicular to the leg orientation. Force was amplified

(Model UA73.202, Sensor Techniques, Cowbridge, UK), digitally converted (USB-230 Series, Measurement Computing Corp., Norton, MA), and recorded using the DAQami software (v.4.1, Measurement Computing Corp., Norton, MA). Knee flexion torque was estimated by multiplying the perpendicular distance between the centre of the force transducer and the femoral lateral condyle. Players performed two 3-5 s duration MVICs in both limbs with a 1 min rest between repetitions. The researchers gave verbal encouragement to ensure maximal effort during the test. If the difference between MVICs was higher than 5%, more repetitions were performed. The highest peak force value within the 5% difference range was used for analysis.

7.2.3.3. ***Fatigue task***

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

7.2.3.4. ***Passive and active muscle stiffness***

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

7.2.4. Data analysis

The highest peak torque obtained during the knee flexors' MVIC trials before (pre-) and after (post-test) the fatigue task was used to calculate the fatigue index (i.e., the percentage of knee flexion MVIC torque loss after the fatigue task). Time to exhaustion was determined by the total time (s) performed by each participant in the fatigue task. Shear modulus data from the passive and active assessments were analysed using customised Matlab® routines (version R2022a, The Mathworks, Inc., Natick, MA, USA; available at https://cimt.uchile.cl/mcerda/). Each video clip was extracted from the ultrasound software and converted into .avi format. The Matlab routine allowed users to manually select the largest rectangular ROI in the elastogram window of the video and convert the pixels containing elastogram measurements into elastic moduli values based on the recorded scale (0-800 kPa). These values were then averaged to obtain a representative muscle value and exported to individual Excel files. It is important to consider that the values displayed in the elastogram windows of the Aixplorer's ultrasound scanner represent Young's modulus (E) of the medium. Therefore, to estimate the shear moduli (μ) of the muscles, these values were divided by 3 following the formula: μ=E/3 (174). To ensure that the values were stable at the desired contraction intensity, a manual inspection of the data was performed by excluding the initial 5 s of the contraction onset. Using custom routines in Matlab® (version R2022a, The Mathworks, Inc., Natick, MA, USA), data outliers from each muscle data set were: i) excluded using the interquartile method; ii) interpolated using a fourth-order polynomial function (to impute missing data due to the processing time between ultrasound clips); iii) smoothed using a Savitzky-Golay filter; iv) normalised as a percentage from 0 to 100% contraction time; and v) standardised for uniform length of 100 data points representing the full range of contraction from 1 to 100%.

Additionally, the BFlh/ST ratio was calculated at each data point during the fatigue task. This ratio was obtained by dividing the active stiffness values of the BFlh by the corresponding stiffness values of the ST at each percentage of the contraction duration.

7.2.5. Statistical analysis

Statistical analysis of discrete data was performed on JASP (version 0.17.1.0), while the analysis of continuous data was performed using spm1d (version M.0.4.10, SPM1D package, spm1d.org), i.e., an open-source package for one-dimensional SPM in Matlab® (version R2022a, The Mathworks, Inc., Natick, MA, USA). As the current version of spm1d did not allow unbalanced designs, a similar number of non-injured limbs were selected to match the sample size of injured limbs using a matched pairs design, considering similar characteristics as the injured counterparts (i.e., age, height, BM, playing experience, limb dominance, affected limb right or left, and knee flexors' MVIC relative to BM). Matching accuracy was determined using an independent sample T-Test.

Data distribution was confirmed using the Shapiro-Wilk test. The following statistical approaches were used: a 2-way repeated measures ANOVA (limb [injured, non-injured] x time [pre, post] for knee flexors' MVIC, a 2-way repeated measures ANOVA (limb [injured, non-injured] x muscle [BFlh, ST] for muscles' passive stiffness and muscles' slopes, an independent samples T-tests for fatigue index, and Mann-Whitney U test for time to exhaustion and BFlh/ST ratio slope. Regarding continuous data, a 2-way SPM ANOVA (limb [injured, non-injured] x muscle [BFlh, ST]) was performed to analyse muscles' active stiffness differences between limbs throughout the contraction. A post hoc SPM paired sample two-tailed t-test with Bonferroni's correction was performed if significant main or interaction effects were found. Moreover, an independent sample two-tailed t-test was used to examine BFlh/ST ratio differences between limbs.

Eta squared (η^2) was calculated from the repeated measures ANOVA and categorised as small (0.01-0.06), moderate (0.06-0.14), and large (>0.14) (Cohen, 2013). Cohen's d was used for effect size calculations of multiple comparisons and was interpreted as trivial (<0.2), small (0.2-0.59), moderate (0.6-1.19), large (1.2-

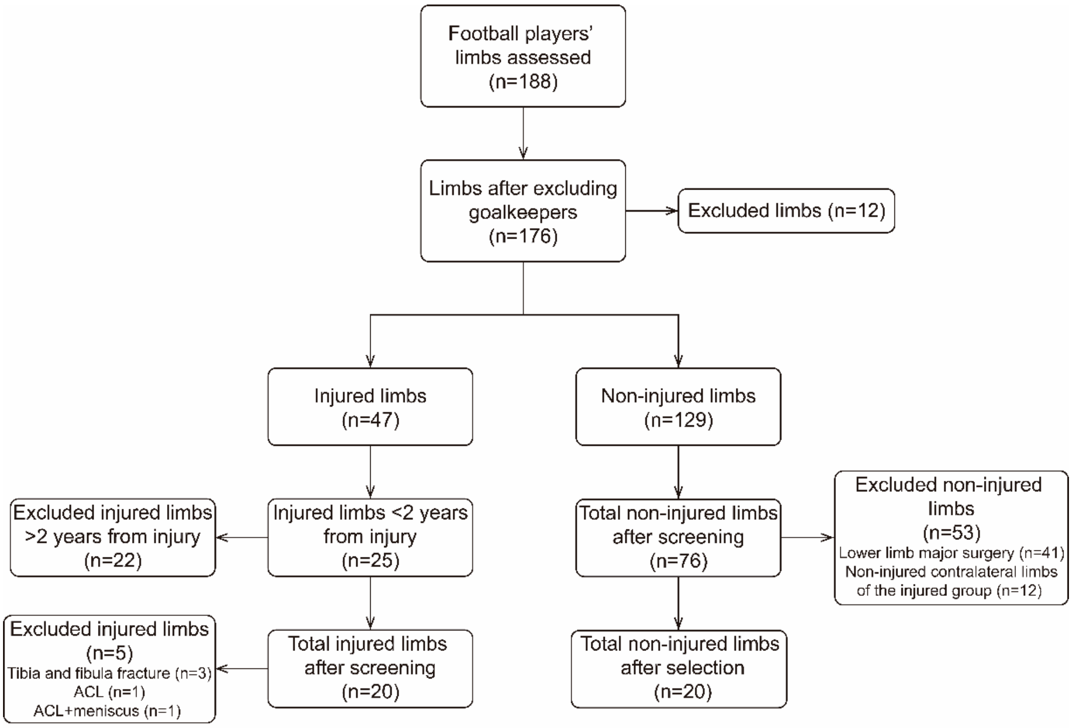
1.99), very large (2-4), and near perfect (>4) (Hopkins et al., 2009). Significance was set at p≤0.05.

7.3. RESULTS

7.3.1. Sample

Four teams accepted to collaborate, and 94 highly-trained national-level (251) male footballers competing in the 2nd (n=50 players) and 3rd (n=44 players) professional divisions (age=24.4±3.8 years; height=180.9±6.5 cm; BM=76.7±6.9 kg; football playing experience=17.0±4.5 years) participated in the study. As shown in Figure 10, 148 limbs were excluded and a total of 40 lower limbs (i.e., 20 with and 20 without previous hamstring injury) were considered for analysis.

Figure 10. *Flow chart of the sample screening.*



7.3.2. Injury data

Descriptive sporting and clinical data from the injured and non-injured limbs can be found in Table 6, where no differences were found for any quantitative variable (p>0.05).

Table 6. *General information of non-injured and injured limbs.*

	Non-injured (n=20)	Injured (n=20)
Age (years)	23.5±3.6	24.0±4.3
Height (cm)	181.7±6.5	183.8±5.7
Body mass (kg)	76.3±7.2	79.7±6.8
Playing experience (years)	16.5±4.4	16.2±5.5
Playing division: 2nd; 3rd (n)	11; 9	5; 15
Centre back (n)	8	1
Full back (n)	0	3
Winger (n)	6	5
Midfielder (n)	4	5
Offensive midfielder (n)	0	0
Forward (n)	2	6
Dominant; Non-dominant limbs (n)	9; 11	9; 11
Right; Left limbs (n)	12; 8	12; 8
Unilateral hamstring injuries (n)		14
Previous HSIs: 1; 2; 3 (n)		14; 4; 2
Injury mechanism: sprint; stretching; COD; unk. (n)		13; 3; 1; 3

Injury context: match; training (n)	9; 11
Match injury incident: ST-1st; END-1st; ST-2nd; END-2nd (n)	1; 2; 1; 5
Time between testing and injury (mo)	12.4±7.1
Time between injury and RTP (wk)	5.5±4.7

Legend: COD, change of direction; END-1st, last 15 min of the first half; END-2nd, last 15 min of the second half; mo, months; HSIs, hamstring strain injuries; ST-1st, first 15 min of the first half; ST-2nd, last 15 min of the second half; unk., unknown; wk, weeks.

7.3.3. Passive stiffness, knee flexors' MVIC, and fatigue results

Table 7 shows the descriptive strength performance results and BFlh and ST passive stiffness outcomes of injured and non-injured limbs.

Table 7. *Performance metrics and passive stiffness of non-injured and injured limbs.*

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

Legend: BFlh, biceps femoris long head; MVIC, maximal voluntary isometric contraction; ST, semitendinosus.

Considering muscles' passive stiffness, significant main effects for muscle factor (p=0.013; η^2=0.09), but not for limb factor (p=0.550; η^2=0.01) or muscle x limb interaction (p=0.885; η^2<0.01) were found. BFlh showed lower passive stiffness levels compared to ST (mean diff.= -0.66 kPa [-1.16 to -0.15 kPa]; p=0.013; ES= -0.54 [-0.98 to -0.10]).

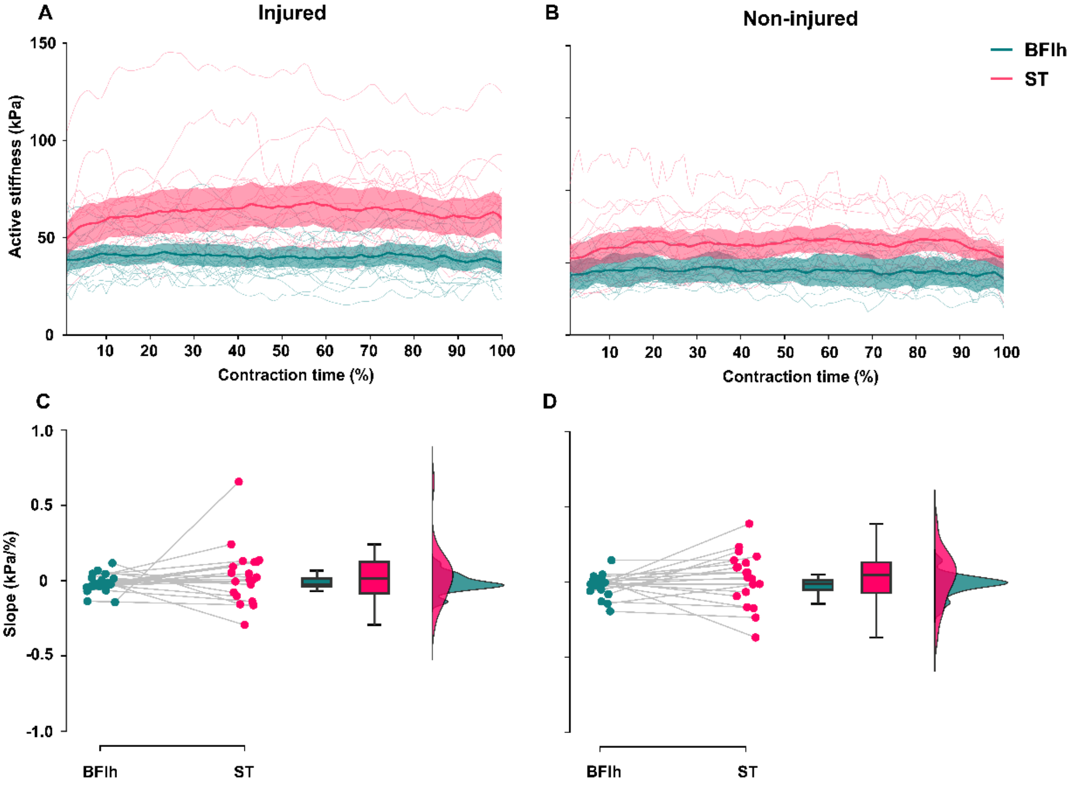
Regarding knee flexors' MVIC, no significant effects were found for limb factor (p=0.322; η^2=0.03) or time x limb interaction (p=0.279; η^2<0.01). However, a significant time factor effect was noted (p<0.001; η^2=0.25) showing a significant knee flexors' MVIC decrease after the fatigue task (mean diff.=17.95 Nm [12.41 to 23.49 Nm]; p<0.001; ES=0.62 [0.34 to 0.90]).

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

7.3.4. BFlh and ST active stiffness

Descriptive data of the active stiffness of BFlh and ST of the injured and non-injured limbs as well as the slope during the fatigue task can be found in Figure 11. No significant main effects for limb factor (p>0.05) or limb x muscle interaction (p>0.05) were found. However, a significant main effect for muscle factor (p<0.001) was noted from 0 to 100% contraction time with the ST muscle showing higher active stiffness compared to BFlh throughout the full contraction duration (p<0.001) (Appendix 6). The slope analysis revealed no significant effects for muscle factor (p=0.116; η^2=0.04), limb factor (p=0.809; η^2<0.01), or limb x muscle interaction (p=0.948; η^2<0.01).

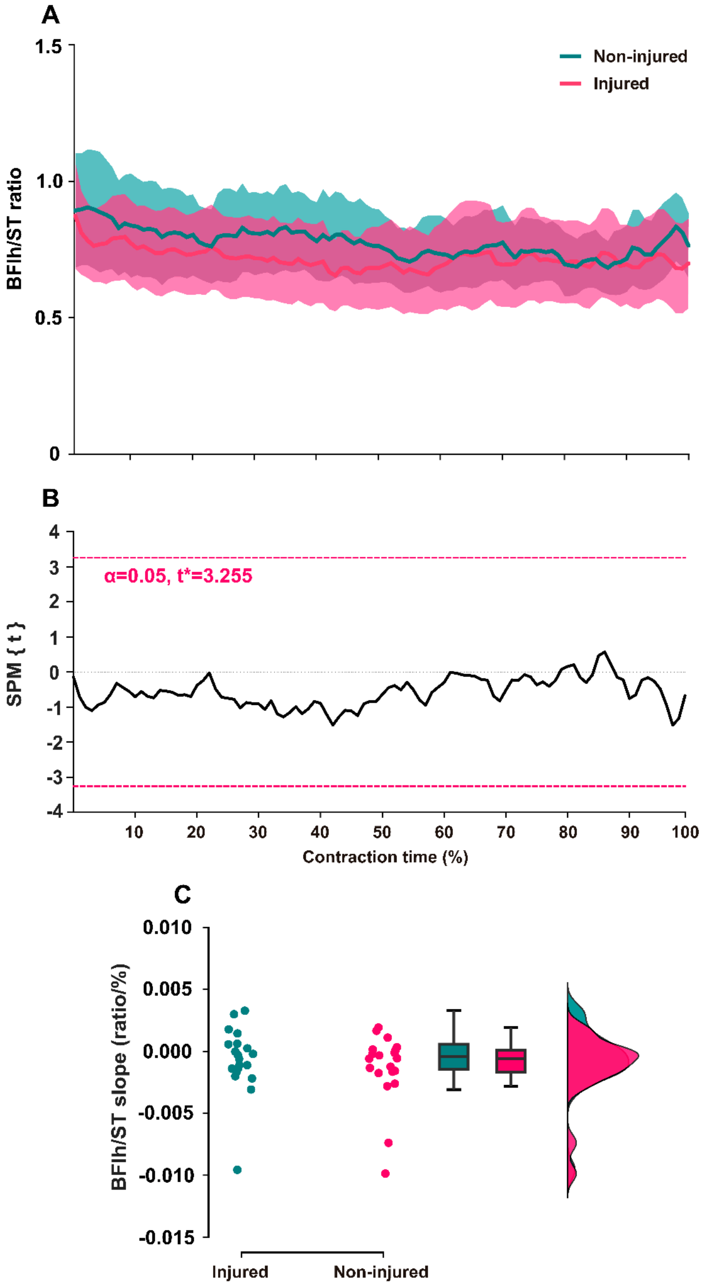
Figure 11. Injured (panel A) and non-injured (panel B) mean active stiffness (solid line) of biceps femoris long head (BFlh) and semitendinosus (ST) during the knee flexors' submaximal contraction until exhaustion. Shaded areas represent the upper and lower 95% confidence interval. Individual data points can be seen as shaded lines. Panels C and D show the between muscle comparisons of injured and non-injured limbs slope, respectively.



7.3.5. BFlh/ST ratio

No significant differences were found throughout the contraction between the BFlh/ST ratio of injured and non-injured limbs (p>0.05) (Figure 12A and B). BFlh/ST ratio slope was similar between injured and non-injured limbs (p=0.547; ES=0.24 [-0.38 to 0.86]) (Figure 12C).

Figure 12. *A) Mean (solid line) biceps femoris long head/semitendinosus (BFlh/ST) ratio during the knee flexors submaximal contraction until exhaustion in injured and non-injured limbs. Shaded areas represent the upper and lower 95% confidence interval. B) Statistical parametric mapping (SPM) analysis, i.e., t-statistics (SPM{t}), of the differences in BFlh/ST ratio between injured and non-injured limbs. The pink dashed lines represent the critical thresholds. C) Between limb comparison of BFlh/ST ratio slope.*



7.4. DISCUSSION

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

During the fatigue task, our analysis showed that active stiffness levels of the BFlh and ST muscles were consistent across injured and non-injured limbs, a result that diverges from the findings of Freitas et al. (121) yet aligns with those in another study by the same research group (236). Despite applying a similar methodological procedure but with a higher contraction intensity (i.e., 40% of MVIC) than that used by Freitas et al. (121) (i.e., 20% of MVIC), no significant differences in BFlh active stiffness across limbs were detected during the fatigue task. These findings might be partly explained by the different training statuses between the samples (elite vs. highly-trained football players) and the higher contraction intensity used. Martínez-Serrano et al. (252) investigated the same contraction intensity (i.e., 40% of MVIC) in recreationally active individuals, and concluded that to induce changes in the shear modulus of the BFlh and ST, longer contraction durations at reduced intensities were required. This could elucidate the lack of discernible differences between injured and non-injured limbs, given that the average contraction duration for all limbs was 102.9±42.1 s, even shorter than the duration observed in the abovementioned study (153.1±40.0 s). Notably, Evangelidis et al. (138) demonstrated that intermittent contractions at 50% of MVIC for a total duration of 494 s in healthy individuals could elicit active stiffness alterations in the ST and BFlh muscles. Hence, future research aiming to identify differences between injured and non-injured players under isometric conditions may consider longer muscle contraction durations, although the efficiency of testing sessions, particularly in pre-season assessments of full squads, could be affected.

Nevertheless, incomplete knowledge surrounding near maximal isometric contractions or explosive dynamic contractions in SWE measurements is still present, mainly due to the saturation of the elastogram (253) and the low sample rate of the ultrasound devices (254). This technological constraint hinders the understanding of the mechanical properties of the muscle under more realistic conditions. Finally, another contributing factor could be the level of NMF induced by the fatigue protocol. In our study, the overall strength loss was 12.4±10.9%, whereas previous research has documented reductions in knee flexion torque typically ranging from ~4% to ~8% (121,137). Notwithstanding, despite having a lower fatigue index, they observed within-muscle stiffness changes during the contraction until exhaustion at 20% of MVIC, suggesting that the level of NMF might not substantially influence muscle active stiffness changes compared to increased intensity levels.

The evaluation of load-sharing patterns revealed similar BFlh/ST ratios between injured (0.72±0.3 ratio) and non-injured (0.78±0.28 ratio) limbs throughout the contraction duration, which contradicts our initial hypothesis. Particularly, load-sharing strategies appeared highly individualised, as evidenced by the distinct BFlh/ST ratios during contraction in injured (CV=42.3%) and non-injured limbs (CV=35.5%) suggesting that players might have adopted diverse strategies to sustain torque production at the predetermined intensity, mainly by altering the ST behaviour rather than the BFlh. Moreover, it is important to note that between 40 to 60% of knee flexors' MVIC, the change in the shear modulus of these muscles stabilises, probably due to the increased participation of other synergistic muscles (e.g., SM, gastrocnemius, BFsh, gracilis, and sartorius) (183). Due to experimental limitations, this hypothesis could not be directly verified. Thus, future research may explore the contribution of these synergistic muscles as well as including hip extension contractions.

On the other hand, inconsistent evidence regarding the differences in passive mechanical properties measured via SWE in previously injured hamstrings has been found. Some studies reported increased passive stiffness in the muscle belly of injured limbs (255), increased passive stiffness exclusively in the BFlh fascia of injured limbs (256), while others observed no differences (236). Our findings indicate similar passive stiffness between injured and non-injured limbs as Freitas et al. (236), although with lower absolute values (mean BFlh=4.3-4.5 kPa and mean ST=4.5-4.7 kPa). Interestingly, our data suggests higher overall passive stiffness in the ST compared to the BFlh (BFlh=6.7±1.3 kPa and ST=7.4±1.1 kPa), which diverges from prior research suggesting that the ST typically exhibits the lowest passive stiffness values within the hamstring group irrespective of hip and knee position (257,258) and athlete training status or sport specialization (259). The underlying causes remain unclear; however, the specific location of the injury may be a contributing factor, which was not evaluated in this study as the clinical data was derived from self-reported information.

Maximal force production capabilities of the knee flexors were similar between injured and non-injured limbs, consistent with the hypothesis that muscle inhibition could predominantly occur in dynamic actions, especially under eccentric contractions (104,111). While some studies present conflicting evidence, it is pertinent to note that such investigations were either conducted with recreationally active males (250) or identified differences exclusively at longer muscle lengths (260). Consequently, it could be assumed that knee flexors' weakness under isometric conditions becomes meaningful only at lengthened positions, similar to those experienced during the early stance and late swing phase of a sprint, where hamstring injuries are more susceptible to occur (95,261). Ultimately, no significant difference in knee flexors' endurance between limbs with and without a history of HSIs was found. Freckleton et al. (262) and Lord et al. (263) found contradictory results, showing reduced endurance capacities in the injured limb; however, both studies used different endurance tests compared to the present study. This underscores the importance of considering the specific functional demands and biomechanical positions when assessing muscle strength and rehabilitation outcomes in hamstring injury management.

This study faces several limitations that require consideration when interpreting its findings. The absence of detailed injury reports from medical records and reliance on players' self-reports introduces potential gaps in our understanding of the precise type and location of injuries. Such details, including the specific muscle injury location (proximal, medial, or distal) and the injury site (myofascial, myotendinous junction/muscular, or tendinous), are critical for fully comprehending the passive and active mechanical properties of the BFlh and ST muscles. Future research should, therefore, incorporate in-depth assessments of injury location through gold-standard imaging (i.e., MRI), considering the specific characteristics of individual injuries to determine variations in stiffness between limbs more accurately. Lastly, our findings revealed heterogeneous responses in BFlh and ST muscle stiffness during the fatigue task, challenging the hypothesis of uniform load-sharing patterns between injured and non-injured limbs. This variability may be attributable to the contributions of synergistic muscles in torque production (242) and the potential co-activation of antagonist muscles at higher contraction intensities (243). However, the complexity of our experimental setup and resource constraints excluded direct measurements of these factors. As a result, caution should be taken in interpreting these findings, emphasising the need for further research to elucidate the mechanical differences in athletes with a history of hamstring injuries, incorporating a more detailed examination of contributing factors to muscle function and injury effects.

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

VIII – DISCUSSION

VIII - DISCUSSION

The main objective of the present compendium of studies was to investigate the NMF of the posterior chain muscles during a competitive football microcycle, compare the effects of increased contraction intensity on the active stiffness responses and load-sharing of BFlh and ST, and compare the mechanical properties and load-sharing strategies of the hamstrings in previously hamstring-injured football players to healthy players. The main results indicated that a) an increase in HSR demands in a typical in-season training session in young elite football players acutely decreased the neuromuscular performance of the posterior chain muscles without affecting perception of effort; b) muscle contraction duration seems to have greater relevance than contraction intensity in altering the active stiffness of the hamstring muscles during fatiguing tasks, particularly in the ST; and c) no alterations in the mechanical characteristics or load-sharing strategies of the BFlh and ST of previously injured limbs at 40% of MVIC.

In study 1, we analyzed the acute and residual effects of different HSR demands (i.e., high and low, as divided through a median split) during a training session in young football players on localized NMF of the KE, posterior chain muscles, and RPE. The acute decrease in posterior chain neuromuscular performance after the training session highlights the importance of considering external load metrics, specifically HSR, when monitoring training-induced fatigue. Considering the multiple variables provided by the GPS, only HSR was sensitive enough to detect acute NMF in the posterior chain muscles. The exposure to large distances of sprinting/accelerations has demonstrated significant decreases in the recovery kinetics of strength performance after a football match (148,264). Interestingly, a systematic review and meta-analysis that aimed to determine which external load metrics during a football match-play more effectively reflect the acute and residual changes in post-match responses found that very high-intensity running (i.e., above 5.5 $m \cdot s^{-1}$) was the only variable that largely correlated with biochemical and neuromuscular markers (209). They showed that very high-intensity running could explain up to ~50% of the post-match neuromuscular state, highlighting the importance of controlling this variable if the goal of the strength and conditioning coach is to avoid significant changes in the players' neuromuscular performance. Although our results showed that posterior chain neuromuscular performance was recovered 6h and 24h after the training session, the group that performed higher HSR demands presented a delayed recovery dynamic throughout the experimental period compared to the group with lower demands (despite the absence of significant differences between groups). In a practical context, limiting HSR demands in the training session could effectively dampen the acute and residual effects of fatigue and, consequently, potentially decrease the probability of a hamstring injury. This becomes especially important in congested competitive periods where recovery time is limited (153,265).

Regarding the KE muscles, no significant NMF changes within the intervention were noted. This was expected when modifying mainly the HSR demands, as the hamstring activation is expected to be higher at increased running velocities compared to KE muscles, which tend to decrease (211). Nevertheless, we were surprised that players who performed high HSR demands had similar internal responses compared to players with lower demands as previous studies have shown a high correlation between these variables (214,266,267). This could be partially explained by the type of external load variable used, as the number of HSR actions indicates volume but not the frequency of these actions. Therefore, it might be possible that these actions were heterogeneously spread across the training session interspersed with extensive recovery time between them, thus reducing players' perception of effort. Notwithstanding, it is important to note that these players presented neuromuscular impairments in the posterior chain muscles of the dominant limb, emphasizing the importance of evaluating subjective information together with specific neuromuscular assessments of the muscles involved.

Then, in study 2, we aimed to compare the effects of contraction intensity during a knee flexors' isometric contraction until exhaustion performed at 20% or 40% of MVIC, on the active stiffness responses and load-sharing of BFlh and ST. Our results showed that at 20% of MVIC, ST active stiffness decreased at the last stage of the contraction but not at 40% of MVIC. In contrast, BFlh active stiffness remained relatively unaltered during both intensity conditions. This led to a greater increase in the BFlh/ST ratio over time in the low intensity condition. The decrease in ST active stiffness that was found only in the low intensity condition could be simply explained by the significantly higher fatigue index produced at 20% of MVIC compared to 40% of MVIC. However, in a previous study by Evangelidis et al. (138), a decreased tendency in the ST active stiffness and an increased tendency in BFlh was noted after 99 intermittent isometric contractions performed at 50% of MVIC, where participants lost 18.4% of knee flexors' MVIC, a similar value that we found at 40% of MVIC (i.e., 19.6%). This would suggest that contraction duration could be more relevant to induce changes in the stiffness of the hamstring muscles. For instance, the total effective contraction duration in Evangelidis et al. (138) was 494 s at 50% of MVIC and we observed a decrease in ST active stiffness at ~561 s onwards at 20% of MVIC, probably explaining why no changes at 40% of MVIC were found due to the short contraction duration (average duration of 153 s). Nevertheless, despite measuring specifically in the muscle that is composed primarily of contractile materials, the non-contractile connective tissues (consisting mainly of collagen) such as perimysium and epimysium could have affected muscle stiffness (268,269) due to a possible stress-relaxation effect (270). Moreover, other connective tissues in the ST, particularly in its long distal and compliant tendon and in the tendinous inscription, could have decreased the tensile stress throughout the contraction (49,271).

Surprisingly, the load-bearing of ST was superior to BFlh during both submaximal isometric contraction intensities, but at some point, became similar. This was interesting, as the PCSA of the ST is significantly lower than the BFlh (25), indicating a lower torque-generation capacity. This load-sharing strategy could be potentially less efficient as if the muscle is activated more than required by the task, its metabolic demand would be higher and would develop fatigue earlier. However, this may reflect the diverse architecture of the hamstring muscles. For example, the fusiform and long fibered ST could transmit force more efficiently and their sarcomere could operate closer to their optimal length over a larger range of motion (24). This behaviour seems consistent, as different studies have found this preferential borne of ST over BFlh and SM, even under different contraction modes (187). Notably, Evangelidis et al. (187) found that the anatomical cross-sectional area of ST was negatively correlated with the peak isometric shear wave velocity of the ST, explaining that subjects with a small ST muscle would have to operate closer to their absolute load capacity and thus be more susceptible to fatigue. This is noteworthy, as it could be hypothesized that BFlh injuries could be the result of an overloaded ST that changes the load distribution among hamstrings during fatigue conditions, creating large intermuscular shearing forces that could damage the extracellular matrix structures (272). This assumption could explain why the Nordic hamstring exercise, which preferentially targets the ST (273,274), is an effective exercise for hamstring strain injury prevention (275,276).

Contrary to the hypothesised, the BFlh/ST ratio was found to be similar between both intensity conditions. We observed high interindividual variability, especially in the ST muscle at 40% of MVIC which could partially explain why no statistical differences were noted. In line with this result, Hug and Tucker (277) explained that muscle coordination strategies are individual-specific and that only reporting average group data may conceal important differences between participants and lead to an understanding that all individuals use similar coordination strategies. This observation has been proved in different simple single-joint tasks such as isometric plantar flexion (278) or KE (279). Regarding the hamstring muscles, Avrillon et al. (233) found that torque-sharing strategies between participants varied significantly during an isometric contraction of the knee flexors at 20% of MVIC, with a torque distribution ranging from 20.2 to 54.8% for BF, 28.3 to 63.7% for SM, and 9.1 to 39.9% for ST. This supports the idea that, instead of finding the best-optimized strategy for a specific task (280), individuals can perform it with a range of "good-enough strategies" (281) following a trial-and-error process. Therefore, providing individual muscle coordination strategies instead of group averages could help researchers identify if some patterns could increase the risk of injury compared to others, as found in Schuermans et al. (118).

In Study 3, the objective was to determine if players with a previous hamstring injury presented modified passive and active stiffness properties and load-sharing strategies in the BFlh and ST, together with a performance decrease in maximal and endurance capacities of the knee flexors. Our findings revealed no alterations in the stiffness characteristics or load-sharing capacities of the BFlh and ST in previously injured limbs, together with similar force and endurance capacities to non-injured limbs. This would suggest that previously injured players may have recovered or maintained BFlh and ST passive and active stiffness. The fact that we did not have access to players' injury reports limited the explanation of this result, as the specific muscle, injury location, or injury site was not considered. Furthermore, and as found in Study 2, a high interindividual variability was found, especially in the ST muscle. Along with the previously mentioned explanation of individual-specific muscle coordination strategies, we believe that the higher intensity used could have increased the co-activation of the antagonist and synergistic muscles, thus creating high variability in torque production. However, we were not able to confirm this as the antagonist and synergistic muscles were not measured. Moreover, considering the results in Study 2, it was possible that the contraction duration was not long enough to produce changes in the active stiffness of the muscles. Interestingly, the fatigue index in the football players was lower than in the recreationally active individuals of Study 2. However, we are unaware if higher fatigue index levels could elucidate higher changes in active stiffness during the contraction as Mendes et al. (137) found that ST active stiffness started to decrease at 40% of the contraction duration and the fatigue index (4 to 8%) was even lower than in our study. This means that the hamstring active stiffness pattern could be modified with minimal fatigue levels, contrary to what has been evidenced in the quadriceps (216,249).

On the other hand, the position used to test and fatigue the hamstring muscles (i.e., prone with 0° hip flexion and 30° knee flexion) could have also explained why no changes were found between limbs. In this position, the hamstrings would be operating on the ascending part of their force-length relationship for torque production (i.e., at a less optimal range for torque development and relatively short muscle lengths). Therefore, it would be possible that the mechanical properties of the BFlh would only be affected at longer muscle lengths, where the injury seems to occur (120). Notably, Kellis and Blazevich (282) found that the contribution to knee flexion torque of BFlh and SM was higher in lengthened positions. This is in line with the specific BFlh and SM muscle hypertrophy increase found after a lengthened state eccentric training that forced greater hip flexion but not during Nordic hamstring training (283). Therefore, further research should then consider measuring at longer muscle lengths by increasing the degree of hip flexion to better load the BFlh over ST. Moreover, it would be interesting to include hip extension contractions, as the hamstrings' maximum moment arms are greater at the hip than the knee, thus increasing force production at the hip (282).

Lastly, it is important to consider that the rehabilitation and return-to-play process of the players was not evaluated. Therefore, it would be possible that players would have completed a comprehensive return-to-play protocol focusing on isometric and eccentric contractions at long muscle lengths, improving the symmetry between limbs (284,285). This may further explain why no changes in maximal force production and endurance capabilities were found between previously injured and non-injured limbs. Nevertheless, following that compelling evidence indicates that neuromuscular muscle inhibition after a hamstring injury may be found preferentially under dynamic eccentric contractions (111), we do not exclude that some differences between previously injured and non-injured limbs may be encountered.

In summary, from an applied perspective and based on the results of the present compendium of studies, strength and conditioning coaches should be aware of the acute reductions of posterior chain neuromuscular performance due to increased HSR demands and include a simple isometric test to evaluate localized NMF as RPE may not necessarily discriminate between players with higher and lower HSR demands and NMF. Moreover, when assessing knee flexors' active stiffness during fatigue conditions with SWE, low-intensity isometric contractions at 20% of MVIC are recommended to evidence stiffness changes, specifically in the ST muscle. Lastly, a higher submaximal contraction intensity at 40% of MVIC did not show that active stiffness of previously hamstring-injured limbs was different to non-injured limbs under fatigue and non-fatigue conditions, suggesting that BFlh and ST mechanical properties and load-sharing may be recovered or maintained. Therefore, further research should use other contraction intensities, muscle-tendon lengths, or contraction types to corroborate this finding.

IX – CONCLUSIONS

IX - CONCLUSIONS

9.1. GENERAL CONCLUSIONS

The results of the present compendium of studies concluded that the maximal force production capacity of the hamstrings is acutely reduced after a training session with high HSR demands and the mechanical properties, as well as the load-sharing between the BFlh and ST, are not affected in players with a history of previous hamstring injuries.

9.2. SPECIFIC CONCLUSIONS

The specific conclusions of the studies included in this thesis are outlined below. It is crucial to note that these conclusions are only applicable to samples with similar characteristics to those described in each study.

Study 1:

- HSR demands had the most influence on the acute effects of NMF in the posterior chain muscles, while no other GPS variable was allowed to discriminate NMF in the KE.
- Posterior chain NMF increased after the training session only in the players who performed high HSR demands. However, performance was recovered at 24 h after the training session.
- Players with different HSR demands perceived the intensity of the training session similarly, although only the player with high HSR demands presented NMF on the posterior chain muscles.

Study 2:

- ST active stiffness only decreased after a specific threshold in the 20% of MVIC session, while BFlh active stiffness remained unchanged despite using a higher contraction intensity. Moreover, ST displayed higher active stiffness than BFlh in both conditions but at some point of the contraction, the intermuscular stiffness became similar.

- The BFlh/ST ratio was similar between intensity conditions, although a steeper increase in the ratio was found at 20% of MVIC, suggesting that the contribution of BFlh increased throughout the contraction due to the decrease in ST active stiffness.

Study 3:

- Similar BFlh and ST active stiffness levels were found between previously hamstring-injured and non-injured limbs, both in fatigued and non-fatigued states.
- Similar load-sharing strategies were found between previously hamstring-injured and non-injured limbs. Moreover, previously hamstring-injured limbs did not show a steeper increase in the BFlh/ST ratio throughout the contraction.
- Similar muscles' passive stiffness, knee flexors' maximal torque and endurance capacities were found between previously hamstring-injured and non-injured limbs.

X – LIMITATIONS

X - LIMITATIONS

Several limitations of the studies that compose this thesis must be addressed:

Study 1:

- KE MVIC could not be measured at all time points, limiting the understanding of KE residual fatigue during the experimental protocol.
- Only the dominant limb was assessed, thus it is unknown whether the increase in HSR demands also affected the non-dominant limb.
- The moment arm was not measured and only force in N was reported. This leads to a significant limitation, as force does not account for the rotational effect produced by the muscle around the joint (286). For example, one individual may generate higher forces but lower torque if their moment arm is shorter.
- Injury history was not considered in the analysis of the results nor were the groups counterbalanced to have an identical number of injured and non-injured players in each group.

Study 2 and 3:

- During the fatigue task, the contribution of the synergist muscles and the possible co-activation of the antagonist was not measured. Therefore, we are unaware of how these factors could have modified our results, maybe explaining the high variability found at the higher submaximal isometric contraction at 40% of MVIC.
- Limited information regarding muscle regional differences in passive and active stiffness can be drawn from the studies as only the mid-region of the muscles was measured. For instance, Vaz et al. (287) found a heterogeneous stiffness response in the BFlh during knee flexion, showing greater stiffness distally than proximally. Moreover, the fact that the size of the ROI was not standardized between muscles (i.e., the largest rectangular ROI in the elastogram window in each muscle was selected, avoiding aponeuroses and tissue artefacts) modified their sampling frequency. For instance, the

sampling frequency for ST (0.7-1.0Hz) was sometimes lower than the BFlh (1.0-1.1Hz). This could have created a higher variability in the elastogram signal.

- The recording duration in SWE mode of the ultrasound was 60s and the processing time between clips was 5s. Despite fitting a 4th-order polynomial function to impute the missing data, the true active stiffness response was lost.
- When analysing torque data during the fatigue task, some individuals showed, at brief instants, torque deviations from the 5% threshold range. Although the torque did not change significantly from the targeted intensity as shown in (Appendix 2), this could have introduced more variability in the elastogram, considering the force-stiffness relationship.
- In Study 2, only the dominant limb was measured in both intensity conditions, thus we are unaware if the load-sharing pattern between the BFlh and ST would be different between limbs.
- In Study 3, only self-reports of players' injury history could be collected, lacking important information such as muscle injured, specific muscle injury location (i.e., proximal, medial, or distal), and injury site (i.e., myofascial, myotendinous junction/muscular, or tendinous). Moreover, the rehabilitation process after the last injury was not provided by the medical team of the clubs.

XI – PRACTICAL APPLICATIONS

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From a practical and applied standpoint, based on the findings of the studies presented in this thesis, strength and conditioning coaches and researchers should consider the following:

- Strength and conditioning coaches should implement fatigue monitoring practices to prevent injury risk factors associated with NMF from various tasks and demands during the competitive microcycle.
- Given that HSR demands significantly impact posterior chain neuromuscular performance, strength and conditioning coaches should be cautious when prescribing high volumes of HSR tasks in consecutive sessions or congested competition periods.
- While RPE is a useful measure of how intense players perceive a session, it may not accurately differentiate between athletes with varying HSR distances and NMF levels. Thus, RPE should be combined with external load tracking and muscle-specific neuromuscular assessments for more comprehensive monitoring of localized fatigue.
- Researchers may use 20% of MVIC to identify changes in ST active stiffness and load-sharing between BFlh and ST throughout a submaximal isometric contraction of the knee flexors.
- Researchers should consider the individualised analysis of load-sharing between synergistic muscles to better represent the highly individual responses during sustained submaximal isometric contractions until exhaustion.
- A neutral hip with a 30° knee flexion in a prone position can be used by researchers if they aim to mainly bear the ST over the BFlh, to analyse its fatigability during sustained submaximal isometric contractions until exhaustion.
- Moderate contraction intensities at 40% of MVIC may not be optimal to discriminate if previously hamstring-injured players have different

stiffness levels and load-sharing strategies compared to healthy players during a sustained submaximal isometric contraction until exhaustion.

XII – FUTURE RESEARCH LINES

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Upon completing this thesis, several future research directions emerge from the obtained results. The following potential research could provide further insight into the topics studied here:

- To analyse how posterior chain neuromuscular performance variates throughout the competitive season, focusing on congested periods where recovery time between stimuli is limited. Moreover, to identify if sudden changes in HSR demands in training and competition are linked with the occurrence of hamstring injuries.
- To determine if previously hamstring-injured players are more sensible to NMF when performing high HSR distances compared to non-injured players.
- To explore if players with longer hamstring fascicles are better protected from the acute and residual effects of NMF after an HSR session.
- To further determine the role of contraction intensity and contraction duration by testing different contraction intensities with the same absolute time achieved at 20% of MVIC.
- As in Studies 2 and 3, the position selected mainly loaded the ST over the BFlh, future research should test the combination of different hip and knee angles (i.e., short, intermediate, and long muscle lengths) and different joint actions (i.e., hip extension, knee flexion or combined movement) to understand which position and joint action better involves the BFlh over other hamstring muscles to identify the muscle fatigability and possible differences between injured and non-injured players.
- To prospectively analyse if players with lower ST stiffness fatigability present a lower injury incidence throughout their career.
- As females seem to present a different injury susceptibility compared to males concerning hamstring injuries, it would be interesting to compare

the passive and active stiffness and load-sharing of the hamstring muscles between sexes.

- To explore if injured and non-injured limbs present different neuromuscular properties such as motor unit recruitment or firing rate.

XIII – MENCIÓN INTERNACIONAL

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Con el fin de cumplir con los criterios establecidos en el Real Decreto 99/2011 para obtener la Mención Internacional en el Título de Doctor, se presentan las conclusiones de este compendio de estudios en un idioma diferente al empleado en el resto de la tesis.

13.1. CONCLUSIONES GENERALES

Los resultados del presente compendio de estudios concluyeron que la capacidad máxima de producción de fuerza de la musculatura isquiosural se ve reducida después de una sesión de entrenamiento con altas demandas de HSR y que las propiedades mecánicas, así como la distribución de la carga entre el BFlh y el ST, no se ven afectadas en jugadores con antecedentes de lesiones previas en la musculatura isquiosural.

13.2. CONCLUSIONES ESPECÍFICAS

Las conclusiones específicas de los estudios incluidos en esta tesis se detallan a continuación. Es crucial indicar que estas conclusiones son aplicables únicamente a muestras con características similares a las descritas en cada estudio.

Estudio 1:

- Las demandas de carrera a alta velocidad tuvieron la mayor influencia sobre los efectos agudos de la fatiga neuromuscular en los músculos de la cadena posterior, mientras que ninguna otra variable de GPS permitió discriminar la fatiga neuromuscular en los extensores de rodilla.
- La fatiga neuromuscular de la cadena posterior aumentó después de la sesión de entrenamiento solo en los jugadores que realizaron altas demandas de carrera a alta velocidad. Sin embargo, los valores de

fuerza se recuperaron a las 24 h después de la sesión de entrenamiento.

- Los jugadores con diferentes demandas de carrera a alta velocidad percibieron la intensidad de la sesión de entrenamiento de manera similar, aunque solo los jugadores con altas demandas de carrera a alta velocidad presentaron fatiga neuromuscular en los músculos de la cadena posterior.

Estudio 2:

- La rigidez activa del semitendinoso solo disminuyó después de un umbral específico en la sesión al 20% de la contracción voluntaria isométrica máxima, mientras que la rigidez activa de la cabeza larga del bíceps femoral se mantuvo sin cambios a pesar de usar una intensidad de contracción mayor. Además, el semitendinoso mostró una mayor rigidez activa que la cabeza larga del bíceps femoral en ambas condiciones, pero en un momento de la contracción, la rigidez intermuscular se volvió similar.
- El ratio cabeza larga del bíceps femoral/semitendinoso fue similar entre las diferentes condiciones de intensidad, aunque se encontró un aumento más pronunciado en el ratio al 20% de la contracción voluntaria isométrica máxima, lo que sugiere que la contribución de la cabeza larga del bíceps femoral aumentó a lo largo de la contracción debido a la disminución de la rigidez activa del semitendinoso.

Estudio 3:

- Se encontraron niveles similares de rigidez activa de la cabeza larga del bíceps femoral y semitendinoso entre las extremidades previamente lesionadas y no lesionadas, tanto en condiciones de fatiga como sin fatiga.
- Se encontraron estrategias de distribución de carga similares entre las extremidades previamente lesionadas y no lesionadas. Además, las extremidades previamente lesionadas no mostraron un aumento más pronunciado en el ratio cabeza larga del bíceps femoral/semitendinoso a lo largo de la contracción.

- Se encontraron niveles similares de rigidez pasiva de los músculos, fuerza máxima de los flexores de rodilla y capacidad de resistencia entre las extremidades previamente lesionadas y no lesionadas.

XIV – REFERENCES

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XV – APPENDICES

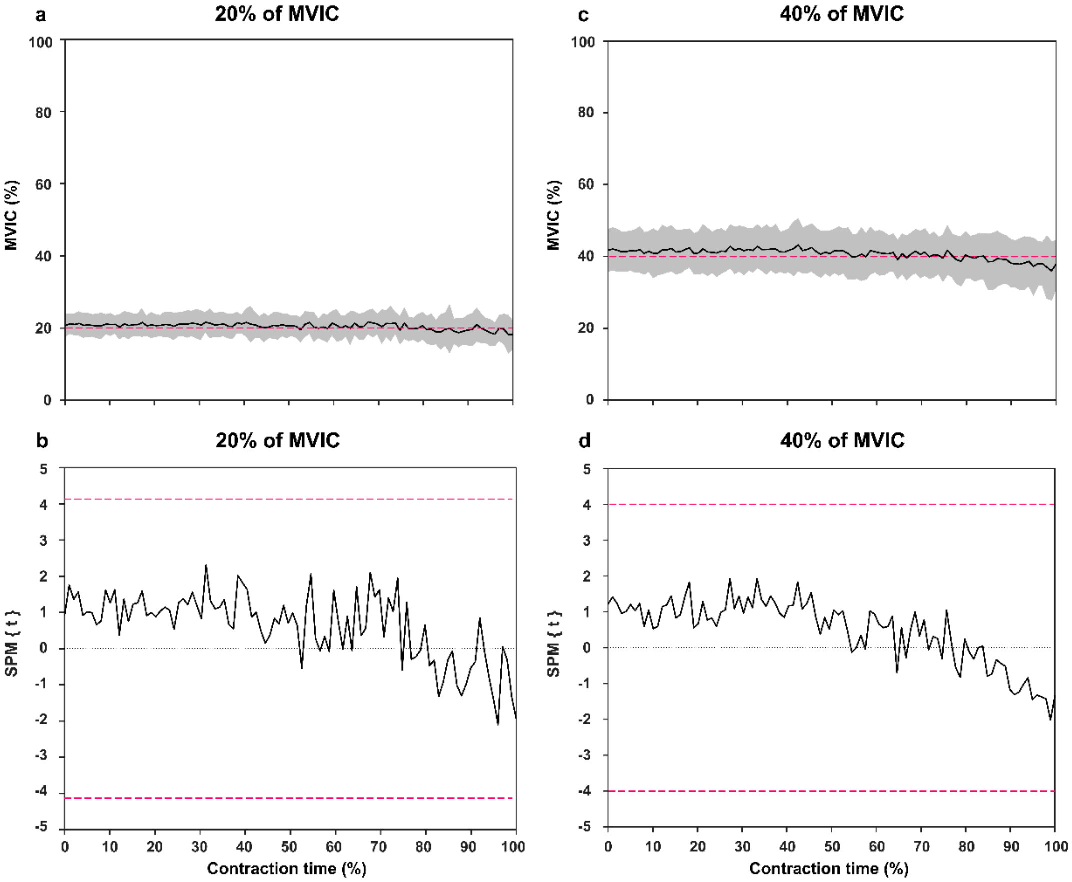
XV - APPENDICES

APPENDIX 1. Multiple comparisons describing the group x time interaction in different sessions.

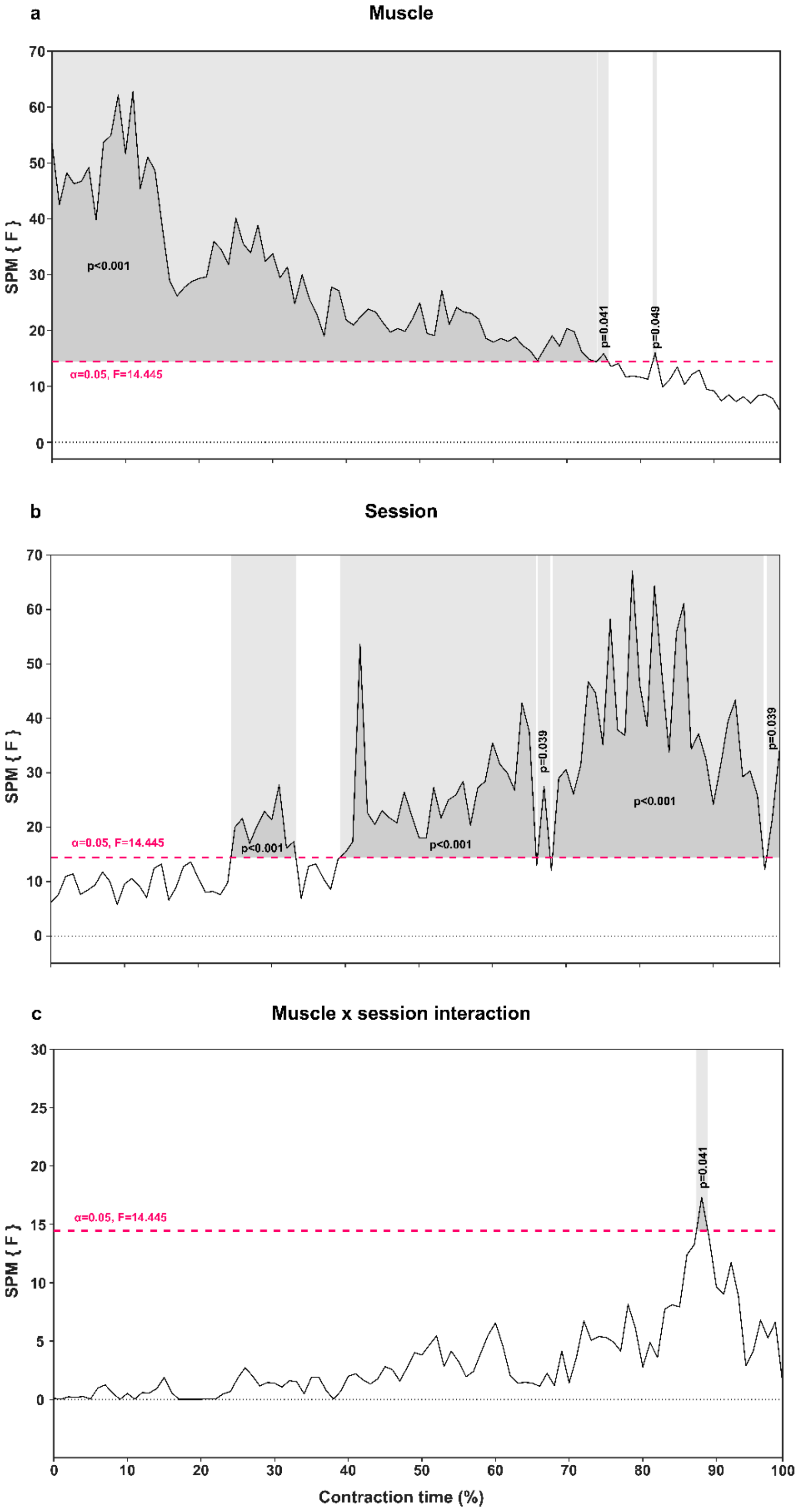
		Tukey’s multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P Value	ES
SSG	**TD**	**HIGH**				
		POST-ACT vs. POST-SSG	6.906	-41.68 to 55.49	0.999	0.10
		LOW				
		POST-ACT vs. POST-SSG	-8.504	-56.13 to 39.12	0.999	0.05
	ACC	**HIGH**				
		POST-ACT vs. POST-SSG	1.017	-46.81 to 48.85	>0.999	0.02
		LOW				
		POST-ACT vs. POST-SSG	-2.504	-51.24 to 46.23	>0.999	0.05
	DEC	**HIGH**				
		POST-ACT vs. POST-SSG	-4.544	-48.46 to 39.37	>0.999	0.04
		LOW				
		POST-ACT vs. POST-SSG	5.312	-48.81 to 59.43	>0.999	0.13
TR1	**TD**	**HIGH**				
		POST-SSG vs. POST-TR1	39.420	-6.756 to 85.60	0.157	0.37
		POST-TR1 vs. POST-6H	-10.450	-58.37 to 37.47	0.998	0.10
		POST-TR1 vs. POST-24H	-12.580	-59.64 to 34.49	0.992	0.12
		LOW				
		POST-SSG vs. POST-TR1	9.451	-36.73 to 55.63	0.999	0.08
		POST-TR1 vs. POST-6H	-10.21	-56.38 to 35.97	0.998	0.08
		POST-TR1 vs. POST-24H	-20.53	-67.56 to 26.50	0.884	0.17
	HSR	**HIGH**				
		POST-SSG vs. POST-TR1	**47.560**	**1.547 to 93.57**	**0.037**	**0.45**
		POST-TR1 vs. POST-6H	-13.430	-61.18 to 34.32	0.989	0.14
		POST-TR1 vs. POST-24H	-18.240	-64.25 to 27.77	0.927	0.17
		LOW				
		POST-SSG vs. POST-TR1	1.776	-45.91 to 49.46	>0.999	0.01
		POST-TR1 vs. POST-6H	-5.178	-52.87 to 42.51	>0.999	0.06
		POST-TR1 vs. POST-24H	3.521	-44.17 to 51.21	>0.999	0.14
	ACC	**HIGH**				
		POST-SSG vs. POST-TR1	17.210	-29.57 to 63.99	0.950	0.14
		POST-TR1 vs. POST-6H	0.440	-47.18 to 48.07	>0.999	0.03
		POST-TR1 vs. POST-24H	-1.644	-48.43 to 45.14	>0.999	0.01
		LOW				
		POST-SSG vs. POST-TR1	31.660	-15.12 to 78.45	0.437	0.32
		POST-TR1 vs. POST-6H	-21.270	-68.88 to 26.34	0.871	0.19
		POST-TR1 vs. POST-24H	-32.510	-81.15 to 16.13	0.453	0.33
	DEC	**HIGH**				
		POST-SSG vs. POST-TR1	26.600	-18.89 to 72.09	0.627	0.25
		POST-TR1 vs. POST-6H	-0.734	-46.98 to 45.51	>0.999	0.03
		POST-TR1 vs. POST-24H	-18.650	-64.14 to 26.84	0.914	0.16
		LOW				
		POST-SSG vs. POST-TR1	22.000	-26.25 to 70.25	0.858	0.18
		POST-TR1 vs. POST-6H	-21.480	-70.66 to 27.70	0.884	0.21
		POST-TR1 vs. POST-24H	-13.760	-64.06 to 36.54	0.991	0.13
TR2	**TD**	**HIGH**				
		POST-PREV vs. POST-TR2	0.337	-30.33 to 31.00	>0.999	0.10
		LOW				
		POST-PREV vs. POST-TR2	22.650	-24.62 to 69.93	0.693	0.18
	HSR	**HIGH**				
		POST-PREV vs. POST-TR2	1.991	-46.64 to 50.62	>0.999	0.04
		LOW				
		POST-PREV vs. POST-TR2	18.050	-29.64 to 65.74	0.942	0.15
	ACC	**HIGH**				
		POST-PREV vs. POST-TR2	16.020	-31.21 to 63.24	0.968	0.15
		LOW				
		POST-PREV vs. POST-TR2	3.069	-46.29 to 52.43	>0.999	0.03
	DEC	**HIGH**				
		POST-PREV vs. POST-TR2	18.300	-28.07 to 64.66	0.929	0.15
		LOW				
		POST-PREV vs. POST-TR2	0.566	-49.84 to 50.97	>0.999	0.05

ACC = acceleration; ACT = activation; DEC = deceleration; HSR = high-speed running; PREV= preventive; SSG = small-sided games; TR1 = training session 1; TR2 = training session 2

APPENDIX 2. Mean torque values during the 20% (a) and 40% (c) of MVIC submaximal isometric contraction of the knee flexors until exhaustion. Panels b and d show the statistical parametric mapping (SPM) analysis, i.e., t-statistics (SPM{t}), of the torque values at 20 and 40% of MVIC, respectively. Legend: i) data in panels a and c are depicted as mean (solid black line), upper and lower 95% confidence intervals (shaded grey areas), and hypothesis criterion (pink dashed line). ii) In panels b and d, the pink dashed line represents the critical threshold with the alpha level set at $p \leq 0.05$.


a
20% of MVIC
c
40% of MVIC
MVIC (%)
100
80
60
40
20
0
b
20% of MVIC
d
40% of MVIC
SPM {t}
5
4
3
2
1
0
-1
-2
-3
-4
-5
0
10
20
30
40
50
60
70
80
90
100
Contraction time (%)

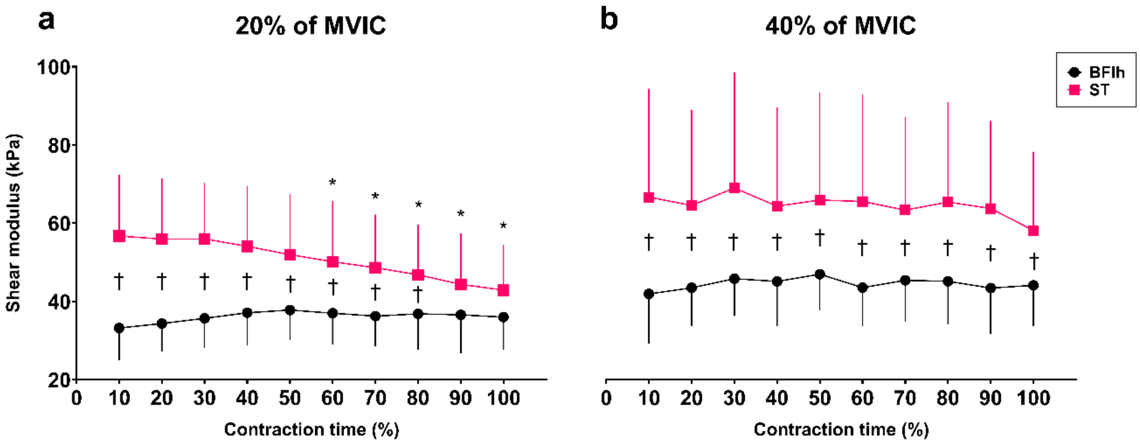
APPENDIX 3. Statistical parametric mapping (SPM) analysis, i.e., F-statistics (SPM{F}), of muscle (a), session (b), and muscle x session (c) interaction effects. The pink dashed line represents the critical threshold. The darker and lighter grey shaded areas represent the portion of the contraction in which significant effects were noted. Alpha level was set at $p \leq 0.05$.


a
Muscle
70
60
50
40
30
20
10
0
SPM { F }
p<0.001
p=0.041
p=0.049
α=0.05, F=14.445
b
Session
70
60
50
40
30
20
10
0
SPM { F }
α=0.05, F=14.445
p<0.001
p<0.001
p=0.039
p<0.001
p=0.039
c
Muscle x session interaction
30
25
20
15
10
5
0
SPM { F }
α=0.05, F=14.445
p=0.041
0
10
20
30
40
50
60
70
80
90
100
Contraction time (%)

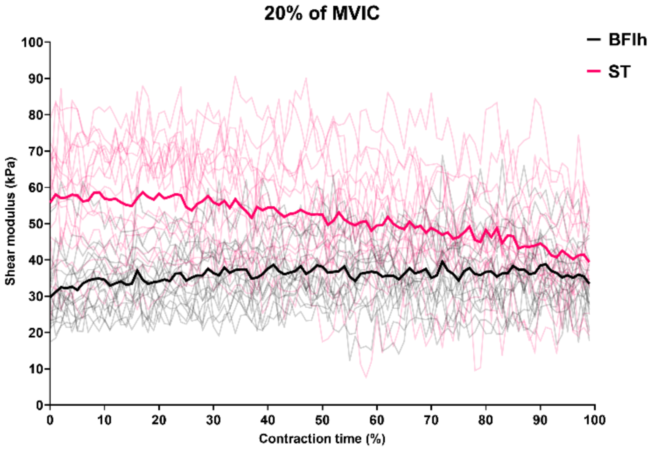
APPENDIX 4. ANOVA statistics and active stiffness of biceps femoris long head (BFlh) and semitendinosus (ST) during the knee flexors submaximal contraction until exhaustion at (a) 20% and (b) 40% of MVIC. †Significant intermuscular differences ($p \leq 0.05$) *Significant intramuscular differences compared to percentile 10th ($p \leq 0.05$).

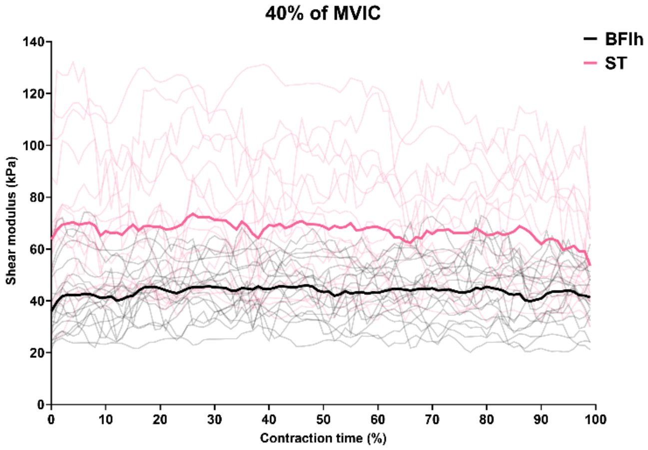
No significant effects were found for time x session (p=0.482; η2=0.002), and muscle x session interaction (p=0.239; η2=0.007). However, significant effects were found for time x muscle x session interaction (p=0.021; η2=0.004), time x muscle interaction (p<0.001; η2=0.015), session (p<0.001; η2=0.122), time (p<0.001; η2=0.012), and muscle (p<0.001; η2=0.296). Overall, a greater active stiffness was seen at 40% than 20% of MVIC (p<0.001; ES = -0.69 [-1.05 to -0.34]), and for ST compared to BFlh (p<0.001; ES= -1.08 [-1.73 to -0.43]).

Post hoc analysis showed that, in the 20% of MVIC session, the muscles had different stiffness values between percentiles 10th and 80th (Figure 3a), while in the 40% of MVIC condition, different stiffness levels were observed for each muscle in all percentiles (Figure 3b). Regarding the time factor analysis for BFlh and ST, no significant effects were found for BFlh at 20% (p=0.190; η2=0.087) or 40% of MVIC (p=0.458; η2=0.054), neither for ST at 40% of MVIC (p=0.082; η2=0.110). Nevertheless, significant effects were found for ST at 20% of MVIC (p<0.001; η2=0.395), showing a significant stiffness decrease from the percentile 60th until the end of the contraction (Figure 3a).

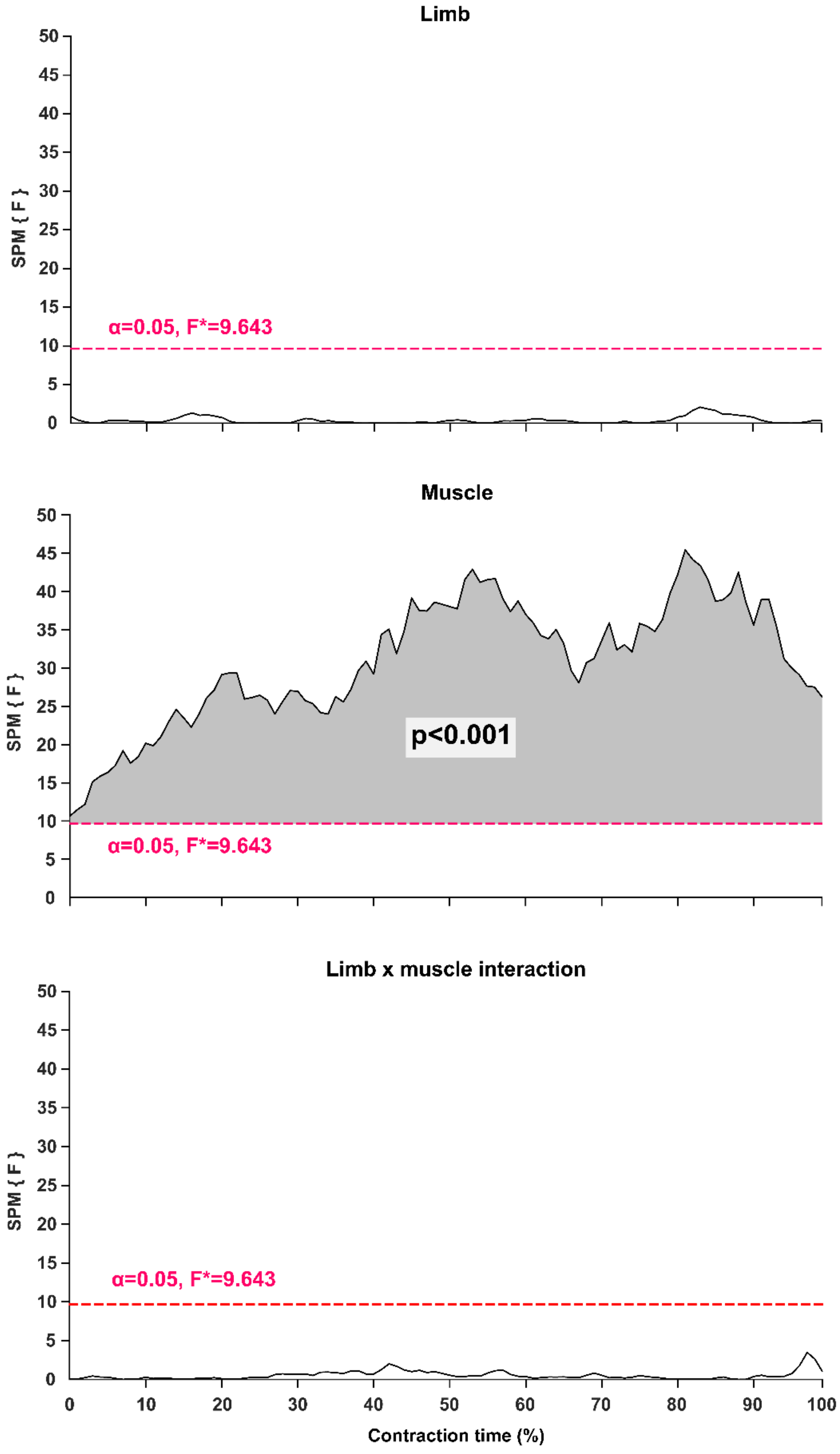


APPENDIX 5. Individuals' biceps femoris long head (BFlh) and semitendinosus (ST) active stiffness responses at 20 and 40% of MVIC sessions. Transparent lines represent single individuals whereas solid lines represent the mean active stiffness responses.


20% of MVIC
BFlh
ST
Shear modulus (kPa)
0
10
20
30
40
50
60
70
80
90
100
Contraction time (%)
0
10
20
30
40
50
60
70
80
90
100


40% of MVIC
BFlh
ST
Shear modulus (kPa)
0
20
40
60
80
100
120
140
Contraction time (%)
0
10
20
30
40
50
60
70
80
90
100

APPENDIX 6. Statistical parametric mapping (SPM) analysis, i.e., F-statistics (SPM{F}), of limb, muscle, and limb x muscle interaction effects. The critical threshold is depicted by the pink dashed line. The shaded area in dark grey indicates the period of contraction where significant effects were observed. The alpha level was established at $p \leq 0.05$.


Limb
SPM { F }
50
45
40
35
30
25
20
15
10
5
0
α=0.05, F*=9.643
Muscle
SPM { F }
50
45
40
35
30
25
20
15
10
5
0
p<0.001
α=0.05, F*=9.643
Limb x muscle interaction
SPM { F }
50
45
40
35
30
25
20
15
10
5
0
α=0.05, F*=9.643
0
10
20
30
40
50
60
70
80
90
100
Contraction time (%)