





Article

Evaluation of Neuromuscular Fatigue According to Injury History in a Repeat Sprint Ability Test, Countermovement Jump, and Hamstring Test in Elite Female Soccer Players

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Abstract: Sprinting is a fundamental component of the professional soccer player's ability to achieve the highest performance in the sport. The aim of this study was to analyze the influence of hamstring injury history on the neuromuscular fatigue produced by an RSA test in elite female football players. Nineteen female elite soccer players of the Second Spanish Soccer Division participated in the study. The participants were divided into: (1) a *Control group* who have not suffered previous muscular injuries and (2) a *Hamstring group* with previous hamstring injury at least one season prior to the protocol. The players performed a protocol consisting of a Repeat Sprint Ability Test (RSA) (6 × 40 m; 30 s rest), and CMJ and Hamstring tests before and after the RSA. The different variables of the study were compared between groups with a two-way ANOVA for repeated measures. The main findings from the present study were that, in subjects with previous hamstring injury, the performance was impaired compared with the control group: (1) in the initial meters of the sprint during an RSA there was a higher percentage difference between Sprint_{TT} and ideal Split in 0–10 m compared to 0–20 m in the hamstring group ($p = 0.006$; ES = 0.51); and in situations of high fatigue there was a higher %Dif1vs6 compared to %Dif1vs5 (percentage difference between the first sprint and fifth sprint) in the hamstring group ($p = 0.005$; ES = 0.54) compared with the control group. It seems that in elite female soccer players with previous hamstring injury, RSA-induced fatigue produces a greater decrease in the performance in the first 10 m of the sprint compared to the control uninjured players.

Keywords: hamstring injury; fatigue; female; soccer; sport

1. Introduction

Soccer, as a sport, requires several physical demands, these include endurance, deceleration, acceleration, maximal sprinting, jumping, and repeated sprinting ability [1]. Sprinting is a fundamental component of the professional soccer player's ability to win duels and defend or create scoring chances [2]. Sprinting generally constitutes between 1 and 10% of the total distance covered (around 1 to 3% of the effective playing time) [3–6]. Moreover, straight-line running is the most frequent action in goal scoring situations, both for the player who assists and the player who scores [7].

Soccer is a team sport played by many athletes worldwide with an estimated 4–26 million female participants [8–11] and approximately 238 million male participants [12]. The number of female football players has increased in recent years by approximately 50%

according to a FIFA report [10,13], and they have started to receive a professional status with higher salary and better team structure—akin to the men’s professional teams. Due to the challenges associated with this rapid increase in the number of participants, it is important to better understand the characteristics of these players, their physiological/physical demands, and their training processes [8,9]. Factors such as gender [14,15] and age [16,17] have been reported to influence, for example, the Repeated Sprint Ability (RSA). In general, being female or younger has been associated with a lower decline score. However, several authors point out that further research is needed to establish whether these differences can be attributed to differences in fatigue or can largely be explained by differences in baseline mechanical performance [18].

The hamstrings muscles are crucial in sprint acceleration performance and maximal sprinting [19]. Moreover, repeated sprint bouts are reported to occur immediately before a goal is scored or conceded, lending credence to the suggestion that the ability or inability to perform repeated sprints may prove critical to the outcome of the match [3]. On the other hand, hamstring strain injuries are common among soccer players and account for 12–16% of all soccer-related injuries [20,21]. Although much attention has been paid to the prevention and treatment of these injuries, the relative number of reported cases has not decreased over time [21,22]. The most cited risk factor for a future hamstring strain injury is a previous injury [20,23]. Additionally, incomplete rehabilitation increases the risk of recurrence and the level of the injury severity [24]. In previous studies, different tests were accomplished to ensure the complete rehabilitation after hamstring strain injury—for example, the hamstrings’ strength and flexibility [24] or the sprint speed [25]. However, to date there is not a gold standard battery test for a safe return to competition and the recurrence rate of hamstring strain injury is up to 33% [25].

From a physiological perspective, RSA correlates with fatigue and motor unit activation [26]. Furthermore, correct motor unit activation is essential to achieve maximal sprint speed [26]. In this regard, good performance will provide the ability to perform repeated sprints [26]. This idea could be related to the strong relationship between PCr resynthesis and recovery of power output after 30 s sprints [27,28]. The RSA test simulates intermittent exercise and identifies the player’s capacity to maintain maximal power production with fast recovery during multiple successive high-speed running or sprinting efforts [29,30]. In addition, there is a significant correlation between decreased performance during repeated sprint exercise and changes in blood pH [31]. Therefore, athletes who are better able to buffer hydrogen ions (H^+) and resist changes in blood pH may have a higher RSA performance [32]. Continuing fatigue [18] during the later stages of a soccer match may cause a higher risk of a hamstring strain injury because of the negative alteration of the sprint biomechanics (i.e., muscle flexibility and/or strength, body mechanics) [33], suggesting that neuromuscular fatigue plays an important role in the risk of hamstring injury [33,34]. It has been observed that when the fatigue level is greater than 10%, a simultaneous decline in mechanical performance and the amplitude of electromyography (EMG) signals has consistently been reported across sprint repetitions [35,36]. Moreover, under fatigue, muscle activation influences sensorimotor control of force [37], which may negatively affect the quality of sport-specific skills and potentially increase the risk of injury (i.e., increased mechanical stress/load on joints) [38]. This suggests that changes in intermuscular coordination (i.e., the coordination pattern between the vastus lateralis and biceps femoris) could contribute to reduced power output under fatigue during RSA [18]. However, it is difficult to understand which determinants are specifically related to RSA. Thus, some uncertainties and non-consensus evidence remain in this regard in women’s soccer. For that reason, it is important to identify the physical capacities that could explain RSA in women’s soccer [39].

Hamstring extensibility is also an important component of physical fitness and spinal health [40]. The lack of extensibility in the hamstring muscle could affect the quality and quantity of the hip’s range of motion (ROM) that is available to perform functional tasks (i.e., running, ball passes, and shots) [41]. In this regard, several studies have found that reduced

hamstring extensibility is associated with an increased risk of spinal disruption, especially in flexed trunk postures [40], low back pain [42], and changes in lumbopelvic rhythm [43], and could lead to patellar tendinosis [44] and patellofemoral pain syndromes [45]. Some authors suggest that soccer players with higher hamstring muscle tension have an increased risk of posterior musculoskeletal hamstring injuries [46,47]. Decreased extensibility has already been identified as a risk factor for developing hamstring strains by passive [46,48] and active leg raises [47]. Dynamic tests of extensibility have been proposed to be more sensitive to the remaining abnormalities and that they are better tools to decide when return to play [49].

On the other extreme, the vertical jump (VJ) is one of the most widely used performance tests. Among them, the countermovement jump (CMJ) performance has been used to: (1) monitor the positive effects of strength, plyometric, resistance, and speed training and (2) to control the mechanical and neuromuscular fatigue status in individual and team sports [50]. In this sense, several researchers have found that CMJ performance is an interesting objective marker of fatigue and overcompensation for athlete performance [50], being one of the factors related to the high incidence of injuries (i.e., muscle overload) in the lower limb muscles [51,52]. Thus, a relationship has been observed between CMJ height loss and metabolic markers, such as lactate or ammonium, in sprinting [53]. This suggests that through the decreases in CMJ mechanical variables, such as the CMJ height, it should be possible to estimate the metabolic stress, neuromuscular fatigue, and athlete overload [53]. Some authors showed an increase in neuromuscular fatigue measured as a reduction in jump height after repeated sprints in players with a prior lower limb injury [54]. Therefore, there is a need for a specific test, with performance and/or clinical validity, capable of identifying those players who are at risk of hamstring injury.

To our knowledge, there is no study investigating the effect of a past hamstring injury on performance variables in elite female soccer players. It was hypothesized that after the RSA, the CMJ height, hamstring extensibility, and the performance variables of the RSA will be more affected in players with previous hamstring injury. Consequently, the aim of this study was to analyze the influence of hamstring injury history on the neuromuscular fatigue produced by an RSA test in elite female football players and how it could contribute to better management for the ones who got a previous hamstring injury.

2. Materials and Methods

2.1. Participants

Nineteen female elite soccer players (20.47 ± 2.67 years, 167.42 ± 7.02 cm, and 59.71 ± 8.77 kg, respectively) of the Second Spanish Soccer Division participated in the study. The participants were divided into (1) a *Control group* (11 players) who have not suffered previous muscular injuries and (2) a *Hamstring group* (8 players) with previous injury to any of the hamstring muscles, up to grade II injury (i.e., partial muscle tear). All players had medical clearance to conduct the study and were completely healthy and uninjured at the time of the data collection. The study was carried out in the 2020–2021 season. The players who participated in the study were both regular starters and substitutes. However, all players had the same training load (i.e., 5 training days and a regular league match per week). The inclusion criteria were: (1) having medical clearance to conduct the study; (2) not having suffered a musculoskeletal injury at least one season before the date of the protocol (i.e., checked through a previous exclusion questionnaire); (3) not being diagnosed with any cardiovascular, metabolic, neurologic, pulmonary, or orthopedic disorder that could affect the participation in the study or limit the performance in the different tests; and (4) not having suffered another injury on the hamstring musculature for at least one season prior to the protocol (criteria for the hamstring group).

All the athletes signed the informed consent form before the study. The study followed the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Universidad Politécnica de Madrid (Madrid, Spain).

2.2. Study Design

The study comprised a pilot familiarization test session with all the test protocols (i.e., sprint, CMJ, and Hamstring test), and a single evaluation session performed on the team's own artificial turf training and playing field. All the participants used their appropriate personal footwear. The test session was on the same schedule to avoid detrimental performance effects associated with circadian rhythm [40] and took place in the middle of the season, specifically on Monday or Tuesday of the training week—depending on match intensity (i.e., there was always 24 h rest after the match for players who played more than 60 min). The sessions began with a 10-min general warm up, always guided by the club's physical trainer, consisting of continuous running, specific running, joint mobility, and ballistic stretching exercises, and followed by a specific pre-test warm-up in which participants performed three progressive sprints with 30 s between each one. After a three-minute rest, the participants started the protocol consisting of an RSA test (6×40 m with 30 s rest between repetitions), and CMJ (with 30 s between trials) and Hamstring test before and after the RSA test (with 30 s between each test) (Figure 1).

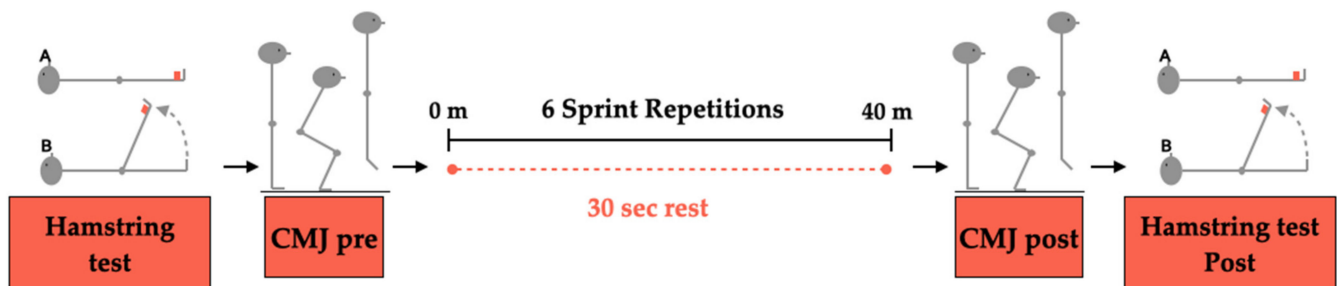


Figure 1. Outline of the protocol procedure.

2.2.1. Hamstring Test

The hamstring test followed the WIMU[®] Hamstring Test guide protocol [55]. The participant was placed lying down in a supine position. A WIMU[®] system (RealTrack Systems, Almería, Spain) was placed over the distal tibia strapped with an elastic band to measure the inclination. The system is a small, wireless device with more than 20 integrated sensors. The sensors include a 1000 Hz 3D accelerometer, a 1000 HZ 3D gyroscope with 2000 degrees per second resolution, a 3D magnetometer, and a barometer that works with an integration of sensors to improve the information. All data regarding the angle reached in each repetition for both legs were sent via Bluetooth to a personal computer, in real time, and were then recorded using SPro software (RealTrack Systems, Almería, Spain). Participants performed five ballistic hip flexions, maintaining the knee extension. The pelvis and the contralateral leg were fixed by a researcher to avoid pelvic movement [55]. The WIMU[®] system simultaneously recorded the hip flexion angle. This protocol was performed on both legs equally. The average of the five repetitions before and after the RSA test of the maximal angulation (deg), maximal velocity (deg/s), average velocity (deg/s), time to maximal velocity (ms), and angle at maximal velocity (deg) of each leg separately and of the average of both legs were analysed.

2.2.2. CMJ Test

All the participants were completely familiarized with the CMJ technique. The participants were always instructed to jump as high as possible. The initial position consists of a static standing position with hands on their hips. From this position, the participants engaged in a continuous and fast triple hip, knee, and ankle flexion movement until they reached $\approx 90^\circ$ of knee flexion, followed by the triple extension of the same joints in a fluid, fast, and continuous way [56]. In this type of vertical jump there is a stretching–shortening cycle (SSC), which takes place during the consecutive eccentric, isometric, and concentric

phases [56]. The participants were asked to take off and land at the same place to avoid lateral or horizontal displacement. During the flight, the participants must keep their hands on their hips and their legs should remain straight, with the ankle as extended as possible and contact the ground with their toes. The mean of the maximum height of the three jumps was analysed [57]. The jumps were evaluated through an Optojump photocell system (Microgate, Bolzano, Italy), which consist of two parallel bars (one receiver and one transmitter unit) that are positioned at the floor level [58]. The Optojump photocell system has been largely used for field-based assessments and for research purposes [59–61], having been tested for validity and reliability [58].

2.2.3. RSA Test

The players were positioned at the start of the marked sprints, 0.5 m behind the first pair of photocells to facilitate the correct registration of the first cut of the photocells [62]. The RSA test consisted of 6 repetitions of 40 m flat sprints with a 30 s rest in between. This distance allows the athlete to reach her maximum speed [2]. The entire sprint course was monitored with a system of five pairs of photocells (Microgate, Bolzano, Italy) placed along the sprint zone to record the sprint time at 10, 20, 30, and 40 m with a sensibility of 0.001 s (Figure 2). The rest were taken in the same place where they finished the sprint, so that the sprints were back and forth. Different variables were calculated to evaluate fatigue during the RSA test (see the variables description in Table 1).

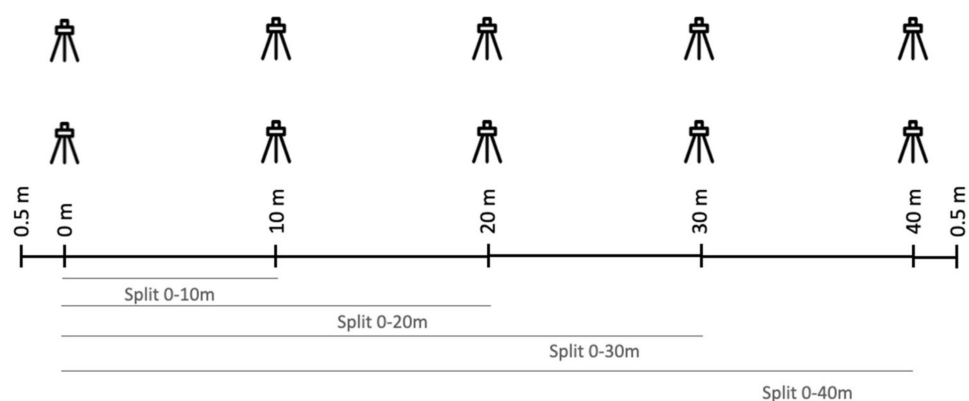


Figure 2. Diagram of the placement of the photocells in the RSA.

2.3. Statistical Analysis

All the data are presented as the means \pm standard deviation (SD). The data were tested for normal distribution with the Shapiro–Wilks test and for homogeneity of variances with Levene’s test. A two-way ANOVA for repeated measures was performed to analyze the effect of previous injury and the time of measurement along with interactions among these factors on the study variables. Additionally, a two-way ANOVA for repeated measures considering the initial 20 m was carried out to analyze the effect of previous injury on the different variables of the RSA test. As in previous studies [19], the initial 20 m of the sprints were specifically analyzed because hamstring muscles implication predominate in the acceleration phase of the sprint [19]. A two-way complementary ANOVA for repeated measures comparing the last three sprints were analyzed to observe if there were differences between the groups or the interaction on the variables of the RSA test. The last three sprints were selected because maximum fatigue occurs there, and hamstring muscles implication is influenced by fatigue [19]. When the Mauchly sphericity assumption was not met, the Greenhouse–Geisser correction was used. Bonferroni post-hoc tests were conducted where significant differences were found in any of the analyzed factors. The effect size of the pairwise comparisons was calculated by Cohen’s d considering $d < 0.5$ as small, $d < 0.8$ as moderate, and $d > 0.8$ as large [63]. The effect size of the ANOVA for repeated measures was calculated by partial eta-squared (η^2_p) and the small, moderate,

and large effects corresponded to values equal or greater than 0.001, 0.059, and 0.138, respectively [64]. The data were analyzed using the SPSS statistic software, version 26.0, for Windows (IBM Corporation; Armonk, NY, USA). The significance level was set at $p < 0.05$.

Table 1. Calculations of the different variables to evaluate fatigue during the RSA test.

Sprint Total Time (Sprint _{TT})	Defined as the Time to Run a Specific Distance of the Sprint	
Ideal Sprint [54]	Defined as the Sprint _{TT} if all the sprints were run as the best of them.	$Ideal\ Sprint\ 1 - X = MIN\ Sprint_{TT}\ 0 - 40m \times 6$ Where X is the number of a specific sprint
Split Total Time (Split _{TT}) [54]	Defined as the time spent to complete a specific distance during the six sprints.	$Split_{TT}0 - Xm = 0 - Xm\ Sprint\ 1 + 0 - Xm\ Sprint\ 2 + \dots + 0 - Xm\ Sprint\ 6$ Where X is the distance of a specific split
Ideal Split [54]	Defined as the Split _{TT} if all the splits were run as the best of them.	$Ideal\ Split\ 0 - Xm = MIN\ Split_{TT}\ 0 - Xm \times 6$ Where X is the distance of a specific split
Percentage difference 1vsX (%Dif1vsX).	Defined as percentage difference between the first sprint and a specific sprint.	$\%Dif = \frac{(Sprint_{TT}X - Sprint_{TT}1)}{Sprint_{TT}1} \times 100$ Where X is the number of a specific sprint
Percentage difference between Sprint _{TT} 0–40 m and ideal Sprint [18].	Defined as percentage difference between the time to run a specific number of sprints and the time if these sprints were run as the best of them.	$\%Dif\ Sprint_{TT}\ and\ Ideal\ Sprint\ 1 - X = \frac{Sprint_{TT} - Ideal\ Sprint}{Ideal\ Sprint} \times 100$ Where X is the number of a specific sprint
Percentage difference between the best time VS worst time of a split (%DifBvsW) [18].	Percentage difference between the best time compared to the worst time to run a split.	$\%DifBvsW\ 0 - Xm = \frac{MAX\ Split_{TT}0 - Xm - MIN\ Split_{TT}0 - Xm}{MIN\ Split_{TT}0 - Xm} \times 100$ Where X is the distance of a specific split
Percentage difference between Split _{TT} and ideal Split (modified from [18]).	Percentage difference between the time to run a specific number of splits and the time if these splits were run as the best of them.	$\%DifSplit_{TT}\ and\ Ideal\ Split\ 0 - Xm = \frac{Split_{TT}0 - Xm - Ideal\ Split0 - Xm}{Ideal\ Split\ 0 - Xm} \times 100$ Where X is the distance of a specific split

3. Results

3.1. CMJ Test

CMJ height was reduced after the RSA test ($F(1.17) = 14.452$; $p = 0.001$; $\eta^2 = 0.459$) in both groups. It was not found to be significantly different between the groups or interaction (Table 2).

Table 2. Results of the Countermovement Jump and the Hamstring Test before RSA (PRE) and after RSA (POST).

CMJ		PRE			POST		
Height (cm)	Control	26.24 ± 4.06			24.53 ± 4.42 *		
	Hamstring	28.04 ± 5.4			26.96 ± 4.92 *		
Hamstring test		Right leg	Left leg	Average	Right leg	Left leg	Average
Max angulation (deg)	Control	88.74 ± 11.75	88.49 ± 16.98	88.62 ± 14.09	84.32 ± 13.33	88.04 ± 19.07	86.18 ± 16.02
	Hamstring	86.04 ± 11.35	84.28 ± 12.41	85.16 ± 10.39	85.55 ± 12.45	89.86 ± 11.58	87.71 ± 11.93
Max velocity (deg/s)	Control	419.23 ± 94.35	367.31 ± 51.43	393.18 ± 63.39	410.15 ± 66.70	373.73 ± 60.6	391.94 ± 56.94
	Hamstring	417.34 ± 118.02	356.01 ± 50.02	386.68 ± 81.51	402.04 ± 87.83	395.16 ± 62.42 #	415.92 ± 95
Avg velocity (deg/s)	Control	258.23 ± 52.78	231.68 ± 42.83	244.95 ± 45.12	263.22 ± 43.27	229.12 ± 54.31	246.17 ± 36.09
	Hamstring	255.78 ± 61.05	235.67 ± 43.58	245.72 ± 49.75	257.24 ± 52.44	267.81 ± 56.1	262.52 ± 53.47
Time to max velocity (ms)	Control	111.18 ± 52.44	121.93 ± 62.38	114.41 ± 50.22	87.64 ± 50.61	106.51 ± 33.13	110.73 ± 69.8
	Hamstring	82.75 ± 67.21	106.5 ± 58.85	94.63 ± 60.64	95.09 ± 56.31	81.95 ± 55.62	88.52 ± 50.88
Angle at max velocity (deg)	Control	43.54 ± 12	42.19 ± 15.57	42.86 ± 12.58	36.5 ± 14.96	41.34 ± 10.24	38.92 ± 11.57
	Hamstring	31.02 ± 13.72	34.97 ± 12.67	32.99 ± 12.82	35.7 ± 14.12	32.92 ± 13.46	34.31 ± 11.57

Values presented as means ± SD. CMJ: Countermovement Jump; Max: Maximal; Avg: Average; Deg: degrees; Control: Subjects without previous hamstring injury; Hamstring: Subject with previous hamstring injury. * Significant different PRE vs. POST without significant difference between groups. # Tendency PRE vs. POST without significant difference between groups.

3.2. Hamstring Test

Maximal velocity of the left leg tended to be higher after RSA ($F(1.17) = 4.358$; $p = 0.052$; $\eta^2 = 0.204$) in both groups, without differences between the groups and interaction. On the contrary, the rest of the variables showed no significant differences before RSA compared to after the RSA between the groups or interaction (Table 2).

3.3. RSA Test

After analyzing the entire 40 m sprint, no significant differences between the groups or interaction were found in the different variables of the RSA (Tables 3 and 4 and Figures 3 and 4).

Table 3. Performance variables of the sprints through RSA.

		Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6
Sprint _{TT} 0–10 m (s)	Control	2.01 ± 0.14	2.04 ± 0.16	2.01 ± 0.16	2.08 ± 0.15	2.07 ± 0.15	2.08 ± 0.16
	Hamstring	2 ± 0.13	2.07 ± 0.15	2.04 ± 0.15	2.11 ± 0.14	2.07 ± 0.16	2.05 ± 0.11
Sprint _{TT} 0–20 m (s)	Control	3.04 ± 0.19	3.48 ± 0.2	3.45 ± 0.24	3.54 ± 0.23	3.54 ± 0.22	3.58 ± 0.23
	Hamstring	3.4 ± 0.17	3.51 ± 0.2	3.47 ± 0.21	3.58 ± 0.19	3.52 ± 0.2	3.45 ± 0.27
Sprint _{TT} 0–30 m (s)	Control	4.75 ± 0.25	4.85 ± 0.27	4.83 ± 0.33 ²	4.97 ± 0.32	4.97 ± 0.33	5.04 ± 0.31
	Hamstring	4.73 ± 0.24	4.85 ± 0.26	4.85 ± 0.29	4.97 ± 0.32	4.91 ± 0.26	4.88 ± 0.3
Sprint _{TT} 0–40 m (s)	Control	6.12 ± 0.35	6.25 ± 0.36	6.27 ± 0.46	6.41 ± 0.43	6.46 ± 0.46	6.56 ± 0.43
	Hamstring	5.96 ± 0.38	6.27 ± 0.36	6.27 ± 0.42	6.44 ± 0.36	6.39 ± 0.34	6.4 ± 0.35
			Sprint 1 vs. 2	Sprint 1 vs. 3	Sprint 1 vs. 4	Sprint 1 vs. 5	Sprint 1 vs. 6
%Dif	Control		2.51 ± 1.75	3.07 ± 2.91	5.81 ± 2.88	5.61 ± 3.84	6.25 ± 5.07
	Hamstring		2.58 ± 1.31	2.33 ± 2.24	4.55 ± 2.43	4.73 ± 2.18	7.71 ± 3.02 *
			Sprint 1–2	Sprint 1–3	Sprint 1–4	Sprint 1–5	Sprint 1–6
%Dif between Sprint _{TT} and ideal Sprint	Control		1.26 ± 0.88	1.99 ± 1.06	2.98 ± 1.41	3.53 ± 1.8	4.07 ± 2.17
	Hamstring		1.29 ± 0.66	1.87 ± 0.69	2.6 ± 1	3.07 ± 0.99	3.89 ± 1.51

Values presented as means ± SD. Sprint_{TT}: Time to run a specific distance of the sprint; %Dif: Percentage difference between the first sprint and a specific sprint; Percentage difference between Sprint_{TT} and ideal Sprint: percentage difference between the time to run a specific number of sprints and the time if these sprints were run as the best of them; Control: Subjects without previous hamstring injury; Hamstring: Subject with previous hamstring injury. * Significantly difference compared to Sprint 1 vs. 5 considering the last two sprints in the analysis.

Table 4. Split_{TT} and Ideal Split from Split 0–10 m to Split 0–40 m of the RSA test.

		Split 0–10 m	Split 0–20 m	Split 0–30 m	Split 0–40 m
Split _{TT} (s)	Control	12.37 ± 0.76	21.14 ± 1.13	29.61 ± 1.72	38.34 ± 2.36
	Hamstring	12.16 ± 0.83	20.7 ± 1.12	28.9 ± 1.45	36.6 ± 2.86
Ideal Split (s)	Control	11.82 ± 0.61	20.36 ± 0.83	28.55 ± 1.27	36.82 ± 1.88
	Hamstring	11.29 ± 1.33	19.65 ± 1.47	27.72 ± 1.56	35.39 ± 2.27

Values presented as means ± SD. Split_{TT}: Split Total Time, defined as the time spent to complete a specific distance during the six sprints; Ideal Split, defined as the Split_{TT} if all the splits were run as the best of them; Control: Subjects without previous hamstring injury; Hamstring: Subject with previous hamstring injury.

If the analysis is carried out considering the initial 20 m, the percentage difference between Split_{TT} and Ideal Split was different between 0–10 m and 0–20 m ($F(1.17) = 5.87$; $p = 0.027$; $\eta^2 = 0.257$) without significant differences between the groups. Also, an interaction was found ($F(1.17) = 5.76$; $p < 0.028$; $\eta^2 = 0.253$). The pairwise comparisons showed that the percentage difference between Split_{TT} and Ideal Split was not different between 0–10 m and 0–20 m in the control group, but it was lower in 0–20 m than in 0–10 m in the hamstring group ($p = 0.006$; $ES = 0.51$) (Figure 3).

When the last two sprints were compared, %Dif1vs5 was lower than %Dif1vs6 ($F(1.15) = 10.27$; $p = 0.006$; $\eta^2 = 0.407$) without a significant difference between the groups. An interaction effect was found ($F(1.15) = 4.29$; $p = 0.05$; $\eta^2 = 0.222$). The pairwise comparisons showed that %Dif1vs5 was similar to %Dif1vs6 in the control group, but %Dif1vs5 was lower compared to %Dif1vs6 in the hamstring group ($p = 0.005$; $ES = 0.54$) (Table 3).

The statistical power was calculated on the RSA variable “Percentage difference between Split_{TT} and Ideal Split” with the software G power (Version 3.1.9.7). It was calculated based on the ES of the partial eta-squared (η^2) observed in this variable “0.5819691” (large),

a sample size of 19 subjects, 2 groups, and ANOVA for repeated measures. According to these magnitudes, and assuming a significance level of 0.05, the statistical power calculated was of 99.8%.

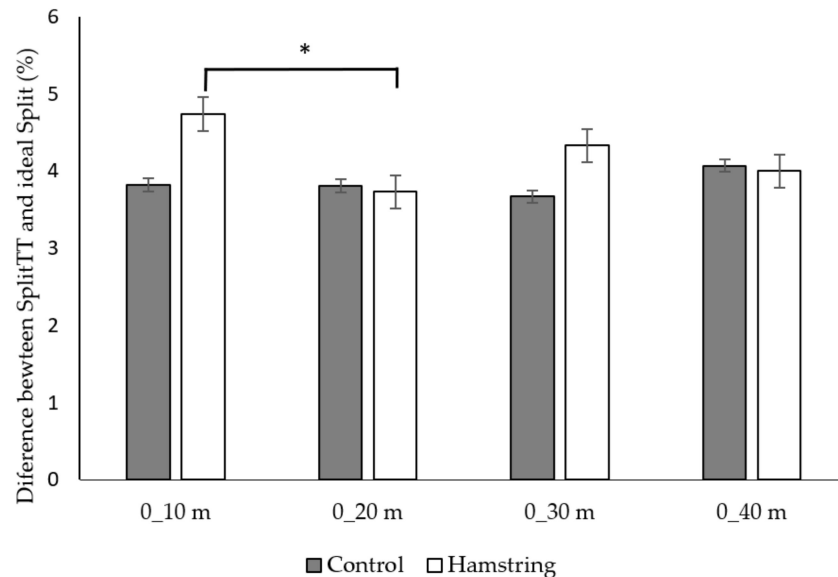


Figure 3. Percentage difference between Split_{TT} and Ideal Split: percentage difference between the time to run a specific number of splits and the time if these splits were run as the best of them. Control: Subjects without previous hamstring injury; Hamstring: Subject with previous hamstring injury. * Significant difference between 0–10 m and 0–20 m in the hamstring group considering the first 20 m of the sprints ($p < 0.005$).

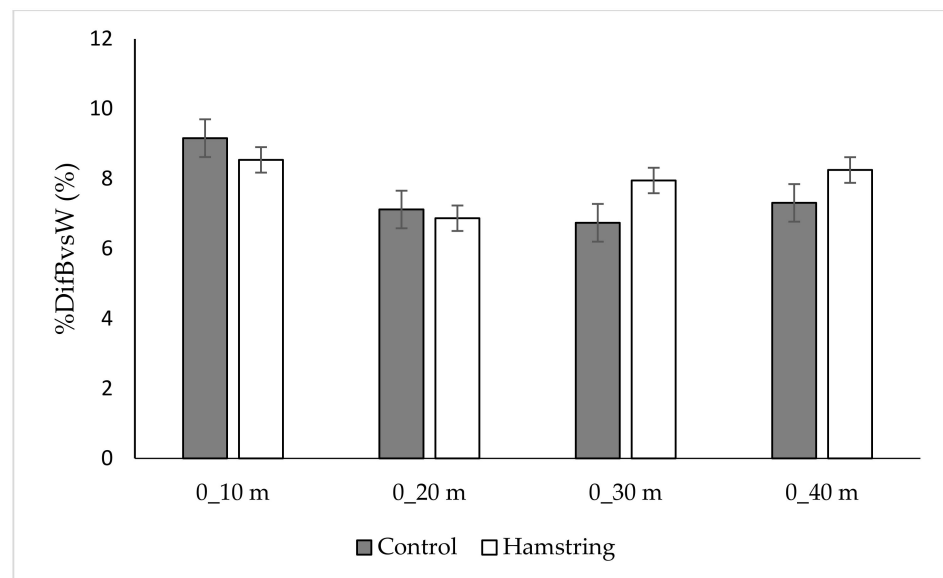


Figure 4. Percentage difference between the best time compared to the worst time to run a split (%DifBvsW) through RSA. Control: Subjects without previous hamstring injury; Hamstring: Subject with previous hamstring injury.

4. Discussion

The objective of this study was to analyze the influence of hamstring injury history on the neuromuscular fatigue produced by an RSA test in elite female football players. For this purpose, the incidence of neuromuscular fatigue was determined through a CMJ test,

hamstring test, and the performance variables of the RSA test itself in subjects according to the hamstring injury history. The main findings from the present study were that, in subjects with previous hamstring injury, the performance was impaired compared with the control group: (1) in the initial meters of the sprint during a RSA there was a higher percentage difference between Sprint_{TT} and Ideal Split in 0–10 m compared to 0–20 m in the hamstring group and (2) in situations of high fatigue there was a higher %Dif1vs6 compared to %Dif1vs5 in the hamstring group compared with the control group.

The results obtained when analyzing the initial 20 m of the sprints showed that the percentage difference between Sprint_{TT} and Ideal Split decreased in 0–20 m compared to 0–10 m in the hamstring group, whilst it remained constant in the control group. In other words, Split_{TT} was further from Ideal Split in 0–10 m than at 0–20 m in the hamstring group, whilst it was similar in the control group. This suggests that performance suffers a larger decline in the first few meters of the sprint in subjects with previous hamstring injuries. Hamstring muscles are known to play a key role during the acceleration phase of the sprint [19]. Therefore, there was a higher decrease in performance in these female elite soccer players with previous hamstring injury in the initial meters of the sprint when acceleration is maximal. This is an important finding because these players had their last hamstring injury at least one season ago and they were theoretically perfectly recovered, however, they may still have a muscular disability. They were not able to apply the same strength and power during the first meters of the RSA test, and they have a lower opportunity to take advantage of the competition and might have a higher re-injury risk [65].

When comparing the last two sprints to observe the evolution of performance under fatigue conditions, the %Dif1vs6 was higher compared to the %Dif1vs5 in the hamstring group, whilst it was not different in the control group. The performance from the 5th to the 6th sprint compared with the 1st sprint was more reduced in the hamstring group and it indicates that performance is more impaired in a situation of fatigue in the hamstring group. This agrees with a previous study [66], which showed a larger decrease in performance in soccer players reporting former hamstring strain injury during an RSA (8 × 20 m) [66]. In this sense, other authors [67] observed a lower biceps femoris activity in the previously injured limb during the final phase of the sprint [68]. Furthermore, since fatigue has been associated with hamstring injuries [33,34], the higher reduction in performance associated to fatigue in subjects with previous hamstring injury might predispose these subjects to a subsequent injury [67].

It is important to underline that the differences were found within groups and in the development of the RSA between groups, indicated by the significant interactions found, which were similar to previous studies [54,67]. This highlights the importance of evaluating subjects with an RSA test over time to know the evolution in performance due to a training program, or the involution because of an injury. In addition, and taking into account the small sample size and its sport and sex specificity, these data might be clinically relevant to ensure an optimal return to play with lower risk to re-injury.

Concerning the hamstring test, the results showed a tendency to increase the maximum velocity of the left lower limb after RSA in both groups. However, no differences between groups or interaction were found. The rest of the variables showed no significant differences between groups or time interaction (i.e., before and after RSA). Therefore, the overall interpretation of this test suggests that RSA-induced fatigue did not affect hamstring flexibility. However, some studies support that muscle tension in the hamstring musculature increases with fatigue and would decrease hamstring flexion values [51,52]. Moreover, it has been shown that female soccer players with less hamstring flexibility have a higher risk of hamstring injury [47]. In contrast, and coinciding with the results obtained in this study, other authors pointed out that ballistic hamstring flexibility evaluated using the hamstring test seems to be unaffected by fatigue [66].

In relation with the CMJ performance, the post-test CMJ height was significantly lower than pre-test values, coinciding with previous authors [53]. However, we did not find

differences between the groups according to their incidence of injury, which coincides with other authors [66]. The CMJ height might not have enough sensibility to know the magnitude of a hamstring recovery in the last phase or after completion (i.e., one season after hamstring injury) because the force produced during a CMJ is oriented vertically and more demanding for the quadriceps, whereas the force produced during the sprint acceleration is oriented horizontally and more demanding for the hamstrings [19]. Moreover, a poor correlation has been observed between the vertical maximum force produced in a CMJ and the horizontal maximum force produced in a sprint, especially in high level athletes [53] such as the subjects in the present study. Further, a gold standard CMJ assessment tool (i.e., two force platforms coordinated) [69] might provide high-precision information regarding the difference in force application between the two lower limbs (i.e., previously injured vs. uninjured), and perhaps found data along the same line as the RSA test realized in the present study.

To delve deeper into the findings found in this study, future research should focus on the electromyography-measured neuromuscular behaviour of previously injured hamstring muscles during an RSA test.

Limitations

During the conduct of this study some limitations were found, including the following: (1) the sample size is limited, however, it was difficult to find elite female soccer players who have had a hamstring injury for at least one season prior to the protocol and who have not relapsed and (2) the sample should have been more homogeneous and have the same injured muscle in the same portion, and with the same degree of damage.

Future research should focus on increasing the sample and a long follow-up (i.e., one or more complete seasons) to determine a fatigue threshold from which it was possible to predict a higher injury risk. Moreover, other muscle groups with high injury incidence should be studied.

5. Conclusions

The present study has demonstrated that in elite female soccer players with previous hamstring injury, RSA-induced fatigue produces a greater decrease in the performance in the first 10 m of the sprint compared to the control uninjured players. Coaches could use this information to struggle against the fatigue influence at these distances by implementing specific training (e.g., single leg muscle power strength training, endurance to maximal power output) to achieve the highest performance and to prevent injury recurrence. The RSA test is a useful tool to evaluate the evolution of performance in athletes with previous hamstring injuries and in the decision to return to play.

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