Article

Not peer-reviewed version

Effectiveness of Electrical Muscle Elongation and Proprioceptive Neuromuscular Facilitation Programs on Muscle Flexibility and Stiffness in Young Adults with Functional Hamstring Disorder: A Randomized Clinical Trial with 4-Week Follow-Up

Carolina Jiménez-Sánchez , Rocío Fortún-Rabadán * , Beatriz Carpallo-Porcar , Paula Cordova-Alegre , Luis Espejo-Antúnez , María Ortiz-Lucas

Posted Date: 21 February 2025

doi: 10.20944/preprints202502.1724.v1

Keywords: muscle disorder; hamstring; electrical stimulation; stretching; flexibility; stiffness

 doi:10.20944/preprints202502.1724.v1

Article

Effectiveness of Electrical Muscle Elongation and Proprioceptive Neuromuscular Facilitation Programs on Muscle Flexibility and Stiffness in Young Adults with Functional Hamstring Disorder: A Randomized Clinical Trial with 4-Week Follow-Up

Carolina Jiménez-Sánchez [1,2], Rocío Fortún-Rabadán [1,*], Beatriz Carpallo-Porcar [1,2], Paula Cordova-Alegre [1,2], Luis Espejo-Antúnez [3] and María Ortiz-Lucas [4]

[1] Department of Physiotherapy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain
[2] Aragón Health Research Institute, Zaragoza, Spain
[3] Department of Medical-Surgical Therapy, Faculty of Medicine and Health Sciences, University of Extremadura, Badajoz, Spain
[4] Faculty of Education, Universidad Europea de Madrid, Madrid, Spain
* Correspondence: rfortun@usj.es

Abstract: Background: Adequate hamstring flexibility is crucial for musculoskeletal health, as increased muscle tone can lead to stretch-type injuries, muscle weakness, dysfunctional neuromuscular control, postural changes, and lower back pain. The aim was to compare the effectiveness of a program based on Electrical Muscle Elongation (EME), Proprioceptive Neuromuscular Facilitation (PNF), and no intervention in improving flexibility and viscoelastic properties of hamstring and quadriceps muscles in active young adults with functional hamstring disorder (type 2B according to the Munich Consensus). Methods: Sixty-five participants (45 male, 20 female) were randomly assigned to three groups: the EME group (n=21) received a simultaneous combination of interferential current and stretching, the PNF group (n=22) underwent active stretching, and the Control group (n=22) received no intervention. Hamstring and quadriceps flexibility and muscle stiffness were measured in both limbs at baseline, post-intervention, and at the 4-week follow-up. Results: The EME group showed significant improvements in hamstring flexibility in the left limb compared to the Control group and in some myotonometric variables of the quadriceps muscle compared to the PNF and Control groups ($p<0.05$). Within-group differences indicated higher improvements in the EME group. Conclusions: This study suggests that EME may offer greater benefits than PNF stretching in young adults with functional hamstring disorder.

Keywords: muscle disorder; hamstring; electrical stimulation; stretching; flexibility; stiffness

1. Introduction

Hamstring muscle injuries are common among people who play sports, especially those involving kicking or a rapid change in running speed [1,2]. According to the latest evidence, they represent up to 25% of all muscle injuries [2], with the myotendinous unit being the site accounting for most of the hamstring injuries [1,3] and rehabilitation needs [4]. Moreover, re-injuries typically occur in this muscle group, requiring a longer period to return to competition [5].

The multifactorial nature of this clinical entity is highlighted. The mechanisms of stretch-type strain injuries at the hamstrings include reaching an extensive hip flexion with an extended knee,

 doi:10.20944/preprints202502.1724.v1

exceeding the limits of the muscle-tendon unit [2]. Among others, several anatomical and neuromuscular aspects such as proprioceptive/neuromuscular deficits, strength imbalances or stiffness are considered risk factors [6,7]. However, reduced flexibility and increased muscle tone along the length of the muscle belly are common findings among individuals who suffer from functional hamstring disorders [8], which also underly most strain injuries [9]. Overall, these facts emphasize the need for optimal hamstring extensibility during eccentric contraction to avoid injuries.

Adequate hamstring flexibility is essential for the correct functioning of the musculoskeletal system, given their clinical relevance in postural control mechanisms [10,11]. Indeed, functional hamstring disorders have been associated with a reduction in muscle strength, impaired quadriceps activation, as well as postural changes leading to low back hyperlordosis and pain [12,13]. Therefore, it seems crucial that efforts are directed towards the design of preventive and management strategies according to the type of hamstring injury [14,15].

Previous studies have suggested that the inclusion of stretching exercises in prevention and rehabilitation programs is beneficial to ensure or restore adequate hamstring function [8,15,16]. During the last decade, different stretching modalities such as static [17] and dynamic [18] stretching, neurodynamic sliding technique [19], electrical muscle elongation (EME) [20] or proprioceptive neuromuscular facilitation (PNF) [21] have been proposed as strategies for immediate increases in hamstring length. However, evidence is very limited regarding the changes induced by stretching techniques in muscle viscoelastic properties such as stiffness [22]. Furthermore, studies investigating their effects in a follow-up period after interventions are lacking, and these seem to be relevant needs given the role of altered neuromuscular control mechanisms in functional muscle disorders [8].

In recent years, EME technique has been postulated as a novel physiotherapeutic intervention that has shown promising results in improving pain, range of movement, and pressure pain threshold in adults with functional hamstring disorders [23]. This electrotherapeutic procedure consists of combining a passive stretching technique with a simultaneous electrical current that stimulates a slight isometric muscle contraction, and contraction of the antagonist muscle group [24]. Therefore, the refractory period of the muscle fiber is delayed, allowing the temperature of the collagen matrix to rise. This may induce provide a greater ulterior muscle relaxation and better gliding capacity, achieving more flexibility of the deeper layers of the muscle connective tissue [25]. By these effects, this innovative way of application could also improve muscle stiffness [22]. However, this technique has not been sufficiently analyzed and its effects, both in the target hamstring muscles presenting functional disorders and in the antagonist muscles, must be investigated.

Therefore, this study aimed to assess the effectiveness of a stretching program based on EME compared to PNF stretching and no intervention on muscle extensibility and viscoelastic properties of hamstring and quadriceps muscles in active young adults with functional hamstring disorder.

2. Materials and Methods

2.1. Study Design

The study was a randomized, single-blinded controlled clinical trial conducted in accordance with The Declaration of Helsinki. This study was approved by the Ethics Committee of Aragon (N°PI16/0033) and registered at ClinicalTrials.gov Protocol Registration System (reference: NCT03084341) following the CONSORT (Consolidated Standards of Reporting Trials) guidelines [26].

2.2. Participants

Young adult volunteers from the local community with limited hamstring muscle extensibility were recruited to participate in this study. All participants provided signed informed consent prior to the start of the procedure.

 doi:10.20944/preprints202502.1724.v1

The inclusion criteria were: (1) physically active participants over 18 years of age (2) with hamstring disorder classified as 2b according to the consensus of Munich [8] (diagnosed by a doctor) at this time, and (3) presenting less than 60° of knee extension in the Active Knee Extension (AKE) test, as well as a Straight Leg Raise (SLR) test of ≤80°. The exclusion criteria were: (1) participation in an organized hamstring stretching program, (2) pain or musculoskeletal injuries or recent surgery in the abdominal or lumbar spine and/or lower limbs, and (3) no evidence of neurological disorder.

The sample size was previously calculated to detect a difference of 10.5° with a standard deviation of 10.1° on the AKE test. The minimal number of subjects required to achieve a power of 0.8 and an alpha level of 0.2 was calculated to be 19 in each group including, 20% more to cover possible losses.

2.3. Randomisation and Blinding

A researcher who was not involved in recruitment used a website (www.randomizer.org) (accessed March 2017) to randomly assign participants (using block randomization, 1:2) to one of the following groups: Electrical Muscle Elongation (EME) group, Proprioceptive Neuromuscular Facilitation (PNF) group, and Control (CT) group. Randomization was performed in numbered, sealed and opaque envelopes.

The study was conducted by two physiotherapists with more than 15 years of experience: One blinded researcher recorded the measurements at baseline, after the intervention and at the end of the follow-up period, while another researcher performed the interventions.

The allocation process was conducted in a protected area to ensure that both the examiner and the intervention provider remained blind.

2.4. Procedure

At the beginning of the study, participants completed a socio-demographic questionnaire. All outcomes were measured at baseline (T0), after the last intervention (T1), and 4 weeks after the last intervention (T2).

Before each session, hamstring flexibility was assessed according to the criteria previously described (<60º AKE) [27]. In case there was shortening, the corresponding intervention was performed until the values considered as normal (≥60° in the Active Knee Extension (AKE) test and ≥80° in the Straight Leg Raise (SLR) test) were reached, with a maximum of 8 sessions (2 per week). Otherwise, if no shortening occurred, the intervention was finished at that point.

Each testing session lasted between 45 and 60 minutes and was conducted at approximately the same time slot for each participant. The interventions were implemented in accordance with the recommendations of the Template for Intervention Description and Replication (TIDieR) checklist [28].

2.5. Outcome Measures

All measurements were carried out by the same trained physiotherapist. At the beginning of the first session, demographic and clinical data including age, sex, height, weight, body mass index (BMI), and level of regular physical activity using the International Physical Activity Questionnaire (IPAQ, short version) were recorded. The participants did not perform sport, warm-up or stretching exercises before the assessment and were blinded to all measurements. All verbal instructions and explanations were standardized.

2.5.1. Hamstring Flexibility

For the Active-Knee-Extension (AKE) test, the participants were assessed in a supine position on a table, facing a rectangular wooden frame attached to the table. The thigh of the tested limb was in contact with the wooden frame, with the hip and knee joints flexed at 90° and the ankle joint in a neutral position. The contralateral lower limb was secured extended and in neutral rotation to the table using a strap across the thigh. A standard universal goniometer was placed over the lateral condyle of the femur with the proximal arm aligned along the thigh in the direction to the greater

 doi:10.20944/preprints202502.1724.v1

trochanter and the distal arm aligned over the leg in the direction to the lateral malleolus. After being positioned, participants were asked to extend the knee until they felt a strong resistance and to hold this final position for 2 to 3 seconds to allow the goniometric reading. The recorded result corresponded to the amplitude of the knee maximum extension in degrees, starting from the initial test position (knee flexed at 90° which corresponded to the goniometric value 0°) [29–31].

The AKE test has an intraclass correlation coefficient (ICC) of 0.87-0.94, a standard error of measurement (SEM) of 2.6-2.9°, and a minimal detectable difference (MDD) of 7-8° [31].

The Straight-Leg-Raise (SLR) test was carried out with the participants lying supine on a table. The contralateral limb was secured with a strap over the thigh to maintain it extended and in neutral rotation. A standard universal goniometer was placed over the greater trochanter of the tested limb, and the goniometer arms were aligned along the midline of the pelvis and with the lateral femoral condyle. Then, the participants raised the tested lower limb with the knees fully extended and the foot in a neutral position slowly to the point that felt a strong resistance in hamstring muscles or when pelvic rotation was observed. When the participant reached the maximum hip flexion with the knee extended, the angle of the hip joint was measured. As described for the AKE test, the participants also held the final position of the SLR test for 2 to 3 seconds to allow the goniometric reading [20].

The SLR test has an intraclass correlation coefficient (ICC) of 0.93-0.97, a standard error of measurement (SEM) of 2.2-2.6°, and a minimal detectable difference (MDD) of 6-7° [31].

The difference in the flexibility of the right/left hamstrings was calculated to measure the asymmetry in the length of the hamstrings. Differences of more than 10-15% between limbs are considered an injury risk factor [32].

2.5.2. Quadriceps Flexibility

Quadriceps flexibility was assessed in a prone position on a table. A standard universal goniometer was placed over the lateral condyle of the femur with the proximal arm aligned along the thigh in the direction to the greater trochanter and the distal arm aligned over the leg in the direction to the lateral malleolus. The assessor slowly bent the participant's knee so that the heel approached the buttock. Attention was taken to ensure that there was no movement of the lumbar spine or pelvis or cramping of the hamstrings and that the thighs remained parallel. The subject was asked to report as soon as the first stretch sensation was experienced in the quadriceps muscle. The recorded result corresponded to the amplitude of the maximum flexion of the knee in degrees (°), starting from the initial test position (0°) Moreover, in this final position, the closest distance from the relaxed buttock to the heel with the ankle passively plantar flexed were measured with a tape measure (centimetres) [31,33].

2.5.3. Muscle Stiffness

Myotonometric parameters were assessed using the MyotonPRO device (Müomeetria AS, Estonia). The device provides a controlled preload of 0.18 N for pre-compression of the tissues and then exerts an additional 15 ms impulse of 0.40 N of mechanical force, which induces a damped natural oscillation of the tissues. Recorded parameters by the testing probe were: 1) oscillation frequency (Hz) as an indicator of muscle tone, which characterizes the resting level of tension in the tissue; 2) logarithmic decrement (arbitrary unit), which is inversely proportional to elasticity, is considered to be the ability of the muscle to restore its initial shape after being deformed; and 3) stiffness (N/m), which reflects the resistance of the tissue to the force that changes its shape [34].

Each testing sites on the muscle belly were located using a tape measure and marked using a permanent dermographic pencil. In the supine position, the rectus femoris (RF) site was located and marked at two thirds of the way between the anterior superior iliac spine and the superior pole of the patella. In the prone position, the biceps femoris (BF) site was located and marked midway between the ischial tuberosity and the head of the fibula [35].

 doi:10.20944/preprints202502.1724.v1

A measurement of 10 consecutive single impulses (multiscan mode) with an interval time of 1 s was completed in each site. The mean data of each series were accepted if the coefficient of variation of the measurement set was inferior to 3% [34].

The MyotonPRO has an intraclass correlation coefficient (ICC) of 0.99 for RF and BF [36].

2.6. Interventions

2.6.1. Proprioceptive Neuromuscular Facilitation Technique

The PNF group carried out the PNF stretching technique. Participants were supine on a table and secured with straps over the contralateral lower limb, which was extended and in neutral rotation, and over the anterior superior iliac spine. A lumbar roll was placed under the participants lower back during the stretching intervention to maintain anterior pelvic tilt during the procedure [21]. The stretching movement was performed by maintaining this knee position with the ankle in a relaxed position and increasing the hip flexion until a feeling of resistance appeared. After that, the participant should perform a concentric contraction of the antagonist muscle (quadriceps), resisted by the physiotherapist for 3 seconds. The elongation process was continued until the participant felt a new stretching sensation. The PNF procedure was repeated four times [37].

2.6.2. Electrical Muscle Elongation Technique

The participants assigned to the EME group received a bipolar interferential current application with a frequency of 4 kHz and a frequency modulation amplitude of 100 Hz using an electrotherapy equipment (Sonopuls 692®) and following the procedure described by Espejo (2022) [25]. Two self-adhesive electrodes (10 × 12 cm, and 75 cm^2 surface area) type Pals Platinum©, Axelgaard Manufacturing Co. Ltd., Fallbrook, CA, USA were placed longitudinally along the hamstring muscles and covered the biceps femoris and semitendinosus muscles. Participants were placed supine on a table and secured with straps over the contralateral lower limb which was extended and in neutral rotation and over the anterior superior iliac spine. During the stretching intervention, a lumbar roll was placed under the participant's lower back to maintain anterior pelvic tilt during the procedure [21]. The stretched limb was placed over the physiotherapist's shoulder with the hip joint flexed and the knee slightly bent to avoid nerve strain. The stretching movement was performed by maintaining the knee position with the ankle in a relaxed position and increasing the hip flexion until a sensation of resistance was felt. At this point the intensity of the electrical current was increased until a tolerable contraction was produced. The participant should then perform a concentric contraction of the antagonist muscle (quadriceps), resisted by the physiotherapist for 3 seconds. The elongation process was continued until the participant felt a new stretching sensation. At this point, the intensity of the current was increased again until the stretching sensation disappeared. This cycle was repeated four times [20,23,37].

2.6.3. No Intervention

Participants randomized to the CT group received no intervention.

2.7. Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) V.28 (SPSS Inc., Chicago, Illinois, USA). The normal distribution of the quantitative variables was tested using the Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean ± SD or median [interquartile range] for continuous parameters and frequency (%) for categorical data. Baseline measurements were compared between groups using the independent Student's t-test or the Mann–Whitney U-test and the chi-square test.

Between- and within-intervention analyses were conducted using one-way ANOVA and mixed-design ANOVA with Bonferroni post-hoc pairwise comparisons when a normal distribution was found. Assuming a non-normal distribution, non-parametric analyses were performed using the Kruskal-Wallis test and the Mann–Whitney U test for between-intervention comparisons and the Friedman test with the Tukey post-hoc test to highlight within-intervention differences. In the Mann–

 doi:10.20944/preprints202502.1724.v1

Whitney U test, type I error will be divided by the number of tests done. The significance level was established at $p < 0.05$.

Furthermore, the effect size was calculated through Cohen's d coefficient and interpreted as small (d = 0.2), medium (d = 0.5), or large (d > 0.8) [38].

An intention-to-treat (ITT) procedure was carried out.

3. Results

A total of 65 participants (45 males and 20 females; age 23.0 ± 4.4 years; stature 1.76 ± 0.1 m; weight 71.0 ± 13.0 kg; body mass index 22.8 ± 2.8 kg/m²) were included in this study (Table 1). The study flow chart can be seen in Figure 1.

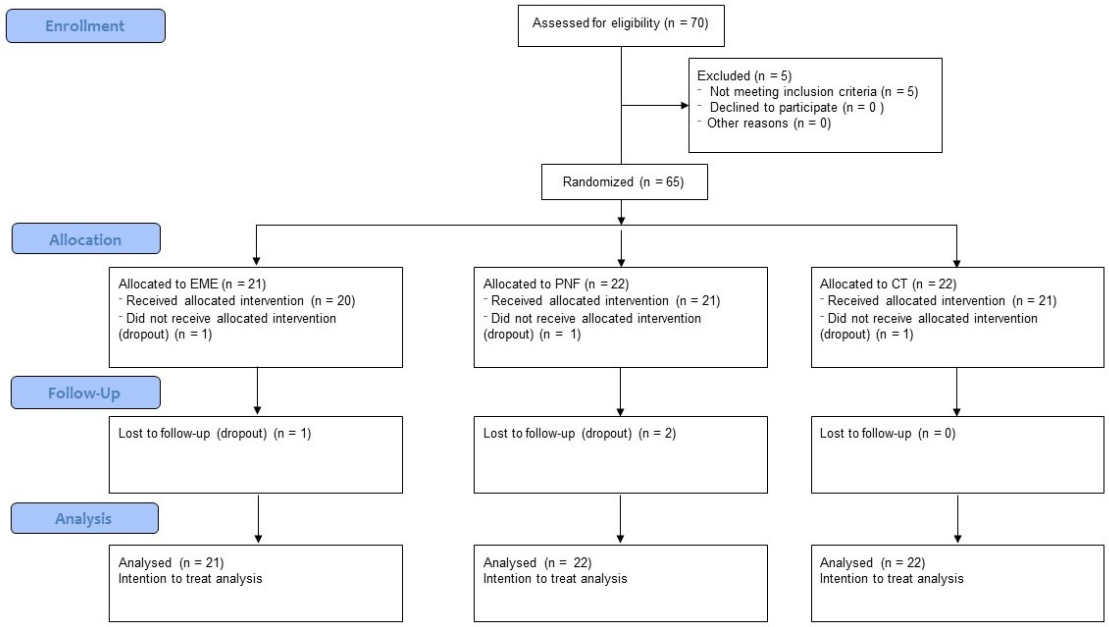

Figure 1. Flow Chart of the Study.

Table 1. Sociodemographic characteristics.

	EME Group (n=21)	PNF Group (n = 22)	CT Group (n = 22)	p-Value
Age (years)	23.45 ± 3.92	21.89 ± 3.58	23.71 ± 5.44	0.338
Gender (male)	16 (76.19%)	16 (72.72%)	13 (59.09%)	0.435
Height (m)	1.77 ± 0.09	1.77 ± 0.10	1.74 ± 0.09	0.647
Weight (kg)	71.95 ± 14.59	72.33 ± 15.58	68.74 ± 8.10	0.614
Body Mass Index (BMI) (kg/m²)	22.77 ± 2.91	22.22 ± 3.07	23.64 ± 2.33	0.257
Dominance (right)	16 (76.19%)	20 (90.90%)	22 (100%)	0.040
IPAQ				
Moderate	11 (52.38%)	5 (22.73%)	6 (27.27%)	0.013
High	10 (47.62%)	17 (77.27%)	16 (72.73%)	

Abbreviations: EME, Electrical Muscle Stretching: PNF, Proprioceptive Neuromuscular Facilitation; CT, Control; IPAQ, International Physical Activity Questionnaire.

In the initial assessment, there were significant differences between groups in terms of dominance, as well as between the right and left limbs in the values of the AKE (right 49.92° ± 8.95; left 48.08° ± 8.37) and SLR (right 62.38° ± 8.15; left 60.54° ± 8.20) tests for all participants (n = 65) ($p < 0.05$). However, these differences were less than 4% and no association was found with the dominance of the participants ($p > 0.05$).

 doi:10.20944/preprints202502.1724.v1

The percentage of participants reporting a high-intensity physical activity level was lower in the EME group compared with PNF and CT groups ($p < 0.05$) (Table 1). The characteristics of the sample are shown in Table 1.

The number of intervention sessions for each group was comparable for the EME and PNF groups, receiving 6.65 ± 1.75 and 6.45 ± 1.76 sessions, respectively. There were no statistically significant differences between the two experimental groups ($p > 0.05$).

3.1. Hamstring Flexibility

In the between-groups analysis, the comparison of pre- and post-intervention values showed a significant increase in the EME group as compared to controls for the AKE test of the left limb ($p = 0.016$), with a large effect size ($d = 0.809$) (Table 2). No other significant difference was found between groups, either for the SLR test values ($p > 0.05$).

Table 2. Hamstring flexibility.

			Descriptive Data			Within-Groups Effect				Between-Groups Effect			
						Post-test vs pre-test		Follow-up vs pre-test		Post-test		Follow-up	
Variable			Pre-test mean ± SD median [interquartile range]	Post-test mean ± SD median [interquartile range]	Follow-up mean ± SD median [interquartile range]	p Value	Effect size	p Value	Effect size	p Value	p Value (significant) -Effect size	p Value	p Value (significant) -Effect size
AKE test [°]	R	EME	46.85 ± 8.53 50 [30–60]	62.89 ± 9.47 65 [50–80]	59.74± 8.89 60 [50–80]	**<0.001**§	**1.527**	**0.002**§	**1.234**		EME-CT 0.622 PNF-CT 0.015 EME-PNF 0.601		EME-CT 0.394 PNF-CT 0.687 EME-PNF 0.308
		PNF	51.11 ± 8.14 50 [40–70]	57.50 ± 8[40–80[40–80.45 60 [40–70]	62.78 ± 10.74 62.5 [40–80]	0.067§	0.817	**<0.001**§	**1.561**	0.305‡		0.429‡	
		CT	53.10 ± 7.15 55 [40–65]	57.62 ± 7.35 55 [50–80]	56.66 ± 6.58 56 [50–80]	0.135§	0.552	0.244§	0.541				
	L	EME	47.10 ± 8.71 50 [30-60]	63.16 ± 10.03 65 [40-80]	62.63 ± 6.32 60 [50-75]	**<0.001**§	**1.326**	**<0.001**§	**1.704**		EME-CT **0.016**˜**-0.809** PNF-CT 0.067˜-0.481 EME-PNF 0.248˜-0.413		EME-CT 0.785 PNF-CT 0.835 EME-PNF 0.166
		PNF	50.56 ± 7.05 50 [40-70]	59.44 ± 7.83 60 [40-70]	63.89 ± 8.67 62.5 [50-80]	**0.003**§	**1.083**	**<0.001**§	**1.444**	**0.032**‡		0.456‡	
		CT	49.05 ± 7.68 50 [30-60]	55.24 ± 9.55 50 [40-80]	56.29 ± 9.52 55 [30-70]	0.651§	0.524	0.124§	0.760				
SLR test [°]	R	EME	61.32 ± 7.23 60 [50-75]	80.26 ± 6.97 80 [70-90]	80.26 ± 7.35 80 [70-90]	**<0.001**§	**1.920**	**<0.001**§	**2.090**		EME-CT 1.316 PNF-CT 0.661 EME-PNF 0.518		EME-CT 1.336 PNF-CT 0.729 EME-PNF 0.430
		PNF	61.94 ± 9.87 60 [40-80]	75.83 ± 9.89 77.5 [50-90]	76.67 ± 9.24 80 [50-90]	**0.008**§	**1.123**	**0.001**§	**1.185**	0.243‡		0.151‡	
		CT	64.29 ± 6.57 65 [45-70]	69.52 ± 9.20 70 [50-85]	70.71 ± 6.94 70 [55-85]	0.180§	0.568	0.102§	0.925				
	L	EME	57.90 ± 9.33 60 [40-75]	78.95 ± 7.37 80 [70-95]	78.95 ± 7.37 80 [70-90]	**<0.001**§	**1.983**	**<0.001**§	**2.658**		EME-CT 0.732 PNF-CT 0.629 EME-PNF 0.518		EME-CT 0.787 PNF-CT 0.568 EME-PNF 0.430
		PNF	61.94 ± 8.07 60 [50-85]	78.61 ± 9.82 80 [60-90]	77.22 ± 8.26 80 [60-90]	**<0.001**§	**2.028**	**0.001**§	**1.436**	0.098‡		0.821‡	
		CT	57.62 ± 7.35 55 [50-80]	57.62 ± 7.35 55 [50–80]	56.66 ± 6.58 56 [50–80]	0.092§	0.728	0.080§	0.809				

 doi:10.20944/preprints202502.1724.v1

	CT	63.57 ± 5.04 65 [50-70]	71.90 ± 11.45 70 [50-90]	71.90 ± 10.30 70 [60-90]							EME-PNF 0.039		EME-PNF 0.221	

Abbreviations: EME, Electrical Muscle Stretching: PNF, Proprioceptive Neuromuscular Facilitation; CT, Control; SD, Standard Deviation; R, right; L, left. †Using mixed-design ANOVA; §Using Friedman test; *Using one-way ANOVA; ‡Using Kruskal-Wallis test; ˜Using Mann–Whitney U test.

In the within-group analysis, the EME group showed a significant increase in the AKE test of 16° for both sides after the intervention, with a large effect size, which was maintained at follow-up ($p < 0.001$). In the PNF group, the AKE test improved only on the left side after the intervention ($p = 0.003$; $d = 1.083$), while at follow-up significant increases were found on both sides compared to baseline ($p < 0.001$) with large effect sizes (Table 2). Regarding the SLR test, EME and PNF groups showed improvements in both sides after the intervention, with large effect sizes, which persisted at follow-up ($p < 0.01$) (Table 2).

3.2. *Quadriceps Flexibility*

No significant changes were found between the groups in the flexibility of the quadriceps in relation to the maximum flexion of the knee and the distance between the buttock and heel ($p > 0.05$) (Table 3).

Table 3. Quadriceps flexibility.

			Descriptive Data			**Within-Groups Effect**				**Between-Groups Effect**			
						Post-test vs pre-test		**Follow-up vs pre-test**		**Post-test**		**Follow-up**	
Variable			**Pre-test mean ± SD median [interquartile range]**	**Post-test mean ± SD median [interquartile range]**	**Follow-up mean ± SD median [interquartile range]**	**p Value**	**Effect size**	**p Value**	**Effect size**	**p Value**	**p Value (significant) -Effect size**	**p Value**	**p Value (significant) -Effect size**
Maximum flexion of the knee [°]	**R**	**EME**	139.21 ± 13.87 140 [105-165]	143.68 ± 9.40 145 [125-160]	143.16 ± 10.17 140 [125-160]						EME-CT 0.375 PNF-CT 0.210		EME-CT 0.044 PNF-CT 0.167
		PNF	141.67 ± 9.39 142.5 [125-160]	142.11 ± 7.95 140 [130-160]	142.78 ± 6.69 142.5 [130-155]	0.069§ 0.803§ 0.076§	0.397 0.057 0.449	0.186§ 0.453§ 0.054§	0.468 0.164 0.427	0.302‡		0.777‡	
		CT	145.00 ± 9.22 150 [120-160]	140.48 ± 7.57 140 [130-160]	141.67 ± 6.58 130 [130-155]						EME-PNF 0.180		EME-PNF 0.174
	L	**EME**	135.79 ± 11.93 135 [115-155]	140.53 ± 10.12 140 [120-160]	140.53 ± 9.70 140 [125-160]						EME-CT 0.382 PNF-CT 0.309		EME-CT 0.227 PNF-CT 0.231
		PNF	139.72 ± 11.04 140[115-160]	139.72 ± 9.15 140 [125-160]	140.28 ± 7.37 140 [125-155]	**0.045**§ 0.617§ 0.076§	**0.774** 0.026 0.427	0.086§ 0.617§ 0.316§	0.382 0.073 0.297	0.327‡		0.673‡	
		CT	140.48 ± 9.86 140 [115-165]	137.14 ± 7.35 135 [125-155]	138.57 ± 7.44 140 [125-155]						EME-PNF 0.084		EME-PNF 0.029

 doi:10.20944/preprints202502.1724.v1

Distance buttock-heel [cm]	**R**	**EME**	13.59 ± 7.96 11.5 [0-33]	9.74 ± 6.94 9.5 [0-22]	9.68 ± 7.09 11 [0-23]	**<0.001‡**	**0.779**	**<0.001‡**	**0.958**		EME-CT 0.224 PNF-CT 0.222 EME-PNF 0.023		EME-CT 0.115 PNF-CT 0.168 EME-PNF 0.038
		PNF	11.40 ± 6.52 11.5 [0-23]	9.89 ± 5.81 10 [0-19.5]	9.42 ± 6.60 10 [0-23]	0.287†	0.470	**0.043‡**	**0.666**	0.604*		0.882*	
		CT	10.58 ± 4.67 10 [0-19]	11.14 ± 5.45 11 [0-21.5]	10.36 ± 4.39 11 [0-18]	0.188†	0.436	0.862†	0.275				
	L	**EME**	15.08 ± 7.71 14 [0-29]	12.03 ± 7.29 10 [0-24]	11.13 ± 7.02 11 [0-26]	**<0.001‡**	**0.831**	**<0.001‡**	**0.982**		EME-CT 0.113 PNF-CT 0.228 EME-PNF 0.090		EME-CT 0.074 PNF-CT 0.205 EME-PNF 0.121
		PNF	12.22 ± 7.57 13.75 [0-28]	11.39 ± 6.91 11.5 [0-25]	10.28 ± 7.05 11 [0-26]	0.999†	0.245	0.089†	0.613	0.555*		0.817*	
		CT	11.79 ± 5.70 12 [0-24]	12.71 ± 5.69 13 [0-23]	11.60 ± 5.41] 13 [0-21]	0.922†	0.226	0.999†	0.051				

Abbreviations: EME, Electrical Muscle Stretching: PNF, Proprioceptive Neuromuscular Facilitation; CT, Control; SD, Standard Deviation; R, right; L, left. †Using mixed-design ANOVA; §Using Friedman test; *Using one-way ANOVA; ‡Using Kruskal-Wallis test; ˙Using Mann–Whitney U test.

Regarding within-group analysis, a significant improvement (p = 0.045) in left knee flexion was found in the EME group with a moderate effect size. Furthermore, statistically significant reductions (p < 0.001) were found for the buttock-heel distance for the EME group after the intervention and follow-up with moderate-large effect sizes, whereas the PNF group only exhibited significant improvements (p = 0.043; d= 0.666) at follow-up compared to baseline (Table 3).

3.3. Hamstring Muscle Stiffness

After the intervention, there were no significant differences between the groups in oscillation frequency, decrement and stiffness variables (p > 0.05) (Table 4). However, there were large effect sizes after the intervention in the stiffness variable in the EME group compared to the CT group (right: d = 0.811; left: d = 1.169) and in the left biceps femoral muscle at follow-up between the EME group and the PNF group (d = 0.859) with higher stiffness in both assessments in the EME group.

The within-group analysis revealed significant differences in frequency and stiffness with higher values for the EME group after the intervention on both sides (p < 0.05) with moderate effect sizes, but these changes were not significant at follow-up. The PNF and control groups did not change significantly over time (p > 0.05) (Table 4).

Table 4. Hamstring Muscle Stiffness.

Descriptive Data						**Within-Groups Effect**				**Between-Groups Effect**			
Variable			**Pre-test mean ± SD median [interquartile range]**	**Post-test mean ± SD median [interquartile range]**	**Follow-up mean ± SD median [interquartile range]**	**Post-test vs pre-test**		**Follow-up vs pre-test**		**Post-test**		**Follow-up**	
						p Value	**Effect size**	**p Value**	**Effect size**	**p Value (significant) -Effect size**		**p Value (significant) -Effect size**	
Oscillation frequency [Hz]	**R**	**EME**	17.48 ± 1.59 17.4 [14.6-21.1]	18.63 ± 1.58 18.7 [16.2-22.5]	18.18 ± 1.48 18.5 [15.4-20.9]	**0.001†**	**0.728**	0.084†	0.285	EME-CT 0.597 PNF-CT 0.047 EME-PNF 0.680	0.172*	EME-CT 0.401 PNF-CT 0.122 EME-PNF 0.553	0.163*
		PNF	17.02 ± 1.90 16.9 [13.4-21.5]	17.39 ± 2.04 17.5 [13.5-20.8]	17.12 ± 2.27 17.2 [12.6-20.4]	0.483†	0.181	0.999†	0.119				
		CT			17.40 ± 2.32	0.095†	0.256	0.290†	0.203				

 doi:10.20944/preprints202502.1724.v1

			16.93 ± 2.14 17.1 [13.0-20.9]	17.49 ± 2.19 17.9 [13.1-20.9]	17.5 [13.4-21.9]								
		EME	17.34 ± 1.40 17.6 [15.3-20.1]	18.19 ± 1.54 17.3 [15.2-20.3]	17.17 ± 1.40 17.2 [14.4-19.9]						EME-CT 0.916 PNF-CT 0.160		EME-CT 0.369 PNF-CT 0.009
	L	PNF	16.64 ± 2.16 16.6 [12.4-20.6]	16.86 ± 1.65 16.9 [13.1-19.9]	16.52 ± 2.20 16.6 [12.1-19.9]	**0.005**‡	**0.552**	0.999‡	0.121			0.346*	
		CT	16.96 ± 2.07 16.5 [12.8-21.2]	16.57 ± 1.97 16.6 [12.9-20.2]	16.50 ± 2.15 16.7 [12.4-20.5]	0.999‡	0.133	0.999‡	0.055	0.177*			
						0.560‡	0.198	0.231‡	0.214		EME-PNF 0.833		EME-PNF 0.353
		EME	1.29 ± 0.14 1.26 [1.1-1.6]	1.28 ± 0.17 1.29 [1.0-1.6]	1.29 ± 0.16 1.21 [1.1-1.5]						EME-CT 0.166 PNF-CT 0.108		EME-CT 0.352 PNF-CT 0.241
	R	PNF	1.24 ± 0.17 1.29 [1.0-1.6]	1.23 ± 0.18 1.21 [1.0-1.7]	1.27 ± 0.15 1.20 [1.1-1.5]	0.702‡	0.059	0.999‡	0.000			0.473*	
		CT	1.24 ± 0.18 1.25 [0.9-1.6]	1.25 ± 0.19 1.28 [0.8-1.6]	1.23 ± 0.18 1.20 [0.9-1.7]	0.921‡	0.056	0.300‡	0.200	0.159*			
Decrement [arbitrary unit]						0.999‡	0.053	0.999‡	0.056		EME-PNF 0.286		EME-PNF 0.129
		EME	1.28 ± 0.17 1.29 [1.0-1.6]	1.30 ± 0.17 1.24 [1.1-1.7]	1.30 ± 0.16 1.24 [1.0-1.6]						EME-CT 0.431 PNF-CT 0.108		EME-CT 0.399 PNF-CT 0.211
	L	PNF	1.23 ± 0.18 1.21 [1.0-1.7]	1.24 ± 0.17 1.24 [1.0-1.6]	1.27 ± 0.19 1.29 [0.9-1.7]	0.900‡	0.118	0.882‡	0.125			0.514*	
		CT	1.25 ± 0.19 1.28 [0.8-1.6]	1.22 ± 0.20 1.21 [1.0-1.7]	1.23 ± 0.19 1.19 [1.0-1.7]	0.999‡	0.056	0.594‡	0.211	0.428*			
						0.892‡	0.150	0.900‡	0.105		EME-PNF 0.353		EME-PNF 0.171
		EME	328.88 ± 36.57 333 [261-399]	354.24 ± 37.10 349 [292-449]	342.76 ± 43.46 341 [264-434]						EME-CT 0.811 PNF-CT 0.058		EME-CT 0.628 PNF-CT 0.110
	R	PNF	314.56 ± 54.05 307 [229-452]	320.74 ± 53.11 311 [227-440]	317.95 ± 54.55 311 [198-422]	**0.002**‡	**0.684**	0.207‡	0.319			0.102*	
		CT	314.57 ± 44.27 314 [219-386]	317.71 ± 51.74 332 [291-394]	311.95 ± 54.06 330 [199-414]	0.899‡	0.116	0.914‡	0.062	0.062*			
Stiffness [N/m]						0.921‡	0.061	0.834‡	0.048		EME-PNF 0.731		EME-PNF 0.503
		EME	324.71 ± 34.33 320 [278-396]	345.53 ± 35.17 341 [263-404]	326.29 ± 41.10 327 [252-445]						EME-CT 1.169 PNF-CT 0.359		EME-CT 0.629 PNF-CT 0.186
	L	PNF	307.32 ± 52.62 300 [195-401]	312.42 ± 41.62 314 [214-410]	307.42 ± 53.76 316 [191-414]	**0.005**‡	**0.592**	0.999‡	0.038			0.153*	
		CT				0.899‡	0.123	0.999‡	0.002	0.102*			
						0.587‡	0.183	0.900‡	0.148		EME-PNF 0.859		EME-PNF 0.394

 doi:10.20944/preprints202502.1724.v1

	305.10 ± 48.03	296.29 ± 48.10	297.87 ± 48.86
	318 [213-420]	296 [199-376]	305 [187-371]

Abbreviations: EME, Electrical Muscle Stretching: PNF, Proprioceptive Neuromuscular Facilitation; CT, Control; SD, Standard Deviation; R, right; L, left. †Using mixed-design ANOVA; §Using Friedman test; *Using one-way ANOVA; ‡Using Kruskal-Wallis test; ˆUsing Mann–Whitney U test.

3.4. Quadriceps Muscle Stiffness

The between-group analysis revealed significant differences with large effect sizes for the decrement variable with higher values after the intervention between the EME group compared to the PNF group ($p = 0.038$; $d = 0.800$) and the CT group ($p = 0.005$; $d = 0.923$) (Table 5). In addition, significant differences with moderate effect sizes in the stiffness variable were observed in both limbs after the intervention between the EME group and the PNF group (right: $p = 0.010$; $d = 0.563$; left: $p = 0.024$; $d = 0.723$) and the EME group and the CT group (right: $p = 0.008$; $d = 0.742$; left: $p = 0.049$; $d = 0.636$). At follow-up, statistically significant differences were only found for the right quadriceps muscle ($p = 0.009$; $d = 0.638$) (Table 5).

In the within-group analysis, there were no significant differences in the myotonometric variables for any inter-group comparisons over time ($p > 0.05$) (Table 5).

4. Discussion

The aim of this study was to evaluate the effects of an 8-week program based on EME versus PNF techniques, and no intervention, on the viscoelastic properties of the hamstring and quadriceps muscles in young adults with functional hamstring disorder. Additionally, the maintenance of these effects was studied after a 4-week follow-up period.

Stretching techniques constitute the most recommended intervention to improve hamstring neuromuscular muscle disorders by means of increasing flexibility [15]. However, to our knowledge this is the first study to analyze the effects of different stretching modalities on a variety of relevant muscle properties, also measuring the impact on antagonist muscles. Furthermore, a non-intervention control group and a follow-up assessment were accomplished.

The between-groups comparison of hamstring extensibility showed a significant improvement in AKE for the EME group compared with the non-intervention controls, which was not achieved by the PNF group. Nevertheless, this difference was only significant for the left limb. The minimum asymmetries evidenced at baseline (pre-test measures) could have influenced the results. Alternatively, as observed in other work [39], the dominance of the participants may be a more plausible explanation for this finding since most participants were right-dominant and the left side showed the most significant improvements in flexibility.

On the other hand, the increased hamstring flexibility achieved in the EME group in contrast with non-intervention controls after the intervention was not significantly maintained in the follow-up. It was previously demonstrated that physical activity levels can influence muscle viscoelastic properties, with more physically active individuals presenting higher levels of flexibility and lower levels of muscle stiffness [40,41]. In the present study, the EME group mostly performed moderate-intensity physical activity, while the PNF and control groups performed significantly higher-intensity (vigorous) physical activity. Then, it is reasonable to hypothesize that the increases in flexibility observed in the PNF and non-intervention groups could have been enhanced for this reason, partially diluting the relative achievements of the EME group. In contrast, EME and PNF techniques have been analyzed in 30 subjects without hamstring disorders finding no influence on physical activity in hamstring flexibility [42]. In view of this, our results suggest the need for further research to elucidate whether physical activity can impact the effectiveness of stretching programs conducted to restore muscle flexibility in functional hamstring disorders.

 doi:10.20944/preprints202502.1724.v1

The within-group results demonstrated that both EME and PNF can increase the flexibility of shortened hamstring muscles requiring a similar number of sessions, although some aspects need to be considered. First, the EME program produced a significant and bilateral increase in hamstring flexibility as measured by the AKE and SLR tests. The efficacy of EME by an interferential current stimulation has been previously contrasted, whereby attributed to a decrease in the orthosympathetic activity and an increase in temperature in the collagen matrix [23,43]. Both underlying effects were pointed out to promote slippage of the muscle connective tissue, thus increasing the hamstring flexibility when combined with a stretching technique. The findings shown here support and reinforce this evidence by also reporting an increase in hip range of motion through the SLR test. Moreover, these significant benefits remained during the follow-up period up to 4 weeks after the intervention, which is a novel finding for the EME applied through interferential current. Other study [44] also reported increases in hamstring muscle flexibility after follow-up by means of EME, whereas applying transcutaneous electrical nerve stimulation (TENS). Low frequency (TENS) and medium frequency (interferential) currents have the same main effects at the neuromuscular level, such as the ability to induce neuromuscular relaxation, as well as activating sensory fibers leading to higher pain thresholds [45,46]. Through these mechanisms, EME could help achieve greater ranges of motion during stretching, which added to the capacity of the electrical current to modulate viscoelastic properties would explain the effects after the intervention, as well as their maintenance in the follow-up period, as observed in this study. Taken together, these results recommend the application of EME during the hamstring muscles stretching to enhance their flexibility [20,45].

The PNF group showed significant improvements in the AKE test after the intervention in the left limb and increases were observed in both limbs in the SLR test. However, at the follow-up, both limbs evidenced higher flexibility in both the AKE and SLR tests. The mechanism of action of PNF has been attributed to the reciprocal inhibition of antagonist muscles, which allows greater stretching of the target muscle [47]. The latest systematic review [48] exploring the effects of PNF was only focused on its acute effects (i.e., the measurements were taken immediately after a single technique or session). Despite the temporal aspects differing from those evaluated here, it was concluded that a single PNF stretching session is sufficient to achieve an improvement in hamstring flexibility. Indeed, previous PNF programs developed with this aim have achieved success in the short, medium and long term, which is consistent with the present findings [42,49–51].

A secondary aim of this study was to explore how the quadriceps muscles adapt to the stretching interventions that focus on their shortened antagonists. According to previous evidence [43], it was hypothesized that there was an increase in the quadriceps flexibility following EME for stretching hamstrings. The present results confirmed this, also finding its maintenance in the follow-up, which is a novel report. Although the differences between groups were not statistically significant, the pattern observed for the increase in hamstring muscle flexibility was mostly replicated by the quadriceps: EME intervention showed generalized effectiveness, while partial (only distance buttock-heel) and unilateral (only left limb) effects were achieved after the PNF intervention, and both groups presented similarly increased quadriceps flexibility in the follow-up. The phenomenon of reciprocal inhibition [37], the relaxation of the connective tissue and the decrease in the mechanical stiffness of shared joints [52], as well as the peripheral and central neural plasticity [53] likely contributed to the parallel neuromuscular changes observed in the quadriceps. These findings are of particular interest and extend beyond hamstring injuries given the clinical relevance of both muscle groups disorders in postural control [54], back pain [55] or sports biomechanics [56], among others.

Viscoelastic properties were also explored in both muscle groups. Regarding the hamstring muscles, despite no significant differences being found in the between-groups analysis, differentiated effects were observed at the intra-group level. The present results are consistent with the systematic review by Freitas et al. [57] showing that PNF alone does not produce changes in hamstring muscles stiffness, whereas this is the first study to assess changes in this structural parameter through a program based in EME for functional hamstring disorders. Specifically, a significant increase in stiffness was obtained after the EME intervention, which also persisted at the follow-up assessment.

 doi:10.20944/preprints202502.1724.v1

It should be noted that, although the recommendations for assessment through myotonometry were followed, several studies showed that stiffness responds in a non-homogeneous way at different hamstring muscles points [58,59]. A recent work [59] testing the biceps femoris in different zones after passive stretching demonstrated that the lower changes in stiffness were obtained in the midpoint site, where the measurements in the present study were taken. Therefore, the significant changes here observed in the EME group gain relevance. While one study highlighted that structural changes in viscoelastic properties leading to an increase in stiffness were washed shortly after the stretching [60], others found that this was achieved after a regular intervention of 4 to 6 weeks [59,61], as was the case in this study. However, according to a recent meta-analysis [18] on the effectiveness of stretching, little is known about the relevance of stiffness at the hamstring muscle bellies since the myotendinous unit is the most common site for injury. In the absence of normative data on this viscoelastic parameter, the present findings present new insights regarding the structural effects of applying EME during active stretching in the recovery of functional hamstring disorders.

Regarding quadriceps muscle, significant differences in viscoelastic properties were only found in the right limb, with a decrease in stiffness in the EME group compared to the PNF and control groups. Reciprocal inhibition, a spinal neurological mechanism whereby the activation and lengthening of one muscle group (hamstrings) induces the relaxation and stiffness reduction of the antagonist group (quadriceps) [62] is a likely explanation to the decrease achieved in quadriceps stiffness while targeting hamstring muscles elongation. In contrast to the PNF intervention, in which only passive stretching and voluntary contractions are acting, the EME approach involves the added effects of the electrical current. The electrical stimulation may have triggered deeper neuromuscular circuits, leading to a stronger inhibitory effect on the quadriceps. Overall, the findings regarding stiffness support that mechanical and neuromuscular stretching approaches can have different effects on the viscoelastic properties and recommend systematically assessing these structural changes both in the targeted and antagonist muscle groups [62, 63].

This study has some limitations. The lack of stratification resulted in a non-homogeneous distribution of physical activity levels and lateral dominance in the study groups. In addition, the neurodynamic properties of the sciatic and femoral nerves were not evaluated and could impact hamstring and quadriceps neuromuscular activity. However, several strengths are highlighted. We provide novel evidence of a stretching program based on EME improving hamstring flexibility in people with functional hamstring disorder, further maintaining these effects in the follow-up. Additional effects on hamstring stiffness and quadriceps flexibility are informed. Given the feasibility and potential usefulness of EME, these findings need to be supported by clinical studies assessing their effectiveness in preventing the occurrence and recurrence of injuries.

5. Conclusions

The application of EME technique can enhance the benefits of active stretching modalities such as PNF in young people with functional hamstring disorder when incorporated into a regular program. Improvements in muscle flexibility as well as viscoelastic changes were evidenced in the hamstring and quadriceps muscles and persisted 4 weeks after the intervention.

Author Contributions: Conceptualization, C.J.-S. and M.O.-L.; methodology, M.O.-L. and R.F.-R.; software, C.J.-S. and M.O.-L.; validation, M.O.-L.; formal analysis, C.J.-S.; investigation, C.J.-S., M.O.-L. and R.F.-R.; resources, C.J.-S. and M.O.-L.; data curation, C.J.-S. and M.O.-L.; writing—original draft preparation, C.J.-S., R.F.-R., B.C.-P., P.C.-A., and L.E.-A.; writing—review and editing, C.J.-S., R.F.-R., B.C.-P., P.C.-A., and L.E.-A.; supervision, C.J.-S., M.O.-L., R.F.-R., and L.E.-A; project administration, C.J.-S, M.O.-L. and R.F.-R.; funding acquisition, C.J.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Gobierno de Aragón (Number grant: B61_23D).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Aragon (N°PI16/0033; 24 February 2016) for studies involving humans.

 doi:10.20944/preprints202502.1724.v1

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

Data Availability Statement: The data can be provided upon a reasonable request to the corresponding author.

Acknowledgments: We would like to thank all participants and collaborators for their significant contributions to this project.

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

References

1. Maniar, N.; Carmichael, D. S.; Hickey, J. T.; Timmins, R. G.; San Jose, A. J.; Dickson, J.; Opar, D., Incidence and prevalence of hamstring injuries in field-based team sports: a systematic review and meta-analysis of 5952 injuries from over 7 million exposure hours. *British journal of sports medicine* **2023,** *57* (2), 109-116. 10.1136/bjsports-2021-104936.
2. Danielsson, A.; Horvath, A.; Senorski, C.; Alentorn-Geli, E.; Garrett, W. E.; Cugat, R.; Samuelsson, K.; Hamrin Senorski, E., The mechanism of hamstring injuries - a systematic review. *BMC musculoskeletal disorders* **2020,** *21* (1), 641. 10.1186/s12891-020-03658-8.
3. Chavarro-Nieto, C.; Beaven, M.; Gill, N.; Hébert-Losier, K., Hamstrings injury incidence, risk factors, and prevention in Rugby Union players: a systematic review. *The Physician and sportsmedicine* **2023,** *51* (1), 1-19. 10.1080/00913847.2021.1992601.
4. Ekstrand, J.; Bengtsson, H.; Waldén, M.; Davison, M.; Khan, K. M.; Hägglund, M., Hamstring injury rates have increased during recent seasons and now constitute 24% of all injuries in men's professional football: the UEFA Elite Club Injury Study from 2001/02 to 2021/22. *British journal of sports medicine* **2022,** *57* (5), 292-8. 10.1136/bjsports-2021-105407.
5. Al Attar, W. S. A.; Soomro, N.; Sinclair, P. J.; Pappas, E.; Sanders, R. H., Effect of Injury Prevention Programs that Include the Nordic Hamstring Exercise on Hamstring Injury Rates in Soccer Players: A Systematic Review and Meta-Analysis. *Sports medicine (Auckland, N.Z.)* **2017,** *47* (5), 907-916. 10.1007/s40279-016-0638-2.
6. Opar, D. A.; Williams, M. D.; Shield, A. J., Hamstring strain injuries: factors that lead to injury and re-injury. *Sports medicine (Auckland, N.Z.)* **2012,** *42* (3), 209-26. 10.2165/11594800-000000000-00000.
7. Wolski, L.; Pappas, E.; Hiller, C.; Halaki, M.; Fong Yan, A., Is there an association between high-speed running biomechanics and hamstring strain injury? A systematic review. *Sports biomechanics* **2024,** *23* (10), 1313-1339. 10.1080/14763141.2021.1960418.
8. Mueller-Wohlfahrt, H. W.; Haensel, L.; Mithoefer, K.; Ekstrand, J.; English, B.; McNally, S.; Orchard, J.; van Dijk, C. N.; Kerkhoffs, G. M.; Schamasch, P.; Blottner, D.; Swaerd, L.; Goedhart, E.; Ueblacker, P., Terminology and classification of muscle injuries in sport: the Munich consensus statement. *British journal of sports medicine* **2013,** *47* (6), 342-50. 10.1136/bjsports-2012-091448.
9. Witvrouw, E.; Danneels, L.; Asselman, P.; D'Have, T.; Cambier, D., Muscle flexibility as a risk factor for developing muscle injuries in male professional soccer players. A prospective study. *The American journal of sports medicine* **2003,** *31* (1), 41-6. 10.1177/03635465030310011801.
10. Erol, E.; Bulut, B. N., Acute effect of instrument-assisted soft tissue mobilization on hamstring flexibility via fascial chain. *BMC musculoskeletal disorders* **2024,** *25* (1), 1046. 10.1186/s12891-024-08182-7.
11. O'Sullivan, K.; Murray, E.; Sainsbury, D., The effect of warm-up, static stretching and dynamic stretching on hamstring flexibility in previously injured subjects. *BMC musculoskeletal disorders* **2009,** *10*, 37. 10.1186/1471-2474-10-37.
12. Sadler, S. G.; Spink, M. J.; Ho, A.; De Jonge, X. J.; Chuter, V. H., Restriction in lateral bending range of motion, lumbar lordosis, and hamstring flexibility predicts the development of low back pain: a systematic review of prospective cohort studies. *BMC musculoskeletal disorders* **2017,** *18* (1), 179. 10.1186/s12891-017-1534-0.
13. Hori, M.; Hasegawa, H.; Takasaki, H., Comparisons of hamstring flexibility between individuals with and without low back pain: systematic review with meta-analysis. *Physiotherapy theory and practice* **2021,** *37* (5), 559-582. 10.1080/09593985.2019.1639868.

 doi:10.20944/preprints202502.1724.v1

14. Jankaew, A.; Chen, J. C.; Chamnongkich, S.; Lin, C. F., Therapeutic Exercises and Modalities in Athletes With Acute Hamstring Injuries: A Systematic Review and Meta-analysis. *Sports health* **2023,** *15* (4), 497-511. 10.1177/19417381221118085.
15. Rudisill, S. S.; Varady, N. H.; Kucharik, M. P.; Eberlin, C. T.; Martin, S. D., Evidence-Based Hamstring Injury Prevention and Risk Factor Management: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *The American journal of sports medicine* **2023,** *51* (7), 1927-1942. 10.1177/03635465221083998.
16. Poursalehian, M.; Lotfi, M.; Zafarmandi, S.; Arabzadeh Bahri, R.; Halabchi, F., Hamstring Injury Treatments and Management in Athletes: A Systematic Review of the Current Literature. *JBJS reviews* **2023,** *11* (11). e23.00161. 10.2106/jbjs.Rvw.23.00161.
17. Medeiros, D. M.; Cini, A.; Sbruzzi, G.; Lima, C. S., Influence of static stretching on hamstring flexibility in healthy young adults: Systematic review and meta-analysis. *Physiotherapy theory and practice* **2016,** *32* (6), 438-445. 10.1080/09593985.2016.1204401.
18. Cai, P.; Liu, L.; Li, H., Dynamic and static stretching on hamstring flexibility and stiffness: A systematic review and meta-analysis. *Heliyon* **2023,** *9* (8), e18795. 10.1016/j.heliyon.2023.e18795.
19. Castellote-Caballero, Y.; Valenza, M. C.; Martín-Martín, L.; Cabrera-Martos, I.; Puentedura, E. J.; Fernández-de-Las-Peñas, C., Effects of a neurodynamic sliding technique on hamstring flexibility in healthy male soccer players. A pilot study. *Physical therapy in sport : official journal of the Association of Chartered Physiotherapists in Sports Medicine* **2013,** *14* (3), 156-62. 10.1016/j.ptsp.2012.07.004.
20. Espejo-Antúnez, L.; López-Miñarro, P. A.; Garrido-Ardila, E. M.; Castillo-Lozano, R.; Domínguez-Vera, P.; Maya-Martín, J., Acute Effect of Electrical Muscle Elongation and Static Stretching in Hamstring Muscle Extensibility. *Science & Sports* **2015,** *31* (1), e1-e7. 10.1016/j.scispo.2015.05.004.
21. Puentedura, E. J.; Huijbregts, P. A.; Celeste, S.; Edwards, D.; In, A.; Landers, M. R.; Fernandez-de-Las-Penas, C., Immediate effects of quantified hamstring stretching: hold-relax proprioceptive neuromuscular facilitation versus static stretching. *Physical therapy in sport : official journal of the Association of Chartered Physiotherapists in Sports Medicine* **2011,** *12* (3), 122-6. 10.1016/j.ptsp.2011.02.006.
22. Freitas, S. R.; Mendes, B.; Firmino, T.; Correia, J. P.; Witvrouw, E.; Oliveira, R.; Vaz, J. R., Semitendinosus and biceps femoris long head active stiffness response until failure in professional footballers with vs. without previous hamstring injury. *European journal of sport science* **2022,** *22* (7), 1132-1140. 10.1080/17461391.2021.1910347.
23. Espejo-Antúnez, L.; López-Miñarro, P. A.; Garrido-Ardila, E. M.; Castillo-Lozano, R.; Domínguez-Vera, P.; Maya-Martín, J.; Albornoz-Cabello, M., A comparison of acute effects between Kinesio tape and electrical muscle elongation in hamstring extensibility. *Journal of back and musculoskeletal rehabilitation* **2015,** *28* (1), 93-100. 10.3233/bmr-140496.
24. Espejo-Antúnez, L.; Cardero-Durán, M. A.; Albornoz-Cabello, M., Elongación Muscular Eléctrica. In *Electroestimulación transcutánea y neuromuscular y neuromodulación*, 2nd ed.; Albornoz-Cabello, M., Maya-Martín, J., Ed. Elsevier: Madrid, 2021.
25. Espejo-Antúnez, L.; Cardero-Durán, M. A.; Albornoz-Cabello, M., Elongación Muscular Eléctrica. In *Electroterapia Práctica. Avances en Investigación Clínica*, 2nd ed.; Elsevier: Madrid, 2022.
26. Schulz, K. F.; Altman, D. G.; Moher, D., CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Annals of internal medicine* **2010,** *152* (11), 726-32. 10.7326/0003-4819-152-11-201006010-00232.
27. Bandy, W. D.; Irion, J. M.; Briggler, M., The effect of time and frequency of static stretching on flexibility of the hamstring muscles. *Physical therapy* **1997,** *77* (10), 1090-6. 10.1093/ptj/77.10.1090.
28. Hoffmann, T. C.; Glasziou, P. P.; Boutron, I.; Milne, R.; Perera, R.; Moher, D.; Altman, D. G.; Barbour, V.; Macdonald, H.; Johnston, M.; Lamb, S. E.; Dixon-Woods, M.; McCulloch, P.; Wyatt, J. C.; Chan, A. W.; Michie, S., Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. *BMJ (Clinical research ed.)* **2014,** *348*, g1687. 10.1136/bmj.g1687.
29. Hamid, M. S.; Ali, M. R.; Yusof, A., Interrater and Intrarater Reliability of the Active Knee Extension (AKE) Test among Healthy Adults. *Journal of physical therapy science* **2013,** *25* (8), 957-61. 10.1589/jpts.25.957.

 doi:10.20944/preprints202502.1724.v1

30. Medeiros, D. M.; Miranda, L. L. P.; Marques, V. B.; de Araujo Ribeiro-Alvares, J. B.; Baroni, B. M., Accuracy of the functional movement screen (FMS(TM)) active straight leg raise test to evaluate hamstring flexibility in soccer players. *International journal of sports physical therapy* **2019,** *14* (6), 877-884.
31. Neto, T.; Jacobsohn, L.; Carita, A. I.; Oliveira, R., Reliability of the Active-Knee-Extension and Straight-Leg-Raise Tests in Subjects With Flexibility Deficits. *Journal of sport rehabilitation* **2015,** *24* (4). 10.1123/jsr.2014-0220.
32. Iglesias-Caamaño, M.; Carballo-López, J.; Álvarez-Yates, T.; Cuba-Dorado, A.; García-García, O., Intrasession Reliability of the Tests to Determine Lateral Asymmetry and Performance in Volleyball Players. *Symmetry* **2018,** *10* (9), 416. 10.3390/sym10090416.
33. Mason, M.; Keays, S. L.; Newcombe, P. A., The effect of taping, quadriceps strengthening and stretching prescribed separately or combined on patellofemoral pain. *Physiotherapy research international : the journal for researchers and clinicians in physical therapy* **2011,** *16* (2), 109-19. 10.1002/pri.486.
34. Jiménez-Sánchez, C.; Ortiz-Lucas, M.; Bravo-Esteban, E.; Mayoral-Del Moral, O.; Herrero-Gállego, P.; Gómez-Soriano, J., Myotonometry as a measure to detect myofascial trigger points: an inter-rater reliability study. *Physiological measurement* **2018,** *39* (11), 115004. 10.1088/1361-6579/aae9aa.
35. Agyapong-Badu, S.; Warner, M.; Samuel, D.; Stokes, M., Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device. *Archives of gerontology and geriatrics* **2016,** *62*, 59-67. 10.1016/j.archger.2015.09.011.
36. Lettner, J.; Królikowska, A.; Ramadanov, N.; Oleksy, Ł.; Hakam, H. T.; Becker, R.; Prill, R., Evaluating the Reliability of MyotonPro in Assessing Muscle Properties: A Systematic Review of Diagnostic Test Accuracy. *Medicina (Kaunas, Lithuania)* **2024,** *60* (6). 10.3390/medicina60060851.
37. Sharman, M. J.; Cresswell, A. G.; Riek, S., Proprioceptive neuromuscular facilitation stretching : mechanisms and clinical implications. *Sports medicine (Auckland, N.Z.)* **2006,** *36* (11), 929-39. 10.2165/00007256-200636110-00002.
38. Cohen, J., *Statistical Power Analysis for the Behavioral Science*. 2nd ed.; Lawrence Erlbaum Associates: Hillsdale: NY, USA, 1988.
39. Labata-Lezaun, N.; González-Rueda, V.; Llurda-Almuzara, L.; López-de-Celis, C.; Rodríguez-Sanz, J.; Cadellans-Arróniz, A.; Bosch, J.; Pérez-Bellmunt, A., Correlation between Physical Performance and Tensiomyographic and Myotonometric Parameters in Older Adults. *Healthcare (Basel, Switzerland)* **2023,** *11* (15). 10.3390/healthcare11152169.
40. Gosselin, L. E.; Adams, C.; Cotter, T. A.; McCormick, R. J.; Thomas, D. P., Effect of exercise training on passive stiffness in locomotor skeletal muscle: role of extracellular matrix. *Journal of applied physiology (Bethesda, Md. : 1985)* **1998,** *85* (3), 1011-6. 10.1152/jappl.1998.85.3.1011.
41. Mullix, J.; Warner, M.; Stokes, M., Testing Muscle Tone and Mechanical Properties of Rectus Femoris and Biceps Femoris Using a Novel Hand Held MyotonPRO Device: Relative Ratios and Reliability. *J. Back Musculoskelet. Rehabil.* **2015,** *28* (1), 93-100. 10.3233/BMR-140496.
42. Pérez-Bellmunt, A.; Casasayas, O.; Navarro, R.; Simon, M.; Martin, J. C.; Pérez-Corbella, C.; Blasi, M.; Ortiz, S.; Álvarez, P.; Pacheco, L., Effectiveness of low-frequency electrical stimulation in proprioceptive neuromuscular facilitation techniques in healthy males: a randomized controlled trial. *The Journal of sports medicine and physical fitness* **2019,** *59* (3), 469-475. 10.23736/s0022-4707.18.08356-1.
43. Espejo-Antúnez, L.; Carracedo-Rodríguez, M.; Ribeiro, F.; Venâncio, J.; De la Cruz-Torres, B.; Albornoz-Cabello, M., Immediate effects and one-week follow-up after neuromuscular electric stimulation alone or combined with stretching on hamstrings extensibility in healthy football players with hamstring shortening. *Journal of bodywork and movement therapies* **2019,** *23* (1), 16-22. 10.1016/j.jbmt.2018.01.017.
44. Adhitya, I.; Yu, W. Y.; Bass, P.; Kinandana, G. P.; Lin, M. R., Effects of Kinesio Taping and Transcutaneous Electrical Nerve Stimulation Combined With Active Stretching on Hamstring Flexibility. *Journal of strength and conditioning research* **2022,** *36* (11), 3087-3092. 10.1519/jsc.0000000000004088.
45. Karasuno, H.; Ogihara, H.; Morishita, K.; Yokoi, Y.; Fujiwara, T.; Ogoma, Y.; Abe, K., The combined effects of transcutaneous electrical nerve stimulation (TENS) and stretching on muscle hardness and pressure pain threshold. *Journal of physical therapy science* **2016,** *28* (4), 1124-30. 10.1589/jpts.28.1124.

 doi:10.20944/preprints202502.1724.v1

46. Rocha, C. S.; Lanferdini, F. J.; Kolberg, C.; Silva, M. F.; Vaz, M. A.; Partata, W. A.; Zaro, M. A., Interferential therapy effect on mechanical pain threshold and isometric torque after delayed onset muscle soreness induction in human hamstrings. *Journal of sports sciences* **2012,** *30* (8), 733-42. 10.1080/02640414.2012.672025.
47. Wanderley, D.; Lemos, A.; Moretti, E.; Barros, M.; Valença, M. M.; de Oliveira, D. A., Efficacy of proprioceptive neuromuscular facilitation compared to other stretching modalities in range of motion gain in young healthy adults: A systematic review. *Physiotherapy theory and practice* **2019,** *35* (2), 109-129. 10.1080/09593985.2018.1440677.
48. Behm, D. G.; Alizadeh, S.; Daneshjoo, A.; Anvar, S. H.; Graham, A.; Zahiri, A.; Goudini, R.; Edwards, C.; Culleton, R.; Scharf, C.; Konrad, A., Acute Effects of Various Stretching Techniques on Range of Motion: A Systematic Review with Meta-Analysis. *Sports medicine - open* **2023,** *9* (1), 107. 10.1186/s40798-023-00652-x.
49. Zaidi, S.; Ahamad, A.; Fatima, A.; Ahmad, I.; Malhotra, D.; Al Muslem, W. H.; Abdulaziz, S.; Nuhmani, S., Immediate and Long-Term Effectiveness of Proprioceptive Neuromuscular Facilitation and Static Stretching on Joint Range of Motion, Flexibility, and Electromyographic Activity of Knee Muscles in Older Adults. *Journal of clinical medicine* **2023,** *12* (7). 10.3390/jcm12072610.
50. Mani, E.; Kirmizigil, B.; Tüzün, E. H., Effects of two different stretching techniques on proprioception and hamstring flexibility: a pilot study. *Journal of comparative effectiveness research* **2021,** *10* (13), 987-999. 10.2217/cer-2021-0040.
51. Yıldırım, M. S.; Ozyurek, S.; Tosun, O.; Uzer, S.; Gelecek, N., Comparison of effects of static, proprioceptive neuromuscular facilitation and Mulligan stretching on hip flexion range of motion: a randomized controlled trial. *Biology of sport* **2016,** *33* (1), 89-94. 10.5604/20831862.1194126.
52. Hindle, K. B.; Whitcomb, T. J.; Briggs, W. O.; Hong, J., Proprioceptive Neuromuscular Facilitation (PNF): Its Mechanisms and Effects on Range of Motion and Muscular Function. *Journal of human kinetics* **2012,** *31*, 105-13. 10.2478/v10078-012-0011-y.
53. Scrivener, K.; Dorsch, S.; McCluskey, A.; Schurr, K.; Graham, P. L.; Cao, Z.; Shepherd, R.; Tyson, S., Bobath therapy is inferior to task-specific training and not superior to other interventions in improving lower limb activities after stroke: a systematic review. *Journal of physiotherapy* **2020,** *66* (4), 225-235. 10.1016/j.jphys.2020.09.008.
54. Hart, J. M.; Kerrigan, D. C.; Fritz, J. M.; Saliba, E. N.; Gansneder, B.; Ingersoll, C. D., Contribution of hamstring fatigue to quadriceps inhibition following lumbar extension exercise. *Journal of sports science & medicine* **2006,** *5* (1), 70-9.
55. Madić, D.; Obradović, B.; Golik-Perić, D.; Marinković, D.; Trajković, N.; Gojković, Z., The isokinetic strength profile of semi-professional soccer players according to low back pain. *Journal of back and musculoskeletal rehabilitation* **2020,** *33* (3), 501-506. 10.3233/bmr-171109.
56. Afonso, J.; Clemente, F. M.; Nakamura, F. Y.; Morouço, P.; Sarmento, H.; Inman, R. A.; Ramirez-Campillo, R., The Effectiveness of Post-exercise Stretching in Short-Term and Delayed Recovery of Strength, Range of Motion and Delayed Onset Muscle Soreness: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Frontiers in physiology* **2021,** *12*, 677581. 10.3389/fphys.2021.677581.
57. Freitas, S. R.; Mendes, B.; Le Sant, G.; Andrade, R. J.; Nordez, A.; Milanovic, Z., Can chronic stretching change the muscle-tendon mechanical properties? A review. *Scandinavian journal of medicine & science in sports* **2018,** *28* (3), 794-806. 10.1111/sms.12957.
58. Miyamoto, N.; Kimura, N.; Hirata, K., Non-uniform distribution of passive muscle stiffness within hamstring. *Scandinavian journal of medicine & science in sports* **2020,** *30* (9), 1729-1738. 10.1111/sms.13732.
59. Russell, A.; Choi, B.; Robinson, D.; Penailillo, L.; Earp, J. E., Acute and Chronic Effects of Static Stretching on Intramuscular Hamstring Stiffness. *Scandinavian journal of medicine & science in sports* **2024,** *34* (6), e14670. 10.1111/sms.14670.
60. Hatano, G.; Suzuki, S.; Matsuo, S.; Kataura, S.; Yokoi, K.; Fukaya, T.; Fujiwara, M.; Asai, Y.; Iwata, M., Hamstring Stiffness Returns More Rapidly After Static Stretching Than Range of Motion, Stretch Tolerance, and Isometric Peak Torque. *Journal of sport rehabilitation* **2019,** *28* (4), 325-331. 10.1123/jsr.2017-0203.

 doi:10.20944/preprints202502.1724.v1

61. Reid, D. A.; McNair, P. J., Passive force, angle, and stiffness changes after stretching of hamstring muscles. *Medicine and science in sports and exercise* **2004,** *36* (11), 1944-8. 10.1249/01.mss.0000145462.36207.20.
62. Miranda, H.; FH, D. E. F.; AA, D. E. O.; Dos Santos Ribeiro, J. S.; JBP, D. E. C.; Alvarenga, R. L.; Willardson, J. M., Effect of Different Numbers of Interset Antagonist Proprioceptive Neuromuscular Facilitation Stretching on the Total Number of Repetitions for the Agonists. *International journal of exercise science* **2022,** *15* (4), 498-506. 10.70252/sjqh8101.
63. Vanderthommen, M.; Triffaux, M.; Demoulin, C.; Crielaard, J. M.; Croisier, J. L., Alteration of muscle function after electrical stimulation bout of knee extensors and flexors. *Journal of sports science & medicine* **2012,** *11* (4), 592-9.