



Article

Females Present Different Single-Leg Squat Kinematics and Muscle Activation Strategies than Males Even after Hip Abductor Fatigue

Pablo Gaviraghi ¹, Francesca Chaida Sonda ², Michele Fernandes Frigotto ¹, Talita Molinari ¹, Luiza Pizarro Chaffe ³, José Luis Flor ³ , Rodrigo Rabello ^{4,*} and Rodrigo Rodrigues ^{3,5}

¹ Exercise Physiology and Physical Assessment Laboratory, Serra Gaúcha University Center, Caxias do Sul 95020-371, RS, Brazil; gaviraghipablo@gmail.com (P.G.); michele.frigotto@gmail.com (M.F.F.); t.molinari@hotmail.com (T.M.)

² Exercise Research Laboratory, Federal University of Rio Grande do Sul, Porto Alegre 90690-200, RS, Brazil; francescasonda@hotmail.com

³ Post-graduate Program in Health Science, Federal University of Rio Grande, Rio Grande 96200-190, RS, Brazil; luizachaffe@hotmail.com (L.P.C.); josefisiops@gmail.com (J.L.F.); rodrigo.esef@gmail.com (R.R.)

⁴ Department of Biomedical Sciences for Health, University of Milan, 20133 Milan, MI, Italy

⁵ Institute of Education, Federal University of Rio Grande, Rio Grande 96203-900, RS, Brazil

* Correspondence: rodrigo.rabello@unimi.it

Abstract: Background: Despite the potential connection between hip and knee muscle control, there is limited research on the effects of hip abductor fatigue on the hip and knee neuromuscular responses in both males and females. This study aimed to investigate the influence of sex on the hip and knee frontal plane kinematics and the EMG responses of the hip abductors and knee extensor muscles during the single-leg squat before and after hip abductor fatigue. Methods: A total of 30 participants (males, $n = 15$; females, $n = 15$) performed single-leg squats before and immediately after a hip abductor fatigue protocol (10° hip abduction position while bearing a 20% load of their estimated 1RM until exhaustion). The frontal plane kinematics (hip adduction and knee frontal plane projection angle) and EMG parameters (amplitude and median frequency) of the gluteus medius (GMed), tensor fascia latae (TFL), vastus lateralis (VL) and vastus medialis (VM) were measured during the single-leg squat. Results: We did not find any effects of hip abductor fatigue or interaction between fatigue and sex on the evaluated parameters ($p > 0.05$). However, compared to males, females had greater values for the hip and knee frontal plane kinematics ($p = 0.030$), GMed EMG amplitude (+10.2%, $p = 0.012$) and median frequency (+10.3%; $p = 0.042$) and lower VL median frequency (−9.80%; $p = 0.007$). Conclusions: These findings establish sex-related differences in the kinematics and hip and knee EMG parameters during the single-leg squat, which were not influenced by the hip abductor fatigue protocol.

Keywords: hip; fatigue; sex; knee; injuries



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1. Introduction

Alterations in the hip muscles have been observed in individuals with knee injuries [1,2]. Among these muscles, the hip abductors have garnered significant attention in rehabilitation programs [3] due to their crucial role in dynamic stability. In the context of stabilizing the pelvis and trunk to maintain balance, the gluteus medius (GMed) and tensor fascia latae (TFL) muscles play pivotal roles [4]. Specifically, the GMed has been identified as a significant contributor to the prevention of valgus moments [5], making it a focal point of strengthening programs [6]. Nevertheless, the contribution of the hip abductors to dynamic stability appears to differ between males and females [7], suggesting potential sex-specific variations in the role of the hip abductors [8]. To investigate the role of the hip abductors in lower limb alignment, several studies have induced weakness in the

hip abductors through local fatigue [9–11] to observe the potential compensations that may occur, helping in understanding the role of these muscles in the prevention of knee injuries.

Interestingly, when previous research has compared males and females in terms of hip abductor fatigability during dynamic contractions, the findings have been somewhat conflicting [8,12,13]. However, despite the absence of significant differences in fatigability between the sexes, females appear to exhibit greater susceptibility to the effects of hip abductor muscle fatigue on the kinematics during weight-bearing tasks [8,13]. Nevertheless, the influence of fatigue on knee injuries remains a subject of limited and contentious investigation [14]. While there is evidence of distal compensations resulting from hip abductor fatigue [9–11], we did not find any information available regarding the influence on the quadriceps, despite previous findings suggesting a potential link between hip muscle control and neuromotor regulation of the vasti muscles [15]. This connection has also been observed in lower limb conditions, such as individuals with patellofemoral pain, who simultaneously showed neural and structural alterations in the GMed [1] and recruitment of the vasti muscles [16].

Furthermore, distinctions in the recruitment patterns of the hip abductors have been observed between the sexes [17], as well as disparities in the activation of the vastus medialis (VM) and vastus lateralis (VL) in healthy individuals [18]. Furthermore, there are other potential differences between males and females that may impact lower limb motor control, such as muscle strength, with females typically exhibiting lower strength [17], and an increased Q-angle [19]. There is existing evidence indicating that females exhibit greater lower fatigue levels than males [20], but there is also an absence of studies examining the compensatory effects of hip abductor fatigue on neural responses in the VM and VL for both males and females, despite the observed connection between alterations in the hip abductors and knee extensors in individuals with knee injuries. Therefore, this study aimed to investigate the influence of sex on the hip and knee frontal plane kinematics and electromyography (EMG) responses (amplitude and median frequency) of the GMed, TFL, VL, and VM during the single-leg squat before and after hip abductor fatigue. This task is recognized as a tool for assessing the potential risk of lower extremity injury and is correlated with other functional tasks [21].

2. Methods

2.1. Experimental Design

All the assessments were conducted in a single session, with participants using their preferred limb, as determined by asking them which lower limb they would use to kick a ball. The evaluation sequence included: (i) participant profiling; (ii) assessment of the Maximal Voluntary Isometric Contraction (MVIC) for the knee extensors and hip abductors; (iii) single-leg squat test; (iv) a ten-repetition maximum test (10RM) in the side-lying hip abduction exercise to estimate the 1RM; (v) the isometric sustained hip abductor fatigue protocol (utilizing 20% of the estimated 1RM); and (vi) the post-fatigue single-leg squat test for comparison purposes.

2.2. Participants

The appropriate sample size was estimated a priori with the G*Power software (version 3.1.9.6; University of Trier, Trier, Germany). The ANOVA repeated measures, within-between interaction, was set with a significance level of $p = 0.05$, power $1 - \beta = 0.80$ and an effect size f of 0.47 (based on the difference in the knee valgus angle of males and females during single-leg landing before and after hip abductor fatigue [13]), which indicated a minimum sample size of 12 for a group. Due to potential sample losses, three additional participants were added to each group. A total of 30 participants (male, $n = 15$; female, $n = 15$) were recruited to participate in the study. Recruitment efforts were carried out through various channels, including social media, local fitness centers, and the university campus. All the participants met specific criteria, including being aged between 18 and 35 years old, having no prior history of lower limb injuries and possessing a minimum

of three months' experience in strength-training programs at the time of data collection. None of them participated in other types of activities involving high training volumes (e.g., running or sports). All other activities were documented to prevent potential interference with the results. This study adhered to the ethical principles outlined in the Declaration of Helsinki. After receiving a comprehensive explanation of the study's procedures, all the participants provided written consent to partake in the research, which had received approval from the university's ethics committee.

3. Procedures

3.1. Maximal Voluntary Isometric Contractions

For the evaluation of the MVIC of the knee extensors, each participant began with a warm-up consisting of 15 repetitions of unilateral submaximal knee extensions. The MVIC was performed during an isometric single-leg knee extension while seated in traditional knee extensor chair equipment commonly found in gyms. Participants exerted force against the fixed arm of the equipment, maintaining a knee flexion of 90°, while their trunk was supported by the chair's backrest [22]. The EMG amplitudes of the VL and VM were recorded.

For the assessment of the MVIC of the hip abductors, participants began with a warm-up comprising 15 repetitions of unilateral side-lying hip abduction exercises. This warm-up involved the use of ankle weights with a self-selected low external load. The task was executed in the side-lying position, with the pelvis and hip maintaining a neutral alignment and the toes pointing forward, and the hip of the dominant side positioned at a 10° abduction angle (verified using a digital inclinometer). Concurrently, the contralateral leg was flexed at both the hip and knee to 90°. To ensure standardized resistance, the distal region of the dominant leg (approximately 5 cm above the lateral malleolus) was firmly positioned against a rigid and fixed structure and the EMG amplitudes of the GMed and TFL were recorded [23]. The MVIC assessments of the knee extensors and hip abductors consisted of three trials, each sustained for a duration of five seconds, with a two-minute interval provided between trials. Verbal encouragement was consistently provided throughout these assessments.

3.2. EMG Data Acquisition

A four-channel electromyographer, sampling at 2000 Hz with 14-bit resolution, 126 dB common mode rejection ratio and low-pass filter of 1 kHz (Miotool-400, Miotec—Biomedical Equipment, Porto Alegre, Brazil), combined with a specific data acquisition software (MiotecSuite—Equipamentos Biomédicos, Porto Alegre, RS, Brazil), connected to a computer (Asus z450U i5) was used. The EMG data of the GMed, TFL, VL and VM during the MVICs and during the single-leg squat test were obtained. After skin preparation, two pre-gelled electrodes with a 15 mm radius (Kendall Mini MediTrace 100—Tyco Healthcare, São Paulo, SP, Brazil) and 20 mm of distance between the centers were attached to the skin on each muscle belly and were positioned following the recommendations from the Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM). In addition, an electrode was also positioned at the tibia and used as a reference.

3.3. Frontal Plane Kinematics during Single-Leg Squat

To be consistent with the landmarks used for the hip and knee frontal plane 2D angular measurements, markers were placed on the anterior superior iliac spines (ASISs), the center of the patella, and the midpoint of the ankle malleoli [24]. Male participants were instructed to wear underwear, while female participants were asked to wear bikinis. All the assessments were conducted with the participants barefoot. The participants were directed to maintain both feet grounded until instructed by the researcher. Upon receiving the command, they were to shift their weight onto their dominant side and flex the knee on the opposite side [25], crossing their arms in front of the chest. Prior to initiating the test, the participants completed three repetitions to familiarize themselves with the procedure.

The pace of the movements was regulated by a metronome (2 beats for the eccentric phase and 2 beats for the concentric phase). Following this, the participants performed five consecutive single-leg squats with a self-selected degree of knee flexion [25].

To measure the hip and knee angles and synchronize the movement phases with the EMG signal, we employed two cameras. The first camera (iPad A1416 32 GB; 30 Hz) was situated three meters away, capturing the frontal plane view. The second camera was integrated into the computer used for EMG signal processing (Asus z450U i5; 30 Hz) and was positioned two meters from the participants, providing a sagittal plane view. The synchronization of the video with the EMG signal was performed through this computer.

3.4. Estimated 1RM

The Lombardi coefficients were employed to predict the one-repetition maximum (1RM) from the load and the number of repetitions completed [26] during the side-lying hip abduction exercise. For example, if a participant was able to perform 10 repetitions with 15 kg, the estimated 1RM would be 20.4 kg, corresponding to $15 \text{ kg (load)} \times 1.36$ (coefficient). In this test, the participants used ankle weights equivalent to 20% of their body mass, based on previous studies with a similar sample that performed a 10-repetition maximum (10 RM) of the side-lying hip abduction exercise [25]. A metronome was set at 60 beats per minute to ensure the exercise was executed at a controlled speed, with 2 beats for each phase.

The participants performed the exercise with their hips in a neutral position and full extension of the knee in the tested limb, while maintaining the contralateral knee in a flexed position. They were guided to execute both the eccentric and concentric phases to the maximum extent of their range of motion. In cases where a participant managed to complete more than 10 repetitions with the initial load, a rest period of 5 minutes was allotted. Subsequently, the load was incremented by 10% for the subsequent trial. If the participant again exceeded 10 repetitions with the new load, additional data collection sessions were scheduled. The 1RM was estimated based on the load and the coefficients derived from the number of repetitions performed (with a maximum of 10) using Lombardi's method [26].

3.5. Isometric Sustained Hip Abductor Fatigue Protocol

Following the determination of the estimated 1RM, the participants were situated in a side-lying position with the hip abducted at 10° , maintaining a neutral pelvic alignment, while keeping the contralateral knee in a flexed position. The distal portion of the leg (approximately 5 cm above the lateral malleolus) was secured against a rigid and stationary structure. An external load, equivalent to 20% of their estimated 1RM, was applied to the ankle region of the participants using ankle weights. They were instructed to sustain this position for as long as possible. The test concluded under two conditions: (i) The participant could no longer maintain the foot touching the fixed structure three consecutive times despite strong verbal encouragement. The required position (foot touching the fixed structure) was visually monitored by one of the investigators. (ii) The participant's voluntary exhaustion. Whenever one of these criteria was met, the participant was considered to have reached exhaustion, and the duration of the trial was recorded as the endurance time. Immediately after the fatigue protocol, the participants were positioned to perform the single-leg squat test, which took approximately 1 min.

4. Data Analysis

The EMG data were processed using a band-pass digital filter (5th order Butterworth) with the cut-off frequencies set at 20–500 Hz. Additionally, the signals were smoothed using the 150 ms moving window root mean square (RMS) method [25]. To determine the median frequency (MDF), spectral analysis was conducted using Fast Fourier Transformation. In the analysis of the single-leg squats, data from all five repetitions were included. The RMS value and MDF for the complete eccentric phases were determined by employing a synchronized computer to accurately identify the initiation and conclusion of each phase

visually (just before the onset of the concentric phase). For the EMG amplitude analysis, the RMS values were standardized against the values recorded during the MVICs and presented as a percentage of the maximal activation [24]. All the data were assessed by a single experienced evaluator using Miotec Suite software (version 1.0, Miotec—Biomedical Equipment, Porto Alegre, Brazil) [24,25].

A single experienced evaluator conducted the analysis of all the kinematic data using Kinovea software (version 0.8.15, Kinovea Organization, France). The measurement of the hip adduction angle (hip ADD) involved determining the angle formed between the femur and the line connecting the two ASIS (apex = dominant side ASIS), while the measurement of the knee frontal plane projection angle (knee FPPA) entailed calculating the angle formed between the femur and the tibia (apex = center of patella). Both measurements were expressed as absolute values, derived from the difference between 90° (for the hip ADD) and 180° (knee FPPA) and the angle recorded at the conclusion of the eccentric phase of each repetition [27]. This angle was considered to represent the moment just before the onset of the concentric phase. Positive values represented displacement toward adduction and toward knee valgus, respectively (Figure 1).

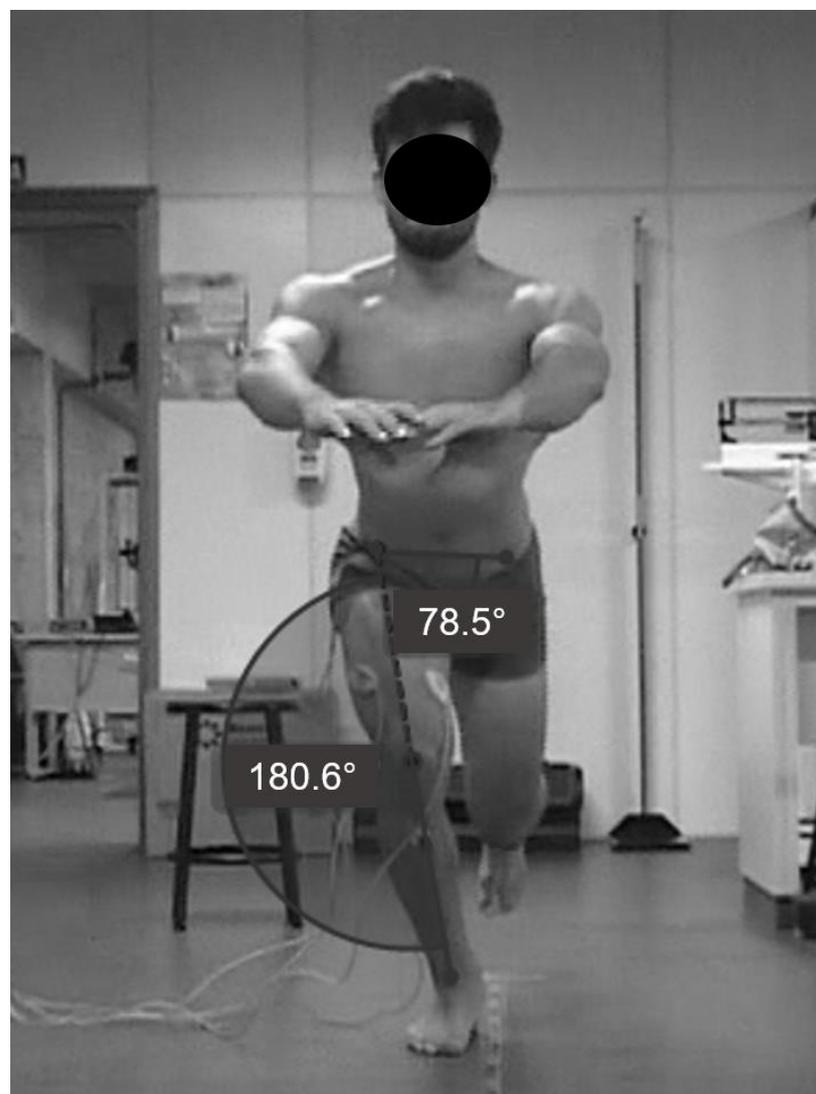


Figure 1. Hip adduction (ADD) and knee frontal plane projection angle (FPPA) measurements were obtained by calculating the deviation from 90° (for hip ADD) and 180° (for knee FPPA), based on the angle recorded at the end of the eccentric phase of each repetition. In this example, the values were 11.5° (for the hip ADD) and 0.6° (for the knee FPPA).

5. Statistical Analysis

Data normality was tested through the Shapiro–Wilk test. Data sphericity was tested by the Mauchly test and the Greenhouse–Geisser correction factor was used when the sphericity was violated. As the normality of our data was not violated, parametric tests were performed. To compare subject characteristics, the estimated 1RM, 20% of the estimated 1RM, and the endurance time between the sexes, an independent Student’s t-test was conducted. To examine the effects of sex and fatigue on the frontal plane kinematics (hip ADD and knee FPPA) and EMG parameters (amplitude and MDF) of the GMed, TFL, VL, and VM during single-leg squats, a factorial ANOVA was employed, considering sex and fatigue (pre and post). Statistical significance was considered at a p -value of <0.05 . All the analyses were carried out using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

6. Results

Males had higher body mass, height, body mass index, 1 RM and 20% of the 1 RM than females ($p < 0.001$). No significant differences were observed between the sexes in terms of age and time to exhaustion (Table 1).

There was not a fatigue * sex interaction for the hip ADD ($p = 0.65$) and knee FPPA ($p = 0.12$), nor a main fatigue effect for the hip ADD ($p = 0.94$) and knee FPPA ($p = 0.91$). We found a main effect of sex for the hip ADD [$F_{(1,28)} = 5.03$; $p = 0.03$] and knee FPPA [$F_{(1,28)} = 5.03$; $p = 0.03$], with females having greater values than males in both of these (Figure 2).

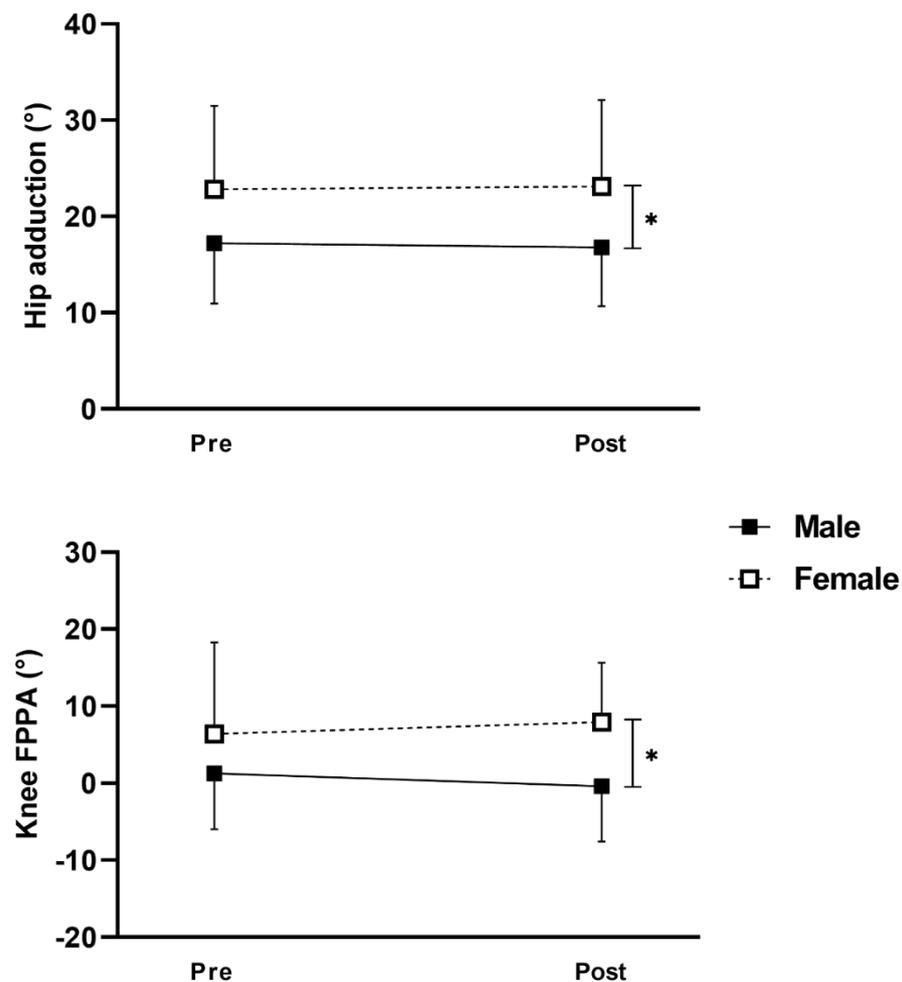


Figure 2. Sex and hip abductor fatigue on the hip and knee frontal plane kinematics (hip ADD and knee FPPA). * Differences between the sexes.

Table 1. Characteristics of participants.

	Male (n = 15)	Female (n = 15)
Age (y)	26.6 ± 4.2	25.6 ± 4.8
Body mass (kg)	85.7 ± 9.2	61 ± 8.8 *
Height (m)	1.77 ± 0.05	1.63 ± 0.09 *
Body mass index (kg/m ²)	27.1 ± 2.2	22.9 ± 2.7 *
Estimated 1RM (kg)	18.8 ± 3.1	12.1 ± 2.3 *
20% of estimated 1RM (kg)	3.8 ± 0.6	2.5 ± 0.5 *
Time to exhaustion (s)	149.9 ± 56.3	159.6 ± 65

* Different between the sexes.

Regarding the EMG amplitude, for the GMed, we did not find a fatigue * sex interaction ($p = 0.49$), nor a main fatigue effect ($p = 0.92$). We found a main effect of sex [$F_{(1, 28)} = 7.15$; $p = 0.012$], with females having greater values than males (+10.2%). For the TFL, we did not find a fatigue * sex interaction ($p = 0.96$), a main effect of sex ($p = 0.31$), or a main effect of fatigue ($p = 0.46$). Finally, we did not find a fatigue * sex interaction for the VL ($p = 0.73$) and VM ($p = 0.47$), a main effect of fatigue for the VL ($p = 0.09$) and VM ($p = 0.52$), nor a main effect of sex for the VL ($p = 0.24$) and VM ($p = 0.95$) (Figure 3).

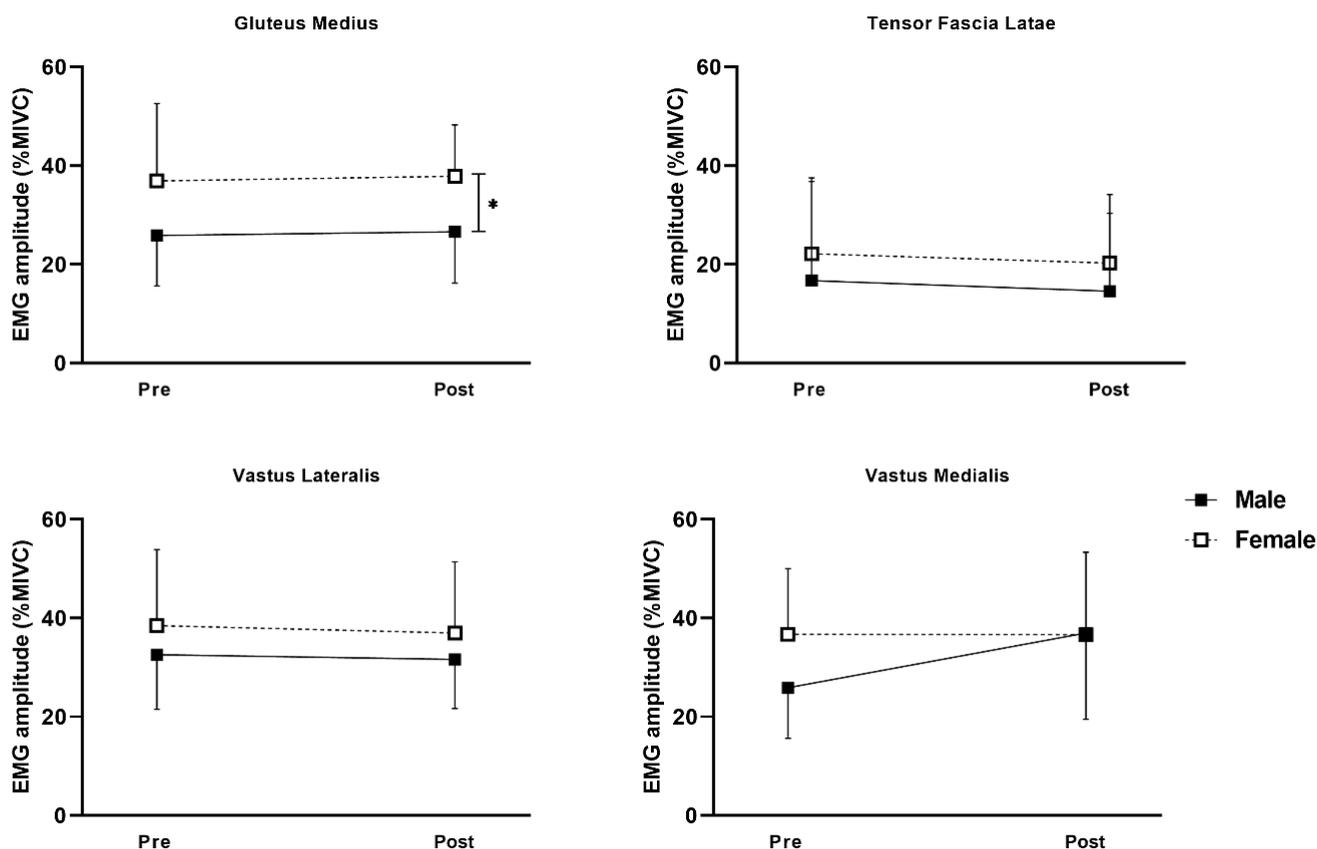


Figure 3. EMG amplitude responses of the hip abductors and knee extensors between the sexes and after hip abductor fatigue. * Differences between the sexes.

Regarding the MDF, for the GMed, we did not find a fatigue * sex interaction ($p = 0.64$), nor a main effect of fatigue, but it approached statistical significance ($p = 0.06$). We found a main effect of sex [$F_{(1, 28)} = 4.53$; $p = 0.042$], with females having greater values than males. For the VL, we did not find a fatigue * sex interaction ($p = 0.92$), nor a main fatigue effect ($p = 0.15$). We found a main effect of sex [$F_{(1, 28)} = 8.38$; $p = 0.007$], with males having greater

values than females. We did not find a fatigue * sex interaction for the TFL ($p = 0.63$) and VM ($p = 0.55$), a main effect of sex for the TFL ($p = 0.93$) and VM ($p = 0.13$), nor a main effect of fatigue for the TFL ($p = 0.18$) and VM ($p = 0.32$) (Figure 4).

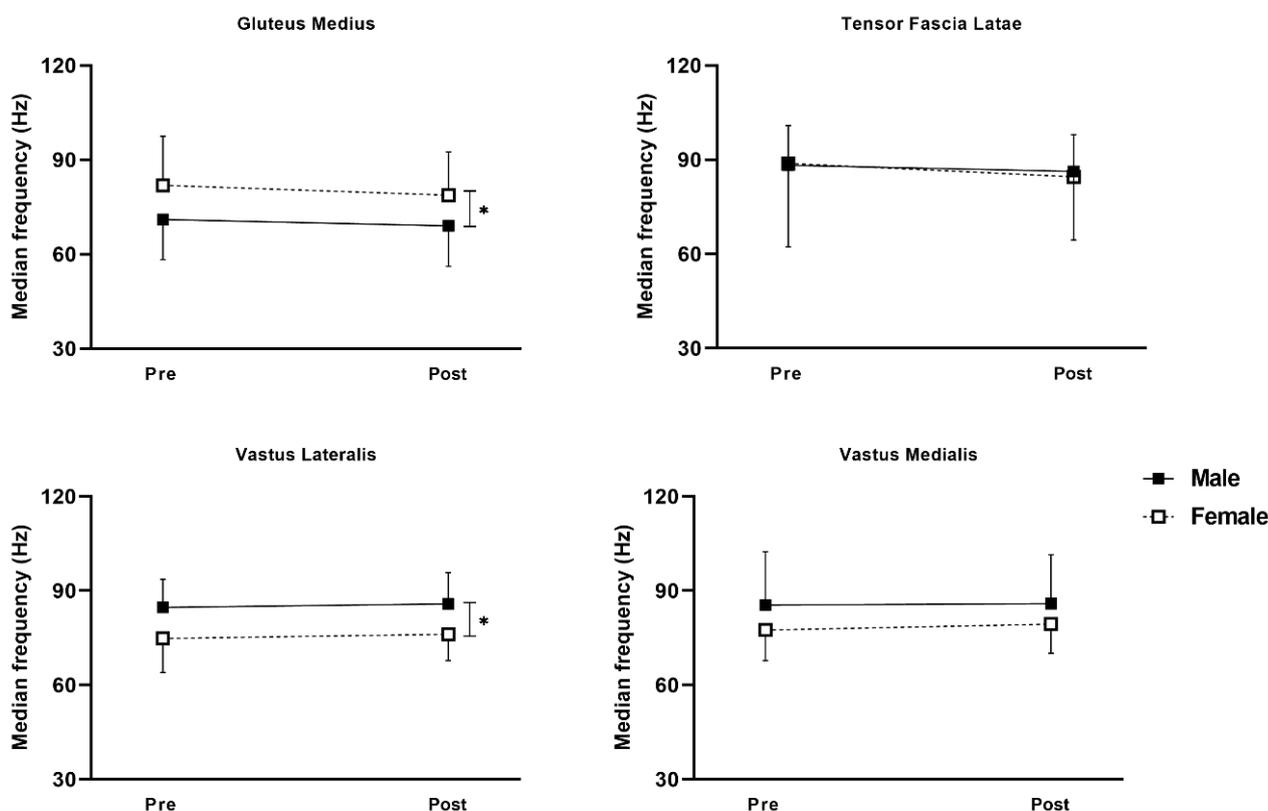


Figure 4. MDF responses of the hip abductors and knee extensors between the sexes and after hip abductor fatigue. * Differences between the sexes.

7. Discussion

Our study revealed that females exhibit greater hip and knee frontal plane kinematics in comparison to males, irrespective of hip abductor fatigue. This finding is consistent with previous research on single-leg squats [17,28]. It appears that females tend to possess lower hip abduction strength than males [17], and this lower strength has been linked to increased hip adduction and knee valgus during several single-leg tasks [7,8]. Our study found a lower hip abduction strength in females, as measured by the non-normalized 1RM, which may help to explain the kinematics results, agreeing with previous studies.

In our study, fatigue of the hip abductors did not lead to any changes in single-leg squat performance. These results go against those found in previous studies that have evaluated cut, jump and running [29], and single-leg landing [8,30]. These discrepancies are likely attributed to the fact that the single-leg squat is a more stable task compared to activities such as landing, running, and cutting, where there is a greater demand on the hip abductors to stabilize the pelvis before the initiation of eccentric contractions. Additionally, neuromuscular changes in the distal joints resulting from hip abductor weakness may become apparent during tasks involving instability [9–11]. Given that we did not find any information available on the impact of hip abductor fatigue on quadriceps activation, it is possible that the effects of hip abductor fatigue may be less pronounced in the context of stable tasks, such as the squat.

In terms of the EMG amplitude, we observed higher values in females than in males for the GMed. Previous studies have reported conflicting results when comparing males and females during single-leg squats [31,32]. A study showed that greater hip adduction and knee abduction angles were associated with a greater GMed EMG amplitude, suggesting a

compensatory strategy by the central nervous system to enhance muscle recruitment and force for lower limb stability [7]. Thus, we hypothesize that as previous studies showed that females have lower hip abductor strength [17] and higher hip adduction compared to males, there was an increase in muscle activation for pelvic stabilization, leading to higher GMed activation, to enhance the stability in the female participants. However, for the TFL, a similar effect did not occur, likely due to its less significant role in pelvis stability during single-leg tasks [33]. Regarding quadriceps activation, a previous systematic review has shown limited neuromuscular differences in the knee muscles between males and females [34]. We found no significant differences between males and females in terms of the vastus muscles' EMG amplitude during the single-leg squat, agreeing with a previous study [35]. This observation suggests that during the single-leg squat, the EMG amplitude of the vastus muscles exhibits a similar pattern in both sexes.

Although a common alteration typically observed in EMG signals during fatigue is a shift in the frequency spectrum toward lower frequencies [36], in our study, hip abductor fatigue did not lead to any changes in frequency during the single-leg squat. However, the changes in the median frequency due to fatigue appear to be more accurate during isometric contractions than dynamic contractions, where other parameters, such as the Dimitrov spectral index of muscle fatigue, explain fatigue more effectively [37]. Additionally, there have been limited studies that have evaluated the frequency component in relation to biomechanical factors associated with knee injuries [24,38,39]. It is essential to point out that the values of the frequency components appear to be linked to the muscle fiber conduction velocity [36], with some authors also attributing this variable to the muscle fiber type [40]. Furthermore, an increase in the frequency components has been associated with increased muscle force, attributable to the progressive recruitment of larger and faster motor units [41]. The comparison of the frequency components during weight-bearing tasks between males and females was addressed in a single study, where the authors noted that females exhibited a lower frequency in the VL and VM compared to males during the initial contact of an unanticipated cutting maneuver [38]. This result is in partial agreement with our findings, as we also observed that females had a lower frequency in the VL but similar in the VM compared to males. One possible explanation for this finding is the greater distribution of fast-twitch muscle fibers (which have a higher muscle fiber conduction velocity) in males compared to females, mainly in the VL [42].

Conversely, we found that females had a higher frequency in the GMed compared to males. Until now, no data for sex differences in the fiber-type composition of the hip abductors have been available. Thus, we believe that the higher frequency in females is consistent with our findings of a higher EMG amplitude of the GMed in females. It is known that the amplitude of the EMG signal is altered due to a potential increase in motor unit recruitment, as well as an increase in the conduction velocity of the muscle fibers [43]. Thus, we believe that the higher values of both EMG variables for the GMed in females (MDF and amplitude) are a result of an increased demand for force production by this muscle in an effort to stabilize the pelvis during the single-leg squat. However, future studies need to confirm this hypothesis.

The present study has some limitations that are necessary to recognize. (i) The 2D hip ADD and knee FPPA are measurements frequently adopted with a reasonable association with 3D motion capture [44], but they neglect changes in the transverse plane. (ii) We did not measure the degree of knee flexion during the single-leg squat, which would have aided in understanding the differences between the sexes and potential compensations induced by the fatigue protocol. (iii) Although the 1RM test is widely used to assess dynamic strength, in our study, it primarily represents the concentric strength of the hip abductors in an open kinetic chain movement. However, our parameters were analyzed during the eccentric phase of the single-leg squat, where the abductors are involved in pelvic stabilization in a closed kinetic chain movement. Therefore, the role of the 1RM result of our study requires caution. (iv) The calculation of the median frequency is limited during dynamic contractions, particularly when the response is related to fatigue. (v)

Our fatigue protocol was isolated to the hip abductors, as we aimed to observe the role of this muscle group in neuromuscular control of the lower limb (which was not found). However, this fatigue protocol has low external validity in real-life scenarios. (vi) Since we did not find previous studies addressing the influence of hip abductor fatigue on the neuromuscular control of the quadriceps during weight-bearing tasks, it is advisable for future research to investigate whether compensatory mechanisms may occur in scenarios with faster movements and higher stability demands, such as landing, cutting, and running.

8. Conclusions

We found that females had greater values for the hip and knee frontal plane kinematics, GMed EMG amplitude and median frequency, but lower VL median frequency, than males. However, hip abductor fatigue did not change the hip and knee frontal plane kinematics, nor the EMG parameters of hip abductors and knee extensors muscles, in both sexes during single-leg squat. These findings validate the presence of sex-related differences in the kinematics and hip and knee EMG parameters during the single-leg squat, which were not influenced by the hip abductor fatigue protocol.

9. Declarations

During the preparation of this work, the author(s) used ChatGPT in order to review and enhance the manuscript's writing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Author Contributions: P.G.: the conception, acquisition of data, analysis and interpretation of data, draft of the article, revising it critically for important intellectual content and final approval of the version to be submitted; F.C.S.: acquisition of data, data analysis, critical revision of the article, final approval of the version to be submitted; M.F.F.: acquisition of data, data analysis, critical revision of the article, final approval of the version to be submitted; T.M.: data analysis, critical revision of the article, final approval of the version to be submitted; L.P.C.: data analysis, critical revision of the article, final approval of the version to be submitted; J.L.F.: data analysis, critical revision of the article, final approval of the version to be submitted; R.R. (Rodrigo Rabello): data analysis, critical revision of the article, final approval of the version to be submitted and corresponding author; R.R. (Rodrigo Rodrigues): the conception and design of the study, study orientation and supervision and critical revision of the article, final approval of the version to be submitted. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and it was approved by the Institutional Review Board (or Ethics Committee) of Serra Gaúcha University Center (Number: 3.446.338).

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to limitations from the approved protocol.

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

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