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Research Article

The Effect of a Fatigue Protocol on EMG Median Frequency during the Swing Phase of Walking in Individuals Following ACL Reconstruction with Different Graft Types

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Abstract

Introduction: Neuromuscular deficits may persist after anterior cruciate ligament reconstruction (ACLR) despite restoration of mechanical stability, and these alterations may become more evident under fatigue. Whether graft type influences fatigue-related changes in EMG spectral behavior during gait remains unclear. This study investigated the effect of an acute fatigue protocol on EMG median frequency (MF) during the swing phase of walking in individuals following ACLR with different graft types.

Method: In this cross-sectional laboratory study, 53 physically active men were included, including 38 individuals with unilateral ACLR reconstructed using single-bundle hamstring tendon autograft (SB-HT, n=12), double-bundle hamstring tendon autograft (DB-HT, n=16), or allograft (n=10), and 15 healthy controls. Participants completed a progressive treadmill running protocol designed to induce systemic fatigue, terminated when Borg rating of perceived exertion exceeded 17 for at least 2 min or heart rate reached at least 85% of age-predicted maximum. Surface EMG was recorded from TA, GC, VL, VM, RF, BF, ST, and GM during walking before and immediately after fatigue. We calculated EMGMF for gait phases, focusing on swing. A two-way mixed-model repeated-measures ANOVA tested the main effects of fatigue and group and their interaction.

Result: No significant main effects of fatigue or group, and no fatigue×group interactions, were observed for the quadriceps (VL, RF, VM), hamstrings (BF, ST), or distal lower-leg muscles (TA, GC) during swing (all interaction $p>0.05$). A trend toward a group effect was observed for semitendinosus ($p=0.065$). In contrast, GM MF increased significantly following fatigue across groups (main effect of fatigue: $p=0.025$, $\eta p^2=0.111$), with no significant group effect ($p=0.437$) or interaction ($p=0.431$).

Conclusion : Acute fatigue did not meaningfully alter GMMF in most lower-limb muscles during the swing phase of walking after ACLR, and these responses did not differ between graft types. The selective post-fatigue increase in GMMF suggests that hip stabilizer activity may respond to fatigue; however, further research is needed before clinical recommendations can be made.

Keywords: Anterior cruciate ligament reconstruction; Fatigue; Electromyography; Median frequency; Gait; Graft Type; Gluteus medius.

Introduction

Injury to the anterior cruciate ligament (ACL) is widely recognized as one of the most frequent and disabling knee injuries, particularly among physically active individuals, and it represents a large proportion of sports‐related trauma worldwide[1]. ACL reconstruction (ACLR) is commonly recommended for young, active patients to re‐establish knee stability and facilitate a safe return to physical activity and sport. However, surgical restoration of the ligament does not necessarily guarantee recovery of normal neuromuscular performance or the reestablishment of typical movement patterns during every day functional tasks[2]. Evidence indicates that individuals may continue to experience functional impairments following ACLR,

often attributed to disturbances in neuromuscular control, which may increase the long‐term likelihood of degenerative joint changes such as osteoarthritis [3-5].

The type of graft used during ACLR is considered an important variable that can influence postoperative outcomes and the recovery process. In clinical practice, commonly utilized grafts include hamstring tendon (HT) autografts applied through single‐bundle (SB‐HT) or double‐bundle (DB‐HT) reconstruction techniques, as well as allografts obtained from donor tissue[6,7]. The DB‐HT method was introduced to more accurately reproduce the anatomical arrangement and biomechanical function of the native ACL, which may improve rotational control of the knee[8]. On the other hand, the SB‐HT technique remains widely used because of its relative simplicity and satisfactory clinical results. Allografts eliminate donor‐site morbidity, although they may show slower biological integration and, in some cases, higher failure rates than autografts[9]. Although numerous investigations have compared these graft options, the literature has not yet clearly established whether one particular graft type leads to superior restoration of neuromuscular function during functional movements following ACLR[10].

Even when surgical reconstruction successfully restores mechanical stability to the knee joint, neuromuscular regulation of movement may remain altered. Previous research has shown persistent reductions in quadriceps strength[11], changes in muscle activation timing, and modifications in inter‐muscular coordination during locomotion in individuals who have undergone ACLR [12,13]. Such alterations may influence how the reconstructed limb contributes to gait, potentially affecting movement efficiency and joint loading patterns during walking. Because walking is one of the most common daily activities, examining neuromuscular behavior during gait provides valuable information about functional knee recovery.

Within the gait cycle, the swing phase represents a critical period in which the limb advances and prepares for the next ground contact. During this phase, appropriate neuromuscular coordination is required to control knee flexion and extension, ensure sufficient foot clearance, and position the limb accurately for the subsequent stance phase. Disturbances in muscle activation during swing can affect limb trajectory and lower-extremity alignment before heel strike, potentially altering joint loading and stability at initial contact. For individuals with a history of ACLR, subtle neuromuscular deficits during this phase may alter limb preparation before weight acceptance. Therefore, focusing specifically on the swing phase offers an important perspective for identifying neuromuscular adaptations that might not be fully captured when evaluating the entire gait cycle.

Surface electromyography (EMG) is widely used to investigate muscle activation and coordination during dynamic tasks such as walking. While many studies have focused on EMG amplitude as an indicator of muscle activation intensity, analyzing the frequency components of the EMG signal can provide additional insight into physiological processes within the muscle. One commonly used parameter is the median frequency (MF) of the EMG power spectrum, which is sensitive to changes associated with muscular fatigue. A shift toward lower EMGMF typically reflects reductions in muscle fiber conduction velocity and changes in motor unit recruitment patterns during sustained or repetitive activity. Consequently, decreases in EMGMF are frequently interpreted as indicators of developing neuromuscular fatigue and reduced efficiency of motor unit activation.

Neuromuscular fatigue can substantially influence motor control and joint stability, particularly in individuals who have undergone ACLR. Fatigue has been shown to degrade proprioceptive acuity, disrupt coordinated muscle activity, and alter muscle recruitment strategies necessary to maintain dynamic joint stability [14, 15]. Individuals following ACLR often display persistent asymmetries in neuromuscular control between limbs and may rely on compensatory activation patterns, such as increased hamstring co‐contraction, to support knee stability [16]. When fatigue occurs, these compensatory mechanisms may change further, potentially modifying muscle activation strategies and neuromuscular efficiency during walking.

Graft selection may also influence long‐term neuromuscular adaptations after ACLR. For instance, harvesting the hamstring tendon for autograft reconstruction may lead to prolonged reductions in hamstring strength and function [17, 18]. In contrast, surgical techniques that more closely replicate the

anatomical configuration of the ACL, such as DB‐HT reconstruction, may influence knee biomechanics and neuromuscular coordination in different ways. These graft‐related differences may interact with fatigue, potentially producing distinct patterns of muscle activation during functional tasks such as walking. However, most previous investigations have focused either on graft-type-related biomechanical outcomes or on the influence of fatigue on neuromuscular control independently. As a result, limited information exists on how fatigue affects the spectral characteristics of EMG signals during gait in individuals reconstructed with different ACL grafts.

Investigating fatigue‐related neuromuscular responses during walking is clinically important because both athletic activities and everyday movements are often performed under conditions in which muscular fatigue progressively develops. Evaluating how fatigue influences muscle activation during the swing phase of gait may provide deeper insight into functional control of the reconstructed limb and the knee's preparation for subsequent loading. Such information may help clinicians better understand graft‐specific neuromuscular adaptations and may contribute to optimizing rehabilitation protocols and return‐to‐sport decision-making .

Accordingly, the purpose of the present study was to evaluate the influence of a standardized fatigue protocol on the EMGMF of lower‐limb muscles during the swing phase of walking in individuals who had undergone ACLR using different graft types (allograft, SB‐HT, and DB‐HT). It was hypothesized that the fatigue protocol would significantly alter EMGMF during walking and that the magnitude of these fatigue-related changes would vary among graft types, reflecting potential differences in neuromuscular adaptations associated with each reconstruction technique.

Methods

2.1. Participants

This cross-sectional study investigated how a standardized fatigue protocol influences the spectral properties of surface EMG signals recorded during constant‐speed walking in individuals who had undergone ACLR with different graft types. A total of 53 physically active men aged between 18 and 30 years volunteered to participate. We recruited only male participants to reduce variability associated with sex‐related hormonal fluctuations, which may influence neuromuscular activation patterns and fatigue responses during locomotor tasks. Of these, 38 participants had previously undergone unilateral ACLR and were classified into three subgroups by graft type: SB‐HT, DB‐HT, and allograft reconstruction. In addition, we recruited a control group of 15 healthy men with similar age and physical activity levels, and no history of lower‐limb injury or surgical intervention, to provide baseline reference data for neuromuscular activation patterns. Participants reported any knee pain or subjective instability during the fatigue protocol. All participants were continuously monitored, and a predefined stopping criterion was established for pain or instability. No participants reported knee pain or instability during or immediately after testing.

Dominant limb determination was conducted using a two‐step approach. First, participants reported their preferred leg for kicking a ball. This self‐reported dominance was then confirmed through a single‐leg hop test for maximal distance [19]. Table 1 presents the distribution of limb dominance across groups. We assessed participants' habitual activity level using the Tegner Activity Scale, which ranges from 0 (severe disability) to 10 (participation in elite competitive sports) [20]. Statistical comparisons indicated no significant differences between groups in demographic variables, anthropometric characteristics, or activity level ($p > 0.05$), suggesting that the groups were comparable at baseline.

Eligibility criteria for the ACLR participants included: (i) a history of primary unilateral ACLR using SB‐HT, DB‐HT, or allograft techniques; (ii) a postoperative period between 6 and 12-months; (iii) completion of a structured rehabilitation program supervised by a licensed physical therapist; and (iv) absence of additional ligament injuries or meniscal resections greater than 50%. In the allograft group, grafts consisted of either tibialis anterior or tibialis posterior tendons, as confirmed by surgical documentation. Participants were excluded if they had a history of lower‐limb fracture, neurological disorders affecting motor control, or cardiovascular conditions that could contraindicate participation in exercise protocols designed to induce fatigue.

2.2. Surgical Procedure Characteristics

Experienced orthopedic surgeons performed all ACLR procedures arthroscopically using standardized techniques corresponding to the selected graft. Participants in the SB‐HT group received an autologous semitendinosus tendon graft configured in a quadruple‐strand construct. In the DB‐HT group, both the semitendinosus and gracilis tendons were harvested to create a double‐bundle graft designed better to reproduce the anatomical structure of the native ACL. Individuals in the allograft group underwent reconstruction using donor tendon tissue, specifically tibialis anterior or tibialis posterior tendons.

Postoperative imaging was used to verify appropriate placement of the femoral and tibial tunnels in all reconstructed knees [21]. Ensuring similar tunnel positioning across the different reconstruction techniques minimized the likelihood that variations in surgical alignment influenced the neuromuscular outcomes assessed in this study. All procedures conducted in this investigation adhered to the ethical principles outlined in the Declaration of Helsinki. The Ethics Committee of the University of Mohaghegh Ardabili, Iran, approved the study (IR.UMA.REC.1404.077). Before data collection, all participants received a full explanation of the experimental procedures and provided written informed consent.

2.2. Fatigue protocol

To induce systemic physiological fatigue before EMG measurements, participants completed a progressive running protocol on a motorized treadmill (Horizon Fitness Omega GT). Subjects wore their usual athletic footwear, and we continuously monitored heart rate using a Polar RS100 heart rate monitor (Polar Electro Oy, Woodbury, NY). The protocol began with running at 6km/h, after which the treadmill velocity increased by 1 km/h every two minutes. At the conclusion of each stage, participants rated their perceived exertion using the Borg 6–20 scale [22]. When participants reported a Borg rating of 13 or higher, the incremental speed increases were stopped and the treadmill velocity was maintained at a constant level to establish a steady-state running phase. During this period, we recorded heart rate and perceived exertion every 30 seconds. We estimated maximal heart rate using the conventional formula (220 − age), which is widely used in exercise testing [23]. In addition, localized fatigue in the lower limbs and subjective awareness of knee discomfort were assessed every minute using a 0–10 numerical scale designed to capture sensations related to muscle fatigue or joint stress [15]. The fatigue protocol ended when participants maintained a Borg score above 17 for at least two minutes or reached 85% or more of their age-predicted maximal heart rate. These criteria were selected to ensure sufficient fatigue, which could reasonably be expected to influence the spectral characteristics of EMG signals in the lower-limb muscles during walking [14, 15]. Treadmill-based fatigue protocols of this type are commonly used to replicate the neuromuscular demands of prolonged activity in individuals with ACLR and to evaluate fatigue-induced shifts in EMGMF during gait [14, 24]. Immediately after the fatigue protocol, participants completed walking trials so EMG data could capture the acute effects of exercise-induced neuromuscular fatigue. Across all participants, the fatigue protocol resulted in a mean Borg score of 18.1 ± 0.7, a mean heart rate of 87.4 ± 3.6% of age-predicted maximal heart rate, and an average protocol duration of 14.2 ± 3.1 minutes. Statistical comparisons showed no significant differences between groups in Borg score, achieved heart rate, or fatigue protocol duration ($p > 0.05$), suggesting comparable fatigue levels across all groups.

2.3. Assessment of lower limb electromyography activity and spectral analysis

Surface electromyography was recorded from eight muscles of the dominant lower limb: tibialis anterior (TA), gastrocnemius (GC), vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), biceps femoris (BF), semitendinosus (ST), and gluteus medius (GM). We selected these muscles because of their important roles in knee stabilization and neuromuscular control following ACLR [25]. We collected data using a wireless EMG system (Biometrics Ltd., UK) with bipolar Ag/AgCl surface electrodes positioned with an inter-electrode distance of 25 mm. Skin preparation followed SENIAM recommendations [26], including shaving of hair, gentle abrasion, and cleaning with 70% ethanol to reduce skin impedance. We sampled the EMG signals at 1000 Hz. We filtered raw data with a band-pass filter (10–500 Hz) to remove movement artifacts and a 50 Hz notch filter to eliminate electrical interference. Because the study focused on neuromuscular behavior during

limb advancement, EMG data were extracted specifically during the swing phase of the gait cycle. Identification of gait events and segmentation of the swing phase were performed using in-shoe footswitch sensors (Biometrics Ltd., UK), which detected toe-off and the subsequent heel contact to delimit the swing interval [27].

Within each identified swing phase, EMG signals were segmented using fixed windows of 250 ms with 50% overlap. We conducted frequency-domain analysis using Fast Fourier Transform (FFT) to estimate the power spectral density of each signal. A 1024-point FFT with a Hamming window was applied to minimize spectral leakage during the frequency analysis. The principal variable of interest was the MF of the EMG spectrum, a reliable indicator of fatigue-related shifts in muscle activity and a reflection of reductions in muscle fiber conduction velocity. MF was selected instead of amplitude-based measures or mean frequency because it is less sensitive to transient amplitude fluctuations and movement-related variability during dynamic tasks such as walking, and it provides a more robust indicator of fatigue-related spectral compression in surface EMG signals. Unlike amplitude-based EMG measures, fatigue-related spectral indices do not require normalization to maximal voluntary isometric contraction (MVIC); therefore, we used raw frequency values in the analysis [28]. A single trained investigator performed all signal-processing procedures. To facilitate comparison between participants and graft types, MF values obtained after the fatigue protocol were normalized relative to the pre-fatigue baseline values.

We selected the swing phase for EMGMF analysis because it provides a mechanically consistent interval with minimal influence from GRFs and impact-related variability. Unlike the stance phase, particularly weight acceptance, where joint stabilization demands, co-contraction, and external loading can introduce substantial variability in EMG spectral content, the swing phase allows clearer assessment of intrinsic neuromuscular fatigue-related changes. Moreover, altered hamstring and GM activation during swing has been reported as a compensatory strategy in individuals following ACLR, making this phase relevant for detecting subtle fatigue-induced adaptations that may not be evident during stance.

2.4. Statistical analyses

We examined data normality using the Shapiro–Wilk test. We evaluated the assumption of sphericity with Mauchly's test, and when it was violated, we applied Greenhouse–Geisser corrections. We used a two-way mixed-model repeated-measures ANOVA to analyze the data, with fatigue condition (non-fatigued vs fatigued) as the within-subject factor and graft type (SB-HT, DB-HT, allograft, healthy) as the between-subject factor. Because group sizes were unequal, we performed a sensitivity power analysis using G*Power to confirm that the study design had sufficient statistical power (≥ 0.80) to detect medium to large effects. The sensitivity analysis indicated that the present sample size provided sufficient statistical power ($1–\beta = 0.80$) to detect effect sizes of approximately $\eta p^2 \geq 0.10$ for the interaction effects. When significant interactions or main effects were identified, Bonferroni-adjusted post hoc comparisons were conducted. Effect sizes were calculated using partial eta squared ($\eta^2 p$) and interpreted according to established guidelines [31]. We set the level of statistical significance at $p < 0.05$. The mixed-model ANOVA approach was selected because the study design involved both a within-subject factor (fatigue condition: pre vs post) and a between-subject factor (group: SB-HT, DB-HT, allograft, healthy), allowing simultaneous evaluation of main effects and potential interaction effects between fatigue and graft type.

Results

3.1 Quadriceps muscles (VL, RF, VM)

For the quadriceps muscle group (VL, RF, and VM), the analysis found no significant main effects of group, fatigue, or group × fatigue interactions for the frequency spectrum variables during the swing phase ($p > 0.05$; Table 2). Specifically, VL frequency showed no significant main effect of fatigue ($p = 0.752$, $\eta p^2 = 0.002$), group ($p = 0.434$, $\eta p^2 = 0.061$), or interaction ($p = 0.501$, $\eta p^2 = 0.053$). Similarly, RF frequency did not demonstrate significant effects for fatigue ($p = 0.353$, $\eta p^2 = 0.020$), group ($p = 0.955$, $\eta p^2 = 0.007$), or the interaction between fatigue and group ($p = 0.119$, $\eta p^2 = 0.126$). For VM, no significant main effects of fatigue ($p = 0.557$, $\eta p^2 = 0.008$), group ($p = 0.431$, $\eta p^2 = 0.061$), or interaction ($p = 0.060$, $\eta p^2 = 0.157$) were identified.

Overall, these findings indicate that the spectral characteristics of quadriceps muscle activity during the swing phase remained relatively stable across groups and were not significantly influenced by the fatigue protocol.

3.2 Hamstring muscles (BF, ST)

For the hamstring muscles, including BF and ST, we observed no statistically significant main effects of fatigue or group during the swing phase ($p > 0.05$; Table 3). BF frequency demonstrated no significant effect of fatigue ($p = 0.975$, $\eta p^2 = 0.000$), group ($p = 0.953$, $\eta p^2 = 0.008$), or group × fatigue interaction ($p = 0.604$, $\eta p^2 = 0.042$). For the ST muscle, fatigue had no significant main effect ($p = 0.511$, $\eta p^2 = 0.010$). However, the main effect of group approached statistical significance ($p = 0.065$, $\eta p^2 = 0.153$), representing a moderate‐to‐large standardized effect size. The interaction between fatigue and group for ST was also not significant ($p = 0.506$, $\eta p^2 = 0.052$). These findings suggest that although ST activity showed a tendency toward group-related differences, the overall spectral characteristics of hamstring muscle activity during the swing phase were not significantly altered following the fatigue protocol.

3.3 Distal lower‐leg muscles (TA, GC)

For the distal lower-leg muscles, including TA and GC, the ANOVA results showed no significant main effects of fatigue, group, or their interaction ($p > 0.05$; Table 4). For TA, the main effect of fatigue ($p = 0.122$, $\eta p^2 = 0.055$), group ($p = 0.857$, $\eta p^2 = 0.018$), and the interaction between group and fatigue ($p = 0.294$, $\eta p^2 = 0.082$) were not statistically significant. Similarly, GC frequency showed no significant effects of fatigue ($p = 0.098$, $\eta p^2 = 0.062$), group ($p = 0.421$, $\eta p^2 = 0.063$), or group × fatigue interaction ($p = 0.131$, $\eta p^2 = 0.122$). Taken together, these results indicate that the fatigue protocol did not significantly modify the spectral characteristics of distal lower‐leg muscles during the swing phase of gait.

3.4 Hip stabilizer muscle (GM)

For the GM muscle, fatigue had a significant main effect ($p = 0.025$, $\eta p^2 = 0.111$). Post‐hoc pairwise comparisons collapsed across groups confirmed that GM median frequency was significantly higher after the fatigue protocol than in the pre‐fatigue condition (Bonferroni‐adjusted $p < 0.05$). Although the increase was relatively modest and varied slightly between groups, the overall direction of change was consistent across participants. However, the main effect of group ($p = 0.437$, $\eta p^2 = 0.061$) and the group × fatigue interaction ($p = 0.431$, $\eta p^2 = 0.061$) were not statistically significant. These findings indicate that fatigue influenced GM spectral characteristics during the swing phase, but this effect did not differ significantly among the ACLR graft groups and the healthy control group.

Table 1. Demographic and anthropometric characteristics of participants

Characteristics	SB-HT	DB-HT	Allograft	Healthy group	p-value
Age (years)	٢٦.6 ± 0.٢	26.1 ± 0.٤	27.٢ ± 0.٤	25.٩ ± 0.١	1.000
Height (cm)	1٧٩.٨ ± 3.٧	179.2 ± ٦.7	١٨٠.1 ± 2.٣	١٨٢.1 ± ٣.1	.٣١٦
Weight (kg)	80.1 ± ٢.1	٨٢.1 ± ١.2	٨٠.9 ± ١.٠	٨١.٢ ± ٢.١	.٢١٥
Body mass index (kg/m²)	٢٢.١0 ± 2.١	٢٢.١٩ ± 1.٢	24.٢٢ ± 2.١٠	23.٠١ ± 3.١٢	.٢٥٦
Time post-ACLR surgery (months)	12±1	11±1	12±1	–	0.124
Tegner activity score	٦,١ ± 0.٣	٦,٢ ± 0.٥	6.٠ ± 1.0	6.٦ ± 0.٨	0.٢١٩
Weekly walking/running (km)	7.9 ± ١.١	٧.4 ± ٠.4	٧.7 ± ٢.١	9.4 ± 3.2	0.١٢٥
Dominant limb	١٦ right foot	١٢ right foot	10 right foot	١٥ right foot	

Group Designations: SB-HT, single-bundle hamstring tendon; DB-HT, double-bundle hamstring tendon.

Table 2. Frequency spectrum variables of quadriceps muscles during the swing phase of walking

Muscles	ACLR group (Allograft)		ACLR group (SB-HT)		ACLR Group (DB-HT)		Healthy group		Main effect of fatigue p (ηp²)	Main effect of group p (ηp²)	Main effect of fatigue *group p (ηp²)
	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue			
VL	32.39±12.88	۲۸.55±۹.96	30.01±15.52	33.72±25.62	25.87±10.64	25.52±6.60	25.32±9.03	23.37±5.90	0.752(0.002)	0.434(0.061)	**0.501(0.053)**
RF	37.45±23.75	30.56±9.69	29.66±15.35	33.10±25.45	33.62±10.12	34.37±10.95	25.51±5.53	37.76±23.05	0.353(0.020)	0.955(0.007)	**0.119(0.126)**
VM	40.98±21.62	32.26±13.31	36.51±15.10	44.70±25.44	31.65±8.67	33.23±14.57	35.91±15.95	28.88±12.91	0.557(0.008)	0.431(0.061)	**0.060(0.157)**

* Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. VL: (Vastus lateralis), RF: (Rectus femoris), VM: (Vastus medialis).

Table 3. Frequency spectrum variables of hamstring muscles during the swing phase of walking.

Muscles	ACLR group (Allograft)		ACLR group (SB-HT)		ACLR Group (DB-HT)		Healthy group		Main effect of fatigue p (ηp²)	Main effect of group p (ηp²)	Main effect of fatigue *group p (ηp²)
	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue			
BF	39.74±13.80	39.79±15.81	36.75±17.70	41.79±23.70	37.33±14.90	35.82±13.79	41.46±18.62	38.19±17.91	0.975(0.000)	0.953(0.008)	0.604(0.042)
ST	43.78±17.07	53.81±20.58	52.00±23.30	56.83±24.05	45.86±19.84	46.32±22.03	38.94±9.86	33.05±17.36	0.511(0.010)	0.065(0.153)	0.506(0.052)

Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. BF: (Biceps femoris), ST: (Semitendinosus).

Table 4. Frequency spectrum variables of distal lower-leg muscles during the swing phase of walking.

Muscles	ACLR group (Allograft)		ACLR group (SB-HT)		ACLR Group (DB-HT)		Healthy group		Main effect of fatigue p (ηp²)	Main effect of group p (ηp²)	Main effect of fatigue *group p (ηp²)
	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue			
TA	74.52±26.05	69.43±13.60	70.58±16.95	74.36±15.75	74.09±13.94	67.47±14.96	73.39±20.39	62.38±11.34	0.122(0.055)	0.857 (0.018)	0.294 (0.082)
GC	46.05±23.61	40.21±16.93	46.17±19.95	51.97±25.10	45.67±22.26	37.95±18.77	61.84±30.36	48.43±23.43	0.098(0.062)	0.421(0.063)	0.131(0.122)

Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. TA: (Tibialis anterior), GC: (Gastrocnemius).

Table 6. Frequency spectrum variables of gluteus medius during the swing phase of walking.

Muscles	ACLR group (Allograft)		ACLR group (SB-HT)		ACLR Group (DB-HT)		Healthy group		Main effect of fatigue p (ηp²)	Main effect of group p (ηp²)	Main effect of fatigue *group p (ηp²)
	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue	Before fatigue	After fatigue			
GM	29.74±7.75	36.80±9.46	32.61±15.30	41.98±23.34	33.46±10.25	33.89±14.33	40.65±17.14	44.15±17.18	**0.025(0.111)**	0.437(0.061)	**0.431(0.061)**

Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. GM: (Gluteus medius).

Discussion

This study investigated the effects of an acute fatigue protocol on EMGMF during the swing phase of walking in individuals with ACLR using different graft types, compared with healthy controls. The primary findings indicated that fatigue did not significantly influence the spectral characteristics of most examined muscles. Contrary to the initial expectation of a fatigue-related reduction in EMGMF, the observed responses were largely unchanged across muscles, except for an increase in GM MF, suggesting that fatigue-related

neuromuscular adaptations during walking may not uniformly manifest as spectral compression. In contrast, MF increased significantly in the GM following the fatigue protocol. These results suggest that neuromuscular responses to fatigue during the swing phase of gait are muscle-specific and may reflect compensatory control strategies to maintain locomotor stability [29-30].

Quadriceps Muscles

A central finding of this study was the absence of significant fatigue-related changes in the quadriceps muscles [31, 32]. Although quadriceps weakness and altered activation patterns are commonly reported following ACLR, the functional demands placed on these muscles during the swing phase of gait are relatively limited. During this phase, the quadriceps primarily control knee extension near terminal swing and prepare the limb for initial contact rather than producing substantial joint torque. Consequently, the mechanical and metabolic demands imposed on the quadriceps during swing may be insufficient to induce detectable spectral changes in EMG signals. Physiologically, reductions in EMGMF are typically associated with peripheral fatigue mechanisms such as decreased muscle fiber conduction velocity, accumulation of metabolic byproducts, and altered motor unit firing patterns [30, 33]. However, because the swing phase represents a low-load and relatively short-duration activation period for the quadriceps, these physiological fatigue mechanisms may not reach a magnitude sufficient to shift the EMG frequency spectrum.

Furthermore, walking is a highly automated locomotor task regulated by spinal central pattern generators and refined by supraspinal control. These well-established motor programs tend to maintain stable activation patterns even under moderate fatigue, which may further explain the stability of quadriceps spectral characteristics observed in the present study [34]. Another possible explanation relates to neuromuscular adaptation following ACLR. Over time, individuals who have undergone ACLR often develop compensatory motor control strategies that enhance knee joint stability during daily locomotor activities [35]. These adaptations may involve more efficient motor unit recruitment patterns or greater reliance on synergistic muscle groups, which could help maintain consistent quadriceps activation even with fatigue.

Hamstring Muscles

The hamstring muscles also did not show significant fatigue-related changes in MF during swing [36, 37]. Functionally, the hamstrings play a crucial role during terminal swing by eccentrically decelerating knee extension and stabilizing the knee joint before heel contact. Despite this important role, hamstring activation during normal walking remains relatively moderate compared with high-demand movements such as sprinting or landing tasks. As a result, the fatigue protocol may not have generated sufficient neuromuscular stress within the hamstrings during the swing phase to produce measurable spectral changes[۱۲]. In addition, load distribution across the posterior kinetic chain may reduce localized fatigue within individual hamstring muscles. Muscles such as the gluteus maximus and other hip extensors help control limb motion, thereby sharing functional responsibilities with the hamstrings. This intermuscular coordination may allow the neuromuscular system to maintain relatively stable activation patterns despite fatigue. Interestingly, the ST muscle showed a group effect approaching statistical significance. Although this effect did not reach statistical significance, it may reflect subtle neuromuscular differences associated with graft harvesting. In many ACLR procedures, the ST tendon is used as an autograft, which can cause long-term morphological changes, including reduced muscle volume, altered architecture, and modified motor unit recruitment patterns [38-40]. These structural adaptations may influence EMG spectral properties during movement. However, the absence of significant results suggests that such changes may not strongly affect muscle activation during low-demand tasks such as walking.

Distal Lower-Leg Muscles

The distal lower-leg muscles (TA and GC) also did not exhibit significant fatigue-related changes in MF during the swing phase [41]. The TA plays a primary role in ankle dorsiflexion to ensure adequate toe clearance, whereas the GC contributes predominantly during the stance phase to generate propulsion. Because the GC is

minimally active during swing, its spectral characteristics would be expected to remain relatively unaffected by fatigue in this phase of gait. Although the TA is active during swing, its activation level during normal walking is typically moderate and highly consistent. The repetitive and cyclical nature of walking may allow this muscle to maintain stable motor unit recruitment patterns even under fatigued conditions [30]. Additionally, locomotor control mechanisms may redistribute mechanical demands across the lower-limb kinetic chain to prevent excessive fatigue accumulation in distal muscles during steady-state gait.

Gluteus Medius

In contrast to the other muscles examined, the GM demonstrated a significant increase in MF following the fatigue protocol [42, 43]. The GM is a key pelvic stabilizer and plays an essential role in maintaining frontal-plane balance during locomotion. Although it peaks during stance, the muscle also contributes to dynamic stabilization and limb positioning during swing, particularly by controlling pelvic alignment and mediolateral stability [44]. The increase in MF after fatigue may reflect a compensatory neuromuscular strategy to preserve pelvic stability when fatigue affects other lower-extremity components. One plausible physiological mechanism is the recruitment of higher-threshold motor units in response to fatigue. As fatigue develops, the central nervous system may increase neural drive to stabilizing muscles, increasing firing rates or recruiting faster motor units, which can shift the EMG power spectrum toward higher frequencies [33]. One plausible physiological mechanism is the recruitment of higher-threshold motor units in response to fatigue. As fatigue develops, the central nervous system may increase neural drive to stabilizing muscles, resulting in increased firing rates or the recruitment of faster motor units, which can shift the EMG power spectrum toward higher frequencies [33]. However, interpret this interpretation with caution, as fatigue-related changes in EMG spectral properties are not always linear. Under certain conditions, changes in muscle fiber conduction velocity and the complex interaction between peripheral and central fatigue mechanisms may also shift the frequency spectrum, including paradoxical increases in median frequency.

Another possible explanation involves proximal compensation mechanisms. Previous studies have suggested that individuals with ACLR often rely more heavily on hip musculature to maintain lower-limb stability and reduce stress on the knee joint [35]. When fatigue challenges the neuromuscular system, this reliance on proximal stabilizers may become even more pronounced. Increased GM activation may therefore serve as a protective strategy to control pelvic motion, maintain step symmetry, and ensure stable limb advancement during swing. The absence of significant group effects for the GM suggests that this compensatory strategy was consistent across all groups, including individuals with different graft types and healthy controls. This finding may indicate that proximal stabilization strategies during fatigued walking are fundamental neuromuscular responses rather than graft-specific adaptations.

۴،۵Limitations.

Several limitations should be considered when interpreting the findings. First, the sample consisted exclusively of male participants. Sex-related differences in neuromuscular control, fatigue response, and gait biomechanics are well documented, particularly in the context of ACL injury and rehabilitation. Therefore, these results cannot be generalized to female populations. Future research should include female participants to investigate potential sex-specific neuromuscular responses to fatigue following ACLR. Second, the fatigue protocol examined only acute effects. Consequently, the results reflect immediate neuromuscular responses rather than long-term adaptations to repeated fatigue exposure. Chronic fatigue or cumulative fatigue across training sessions may influence neuromuscular control differently, and future studies should investigate these longer-term effects. Third, this study relied solely on EMG analysis and did not incorporate knee joint kinematic or kinetic assessment or three-dimensional gait motion capture. Without detailed biomechanical data, it was not possible to determine whether the observed changes in gluteus medius median frequency were associated with alterations in pelvic motion, joint angles, joint moments, or overall gait mechanics. Combining EMG with motion analysis in future studies would provide a more comprehensive understanding of the interaction between neuromuscular activity and biomechanical movement patterns during fatigued gait. Fourth, we did not calculate intra-session reliability of median frequency measurements in the present study. This should be considered when interpreting pre- to post-

fatigue changes, as the reproducibility of spectral measures within the same testing session may influence how much small differences reflect true physiological adaptation rather than measurement variability. Fifth, we did not formally correct for multiple comparisons across the examined muscles and statistical effects. Although the repeated-measures ANOVA framework helps control type I error for the main model effects and interactions, the lack of correction in subsequent pairwise or post-hoc analyses may increase the possibility of false-positive findings. Therefore, interpret the results, particularly isolated significant findings, with appropriate caution.

Limb symmetry index (LSI) between the reconstructed and contralateral limbs was not calculated in the present study. Although LSI is commonly used in ACL research, the contralateral limb cannot always be considered a true healthy reference because bilateral neuromuscular adaptations may occur following unilateral ACL injury. Therefore, the present analysis focused on group-level comparisons and fatigue‐related changes within the reconstructed limb rather than inter‐limb symmetry. Although we observed no significant group × fatigue interaction effects, the smaller sample size in the allograft group may have limited statistical power. A post‐hoc power estimation based on the observed effect sizes indicated moderate statistical power, suggesting that the possibility of type II error for interaction effects cannot be completely excluded.

Clinical Implications

Graft type did not significantly modify fatigue-related EMG responses during the swing phase of walking, suggesting that rehabilitation for gait retraining under fatigued conditions may not need to be differentiated according to graft type .

2) The isolated increase in gluteus medius median frequency should be interpreted with caution and warrants further investigation before specific clinical recommendations can be made.

Conclusion

In summary, this study's findings indicate that an acute fatigue protocol does not significantly alter EMGMF in most lower-extremity muscles during the swing phase of walking in individuals following ACLR. However, the GM demonstrated a significant increase in MF after fatigue, suggesting an adaptive neuromuscular strategy that enhances proximal stabilization to maintain gait control. These results highlight the important role of hip stabilizing muscles in preserving locomotor stability under fatigue and emphasize the potential importance of incorporating targeted hip strengthening and neuromuscular training into rehabilitation programs following ACLR.

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Conflict of Interest Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' Contributions

M. Mousavidizaj and E. Piri: Original Draft, Formal Analysis, Conceptualization. A.A. Jafarnezhadgero: Supervision, Methodology, Investigation, Writing, Review & Editing. M. Dehghani: Methodology. E. Piri: Investigation, Writing, Review & Editing. R. Noktehsanj and M. JabarAli: Supervision, Methodology.

Ethical Statement

This article is a research study, and ethical principles have been observed. The study holds the ethics code from ethics Committee University of Mohaghegh Ardabili (IR.UMA.REC.1404.077). All participants provided written informed consent to participate in this study. We may state that this study was conducted in accordance with the Declaration of Helsinki, 1964, and its subsequent amendments. The informed consent

document clearly states the purpose of the study, procedures, risks, and rights of the participants. The participants are informed that their information will remain confidential and that their participation is voluntary.

Declaration of Generative AI and AI-assisted technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation, writing, or analysis of this manuscript.

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