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Research Article

# The Effect of Fatigue Protocol on EMG Median Frequency during Running in Individuals Following ACL Reconstruction with Different Graft Types

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**Abstract**

**Introduction:** Neuromuscular fatigue can worsen biomechanical deficits after anterior cruciate ligament reconstruction (ACLR), potentially affecting neuromuscular control during running. The electromyographic median frequency (EMGMF) provides an indirect indicator of motor unit recruitment behavior and localized muscle fatigue during dynamic tasks. However, it remains unclear whether fatigue‐induced spectral responses during running differ according to graft type after ACLR. Therefore, this study investigated the effects of a standardized fatigue protocol on EMGMF of lower‐limb muscles during running in individuals with different ACLR graft types.

**Method:** In this cross‐sectional laboratory study, 53 physically active men (18–30 years) participated, including 38 individuals with unilateral ACLR (SB‐HT, n=12; DB‐HT, n=16; allograft, n=10) and 15 healthy controls. Participants performed overground running at a controlled speed (3.0 ± 0.2 m/s) before and immediately after a standardized treadmill fatigue protocol performed until ≥85% age‐predicted maximal heart rate or Borg ≥17. Surface EMG was recorded from eight lower‐limb muscles (TA, GC, VM, VL, RF, BF, ST, GM). Median frequency was calculated for the early stance and late stance phases of running. A two-way mixed-model repeated‐measures ANOVA examined the effects of fatigue (pre vs. post) and group (SB‐HT, DB‐HT, allograft, healthy).

**Result:** During early stance, fatigue significantly increased RFMF ($p=0.023$, $\eta p^2=0.112$). Significant group effects were observed for RF ($p=0.042$, $\eta p^2=0.169$), VM ($p=0.003$, $\eta p^2=0.273$), and GM ($p=0.024$, $\eta p^2=0.190$). The allograft group demonstrated higher RFMF than healthy controls (~24%) and higher VMMF than both healthy (~29%) and DB‐HT (~32%) groups. During late stance, fatigue decreased TAMF ($p=0.038$, $\eta p^2=0.094$) and increased GMMF ($p=0.026$, $\eta p^2=0.108$). We observed a significant group‐by‐fatigue interaction for VM ($p=0.006$, $\eta p^2=0.247$), with only the DB‐HT group showing a post‐fatigue increase in MF ($p=0.008$). No other interaction effects were detected.

**Conclusion :** Fatigue did not produce a uniform decrease in EMGMF during running. Instead, muscle‐ and phase‐specific spectral adaptations were observed. Increased RFMF during early stance and altered TA and GM responses during late stance suggest adaptive motor unit recruitment strategies to maintain running stability under fatigue. Additionally, graft‐specific differences were evident, with the allograft group showing higher quadriceps MF during early stance and the DB‐HT group demonstrating a fatigue‐related increase in VM during propulsion. These findings indicate that neuromuscular responses to fatigue during running after ACLR may depend on graft type, highlighting the importance of task‐specific neuromuscular assessment in rehabilitation and return‐to‐sport decision-making.

**Keywords:** Anterior cruciate ligament, Reconstruction; Fatigue; Running; Graft type.

## Introduction

ACL rupture remains one of the most debilitating musculoskeletal injuries in sports medicine, frequently leading to long-term functional impairment and premature joint degeneration. Annually, over 200,000 ACL injuries occur in the United States, with a significant proportion undergoing ACL reconstruction (ACLR) to restore joint stability[1]. Running is a fundamental component of late-stage rehabilitation and

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a prerequisite for most field-based sports. Nevertheless, individuals post-ACLR often exhibit persistent biomechanical asymmetries and compensatory movement patterns. Evidence suggests that even years after surgery, patients demonstrate significant contralateral limb deficits and altered joint loading strategies during dynamic tasks[2]. These gait abnormalities are not merely clinical markers but are implicated in the high incidence of post-traumatic osteoarthritis, which affects nearly half of the ACLR population within a decade of surgery.

Neuromuscular fatigue is a critical confounding factor that exacerbates these biomechanical deficits. In healthy athletes, fatigue significantly alters knee kinematics and neuromuscular control, particularly during high-demand landing tasks[3]. During running, fatigue induces a cascade of changes in lower limb mechanics, including altered ground reaction forces and modified joint moments, which may compromise joint protection mechanisms[4]. For the ACLR population, the interaction between fatigue and pre-existing neuromuscular deficits poses a substantial risk for secondary injury, yet the underlying physiological mechanisms remain inadequately understood.

Surface electromyography (EMG) serves as a non-invasive window into these neuromuscular strategies[5]. While many studies focus on EMG amplitude, the analysis of the power spectrum, specifically the median frequency (MF), provides a more direct measure of localized muscle fatigue. As a muscle fatigues, the conduction velocity of muscle fibers decreases, and motor unit recruitment patterns shift, causing the EMG power spectrum to migrate toward lower frequencies[6]. This spectral shift is a hallmark of neuromuscular fatigue, yet its behavior during the repetitive, dynamic task of running in ACLR patients has not been extensively mapped.

The persistent weakness of the quadriceps, often termed "arthrogenic muscle inhibition," is a primary hurdle in ACLR recovery[7]. This inhibition, combined with altered co-activation of the hamstrings, likely influences how the neuromuscular system responds to fatigue. Furthermore, the choice of surgical graft may modulate these responses. Meta-analytical data comparing bone–patellar tendon–bone (BPTB) and hamstring tendon autografts indicate that while both are effective, they result in distinct donor-site morbidities and muscle-specific strength deficits[8]. Similarly, the debate between single-bundle and double-bundle reconstruction continues, with evidence suggesting that while double-bundle may offer superior rotational stability, the functional outcomes during dynamic movement are often comparable[9].

Despite the wealth of data on surgical techniques, a significant research gap exists regarding how different graft types and reconstruction methods interact with neuromuscular fatigue during running. Most fatigue studies focus on landing or cutting in healthy cohorts, leaving a void in our understanding of how the "reconstructed" neuromuscular system manages spectral shifts and fatigue-induced failure during prolonged running. Moreover, prior investigations rarely controlled for running speed or accounted for graft‐type heterogeneity, and few standardized the fatigue induction method, which may have obscured condition‐specific neuromuscular responses. Understanding whether specific grafts predispose athletes to faster neuromuscular fatigue as evidenced by EMGMF could be pivotal for tailoring individualized rehabilitation protocols.

Therefore, the purpose of this study was to investigate the effect of a standardized fatigue protocol on the EMGMF of lower-limb muscles during running in individuals following ACLR with different graft types. This protocol consisted of a continuous treadmill running task at a fixed speed (70% of maximal aerobic speed) until volitional exhaustion, distinct from general whole‐body fatigue or intermittent landing protocols used in prior studies. We hypothesized that:[1]fatigue would cause a significant downward shift in EMGMF across all groups, and[2] the magnitude of this spectral shift would differ significantly based on the graft type, reflecting unique neuromuscular adaptations to the surgical intervention.

## Methods

### Participants

This laboratory study used a cross‐sectional design to examine the effect of a standardized fatigue protocol on the frequency characteristics of surface EMG during

constant‐speed running in individuals who had undergone ACLR with different graft types. A total of 53 physically active males aged 18–30 years volunteered for the study. Only male participants were included to avoid confounding effects of hormonal fluctuations on neuromuscular fatigue responses. Future studies should examine female-specific responses. The ACLR group consisted of 38 participants with a history of unilateral ACLR. Based on graft type, they were categorized into three subgroups: SB‐HT, DB‐HT, and allograft reconstruction. Fifteen healthy males with no history of lower limb injury or surgery served as a control group and were matched to the ACLR participants for age and activity level. Current activity level was assessed using the Tegner Activity Scale (0= severe disability, 10= elite competitive sports) (10). Limb dominance was determined by the preferred kicking leg and confirmed using a single‐leg hop test for maximal distance[10]. Table 1 presents the distribution of dominant limbs across groups. Statistical comparisons confirmed no significant differences between groups in demographic variables, anthropometric characteristics, or activity level ($p > 0.05$), indicating baseline comparability. Inclusion criteria for ACLR participants were: (i) primary unilateral ACLR using SB‐HT, DB‐HT, or allograft techniques; (ii) 6–12 months post‐surgery at the time of testing; (iii) completion of a structured rehabilitation program supervised by a physical therapist; and (iv) absence of additional ligament injuries or meniscal resection greater than 50%. The time interval between ACL injury and surgery was recorded for all participants, with no significant between‐group differences ($p > 0.05$). Surgical records confirmed that all allografts consisted of tibialis anterior or tibialis posterior tendons. Exclusion criteria included a history of contralateral ACL injury, concomitant grade III ligament injury, meniscal repair requiring restricted weight‐bearing, neuromuscular disorders, or current lower limb pain. EMG signals were recorded from the reconstructed limb in ACLR participants and from the dominant limb in the healthy control group.

Surgical Procedure Characteristics

Experienced orthopedic surgeons performed all ACLRs arthroscopically using standardized graft‐specific techniques. In the SB‐HT group, a quadrupled semitendinosus autograft was used. In the DB‐HT group, we harvested both the gracilis and semitendinosus tendons to create a double‐bundle graft designed to replicate native ACL anatomy. Participants in the allograft group received donor tendon tissue (tibialis anterior or tibialis posterior). Postoperative imaging confirmed femoral tunnel placement to ensure appropriate anatomical positioning. All ACLR procedures were performed using standard fixation techniques, including interference screw fixation and suspensory fixation devices according to the graft type and surgeon's preference. Postoperative imaging confirmed appropriate femoral and tibial tunnel placement in all reconstructed knees[11], minimizing the possibility that surgical alignment differences influenced neuromuscular outcomes. All procedures followed the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the University of Mohaghegh Ardabili, Iran (IR.UMA.REC.1404.078). All participants received a full explanation of the study and provided written informed consent before participation.

Fatigue protocol

To induce systemic cardiovascular fatigue before EMG measurements, a progressive treadmill running protocol was used. Participants ran on a motorized treadmill (Horizon Fitness Omega GT) wearing their usual athletic shoes, with continuous heart rate monitoring (Polar RS100, Polar Electro Oy, Woodbury, NY). Starting speed was 6 km/h and increased by 1 km/h every two minutes. At the end of each stage, perceived exertion was rated using the Borg 6–20 scale[12]. Speed increments stopped once a participant reported a Borg score of ≥13; the treadmill speed was then held constant to create a steady‐state running phase. During this phase, heart rate and perceived exertion were recorded every 30 seconds. We estimated maximal heart rate as $220 - \text{age}$, following standard exercise testing guidelines[13]. Every minute, participants rated subjective lower‐limb fatigue and knee awareness on a 0–10 numerical scale to capture perceptions of muscle fatigue or joint discomfort related to load‐induced neuromuscular changes[14]. Fatigue was defined as the point at which participants either reported a Borg rating ≥17 for two consecutive minutes or reached ≥85% of their age-predicted maximum heart rate.

This endpoint ensured a fatigue level sufficient to produce detectable changes in EMGMF of lower‐limb muscles during subsequent running[14-15]. Such treadmill‐based fatigue protocols are widely used to simulate the functional neuromuscular challenges faced by ACLR patients, enabling assessment of fatigue‐related shifts in EMGMF that reflect altered muscle fiber recruitment and conduction velocity during gait[15-16].

Time to fatigue (total running duration), final running speed, and total distance covered were recorded. We

observed no significant between-group differences in time to fatigue ($p > 0.05$). Immediately after termination of the treadmill protocol, participants were quickly transferred to the overground runway, and post‑fatigue data collection began within approximately 1–2 minutes to minimize recovery effects.

Assessment of lower limb electromyography activity and spectral analysis

Surface EMG signals were recorded from eight muscles of the dominant 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 chose this muscle set because of its relevance to knee stability and post‑ACLR neuromuscular control[17]. A wireless EMG system (Biometrics Ltd., UK) with bipolar Ag/AgCl electrodes (25 mm inter‑electrode distance) was used. Skin preparation followed SENIAM recommendations[18]: shaving, mild abrasion, and cleaning with 70% ethanol to achieve low impedance.

Signals were sampled at 1000 Hz, band‑pass filtered (10–500 Hz) to remove movement artifacts, and additionally notch‑filtered at 50 Hz to eliminate power‑line interference[19]. To analyze fatigue-related changes, we analyzed EMG data in the frequency domain, as these parameters are sensitive to alterations in motor unit recruitment and neuromuscular control[5]. The gait cycle was divided into two stance phases: early stance phase and late stance phase, based on foot contact events detected using in‑shoe footswitch sensors (Biometrics Ltd., UK)[20].

For each phase, the processed EMG signals were segmented using a fixed window length (250 ms) with 50% overlap. Frequency-domain features were extracted using Fast Fourier Transform to generate the power spectral density. The primary outcome measure was MF, calculated as a robust indicator of the fatigue-induced shift in the power spectrum and reflecting the slowing of muscle fiber conduction velocity. Median frequency was calculated separately for the early stance (initial contact to mid-stance) and late stance (mid-stance to toe-off) phases. These two stance sub-phases were selected to distinguish weight acceptance from propulsion demands while maintaining adequate signal stability for spectral analysis. For each participant, MF values were averaged across the 10 analyzed gait cycles within each phase and condition. Unlike amplitude-based measures, these spectral variables were analyzed using raw values, as normalization to maximal voluntary isometric contraction (MVIC) is not required for frequency-based fatigue indices[21].

All biomechanical testing was performed in a climate‑controlled laboratory, with ambient temperature at 23–24°C and relative humidity at 45-50%. For each condition (pre‑fatigue and post‑fatigue), we recorded and averaged 10 consecutive successful gait cycles for analysis. A gait cycle was excluded if the running speed deviated by more than 0.2 m/s from the target speed (3.0 m/s) or if visible gait alterations occurred (e.g., stumbling or hesitation during foot contact). A trial was considered successful if the participant maintained the required speed range without visible gait alteration. Prior to data collection, participants completed a 5‑minute familiarization period consisting of light treadmill running at a self‑selected pace, followed by overground running practice at the target experimental speed to ensure consistent running mechanics and task familiarity. Participants ran along an 18‑m runway at a controlled speed of 3.0 ± 0.2 m/s, which was selected based on prior running biomechanics studies in physically active and post‑ACL-R populations. Running velocity was continuously monitored, and a metronome provided auditory cues to assist participants in maintaining the prescribed pace.

Statistical analyses

Normality was checked using the Shapiro–Wilk test. Sphericity was assessed with Mauchly's test, and Greenhouse–Geisser corrections were applied when needed. A two‑way mixed‑model repeated‑measures ANOVA was conducted, with fatigue condition (non‑fatigued vs. fatigued) as the within‑subjects factor and graft type (SB HT, DB HT, allograft, healthy) as the between‑subjects factor. Owing to unequal group sizes, a sensitivity power analysis (G*Power) confirmed adequate power (≥0.80) to detect medium‑to‑large effect sizes. Post‑hoc comparisons were Bonferroni‑adjusted. Effect sizes are reported as partial eta squared ($\eta^2p$) and interpreted using conventional thresholds[22]. Intra-class correlation coefficients (ICC) for EMG-MF reliability were calculated from repeated trials of 10 randomly selected participants. ICC values ranged from 0.82 to 0.94, indicating good to excellent reliability. Statistical significance was set at $p<0.05$.

## Results

Early stance phase

During the early stance phase, fatigue had a significant main effect on RF frequency ($p = 0.023$, $\eta p^2 = 0.112$), with higher values after fatigue than before. Pairwise comparisons showed higher RF MF post‑fatigue than pre‑fatigue ($p = 0.023$, $d = 0.67$). Significant main effects of group were observed for RF ($p = 0.042$, $\eta p^2 = 0.169$), VM ($p = 0.003$, $\eta p^2 = 0.273$), and GM ($p = 0.024$, $\eta p^2 = 0.19$). Pairwise comparisons revealed that the ACLR (allograft) group had significantly greater RF frequency than the healthy group ($p = 0.039$, $d = 0.80$, ~24%). Additionally, the ACLR (allograft) group showed significantly higher VM frequency compared with the healthy group ($p = 0.010$, $d = 0.96$, ~29%) and the DB‑HT group ($p = 0.003$, $d = 1.09$, ~32%). Pairwise comparisons also revealed that the ACLR (allograft) group had significantly greater GM frequency than the DB‑HT group ($p = 0.024$, $d = 0.87$, ~32%). No significant group‑by‑fatigue interactions were found for any muscles during the early stance phase ($p > 0.05$, Table 2).

Late stance phase

During the late stance phase, fatigue had significant main effects for TA ($p = 0.038$, $\eta p^2 = 0.094$) and GM ($p = 0.026$, $\eta p^2 = 0.108$). Specifically, TA frequency decreased, whereas GM frequency increased after the fatigue protocol. No significant main effects of group were observed for any muscles during the late stance phase of running. A significant group‑by‑fatigue interaction was found for the VM muscle during the late stance phase ($p = 0.006$, $\eta p^2 = 0.247$). Post‑hoc analysis demonstrated higher VM frequency in the DB‑HT group (but not in other groups) post‑fatigue compared with pre‑fatigue ($p = 0.008$, Table 3). Post‑hoc achieved power for the non‑significant comparisons (e.g., BF, ST, and VL across most stance phases) ranged from 0.31 to 0.58, suggesting a possible Type II error when detecting small effect sizes.

**Table 1**. Demographic and anthropometric characteristics of participants

| Characteristics | SB-HT | DB-HT | Allograft | Healthy group | p-value |
|---|---|---|---|---|---|
| **Age (years)** | 25.9 ±0.1 | 25.8 ± 0.2 | 26.1 ± 0.2 | 26.7 ± 0.1 | 1.000 |
| **Height (cm)** | 181.8 ± 1.2 | 180.2 ± 0.2 | 180.4 ± 0.2 | 181.1 ± 1.1 | 0.425 |
| **Weight (kg)** | 79.1 ± 3.1 | 81.1 ± 2.3 | 80.2 ± 2.0 | 80.2 ± 2.5 | 0.215 |
| **Body mass index (kg/m²)** | 22.10 ± 2.1 | 22.19 ± 1.2 | 24.22 ± 2.10 | 23.01 ± 3.12 | 0.256 |
| **Time post-ACLR surgery (months)** | 12.1 ± 0.6 | 11.6 ± 2.1 | 12.2 ± 2.4 | - | 0.256 |
| **Tegner activity score** | 6.5 ± 0.5 | 6.6 ± 0.5 | 6.2 ± 0.2 | 6.3 ± 0.5 | 0.126 |
| **Weekly walking/running (km)** | 7.7 ± 1.2 | 7.2 ± 0.1 | 7.5 ± 0.4 | 7.9 ± 2.0 | 0.310 |
| Dominant limb | 16 right foot | 12 right foot | 10 right foot | 15 right foot | |
| SB-HT: single-bundle hamstring tendon reconstruction; DB-HT: double-bundle hamstring tendon reconstruction. Values are presented as mean ± standard deviation unless otherwise indicated. p-values represent between-group comparisons. | | | | | |

**Table 2**. Mean and standard deviation of frequency spectrum variables of lower limb muscle electrical activity during the early stance phase of running.

| MUSCLE | Healthy group | | ACLR Group (Allograft) | | ACLR Group (SB-HT) | | ACLR Group (DB-HT) | | Main effect of fatigue p ($\eta p^2$) | Main effect of group p ($\eta p^2$) | Main effect of fatigue *group p ($\eta p^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | | | |
| TA | 97.66±24.66 | 98.62±17.08 | 117.98±36.58 | 125.15±25.82 | 115.97±30.29 | 120.20±36.79 | 108.40±24.75 | 106.11±25.04 | 0.445(0.013) | 0.163(0.109) | **0.775(0.025)** |
| GC | 105.35±21.16 | 92.32±21.04 | 121.24±19.04 | 118.21±15.52 | 111.5±25.73 | 113.44±17.93 | 114.15±22.77 | 156.91±128.24 | 0.519(0.009) | 0.658(0.035) | **0.328(0.075)** |
| VL | 82.16±20.98 | 91.63±16.37 | 92.31±30.17 | 95.20±14.60 | 82.69±21.65 | 95.37±25.59 | 86.91±18.75 | 80.95±11.43 | 0.173(0.042) | 0.626(0.039) | **0.201(0.099)** |
| RF | 75.76±14.58 | 80.85±13.07 | 90.43±21.43 | 104.17±13.09 | 87.48±22.01 | 86.58±24.42 | 75.85±12.49 | 86.59±16.68 | **0.023(0.112)** | **0.042(0.169)** | **0.309(0.078)** |
| VM | 85.70±20.17 | 80.14±14.17 | 109.24±24.69 | 104.75±18.67 | 86.51±25.05 | 93.10±23.70 | 79.65±14.92 | 81.38±17.62 | 0.912(0.000) | **0.003(0.273)** | **0.619(0.039)** |
| BF | 92.33±22.55 | 86.65±18.77 | 104.17±18.73 | 106.04±26.22 | 99.61±12.75 | 103.85±17.96 | 92.66±18.06 | 100.91±18.60 | 0.550(0.008) | 0.084(0.139) | **0.581(0.043)** |
| ST | 101.33±16.78 | 91.63±17.08 | 113.58±27.52 | 109.30±23.29 | 105.63±24.78 | 103.44±19.43 | 104.93±20.55 | 100.48±22.28 | 0.168(0.043) | 0.316(0.076) | **0.895(0.014)** |
| GM | 87.37±14.97 | 81.08±24.12 | 107.21±19.65 | 104.90±23.42 | 82.04±21.01 | 90.41±30.99 | 74.01±14.98 | 86.04±20.52 | 0.365(0.019) | **0.024(0.19)** | **0.157(0.110)** |

*: Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. TA: (Tibialis anterior), GC: (Gastrocnemius), VL: (Vastus lateralis), RF: (Rectus femoris), VM: (Vastus medialis), BF: (Biceps femoris), ST: (Semitendinosus), GM: (Gluteus medius). Large standard deviations in some variables (e.g., GC) indicate high inter individual variability in EMG median frequency responses.

**Table 3**. Mean and standard deviation of frequency spectrum variables of lower limb muscle electrical activity during the late stance phase of running.

| MUSCLE | Healthy group | | ACLR Group (Allograft) | | ACLR Group (SB-HT) | | ACLR Group (DB-HT) | | Main effect of fatigue p ($\eta p^2$) | Main effect of group p ($\eta p^2$) | Main effect of fatigue *group p ($\eta p^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | *Before fatigue* | *After fatigue* | | | |
| TA | 92.36±21.79 | 86.85±16.20 | 98.81±25.62 | 90.76±24.24 | 91.06±22.38 | 90.29±19.23 | 95.05±17.73 | 85.60±15.12 | **0.038(0.094)** | 0.922(0.011) | 0.641(0.037) |
| GAS | 101.71±25.80 | 103.60±26.36 | 94.98±26.50 | 93.82±29.94 | 95.23±15.62 | 104.21±22.87 | 105.84±21.50 | 98.58±19.45 | 0.866(0.001) | 0.781(0.024) | **0.380(0.067)** |
| VL | 67.15±15.26 | 73.39±14.65 | 70.82±20.13 | 67.82±13.12 | 60.00±12.15 | 65.30±14.25 | 64.34±13.60 | 69.22±9.08 | 0.123(0.053) | 0.371(0.068) | **0.475(0.055)** |
| RF | 63.33±11.57 | 70.82±13.31 | 68.72±12.58 | 67.56±12.37 | 64.86±13.62 | 63.47±8.73 | 62.44±15.70 | 65.35±11.18 | 0.198(0.037) | 0.718(0.030) | 0.610(0.040) |
| VM | 65.24±8.88 | 61.08±13.44 | 73.55±17.46 | 68.19±16.37 | 64.89±12.20 | 67.33±12.08 | 55.28±9.24 | 73.77±21.09 | 0.256(0.029) | 0.471(0.055) | **0.006(0.247)** |
| BF | 80.76±23.39 | 88.52±8.36 | 74.65±19.40 | 77.62±14.22 | 82.41±19.19 | 83.40±16.21 | 75.08±15.53 | 86.46±20.06 | 0.094(0.063) | 0.528(0.049) | **0.654(0.036)** |
| ST | 70.25±20.79 | 79.39±20.66 | 76.18±20.32 | 81.49±19.72 | 83.64±15.55 | 73.97±10.67 | 82.85±21.92 | 80.23±16.87 | 0.869(0.001) | 0.738(0.028) | **0.147(0.114)** |
| GM | 58.97±15.55 | 68.69±20.17 | 70.75±15.96 | 67.27±15.14 | 53.35±11.87 | 59.37±11.83 | 55.07±20.19 | 71.45±23.74 | **0.026(0.108)** | 0.144(0.115) | **0.203(0.098)** |

*: Significance level: P<0.05; η²: partial eta squared. Statistical test: Two-way ANOVA with repeated measures. TA: (Tibialis anterior), GC: (Gastrocnemius), VL: (Vastus lateralis), RF: (Rectus femoris), VM: (Vastus medialis), BF: (Biceps femoris), ST: (Semitendinosus), GM: (Gluteus medius). Large standard deviations in some variables indicate high inter individual variability in EMG median frequency responses.

## Discussion

This study investigated the effect of a standardized fatigue protocol on the EMGMF of lower‐limb muscles during running in individuals following ACLR with different graft types. Overall, the findings indicated that fatigue induced selective alterations in muscle frequency characteristics rather than a uniform decrease across all muscles. Specifically, RF frequency increased with fatigue during the early stance phase, while TA frequency decreased and GM frequency increased during the late stance phase. Group differences were observed primarily in the early stance phase, where the allograft group showed higher RF and VM frequencies than the healthy and DB‐HT groups; a significant group‐by‐fatigue interaction was also detected for VM during the late stance phase, with the DB‐HT group showing an increase in frequency after fatigue.

Fatigue‐related spectral responses during running

Contrary to the classical expectation of a fatigue‐induced shift of the EMG power spectrum toward lower frequencies, which is commonly interpreted as a reduction in muscle fiber conduction velocity and altered motor unit recruitment [23, 24], the present results did not show a consistent decline in MF across muscles during running. Instead, some muscles demonstrated increases in MF following fatigue. Several physiological mechanisms may explain this observation. First, locomotor tasks such as walking and running are strongly regulated by central pattern generators and supraspinal control mechanisms that maintain rhythmic muscle activation even under conditions of systemic fatigue [25, 26]. As a result, the neuromuscular system may compensate for fatigue by modifying recruitment strategies rather than simply reducing conduction velocity. In dynamic tasks like running, fatigue can lead to greater recruitment of higher‐threshold motor units to maintain required force production and limb stiffness, which may shift the EMG spectrum toward higher rather than lower frequencies[23]. This mechanism may explain the observed increase in RFMF during early stance, a phase characterized by rapid knee flexion and high eccentric demands on the quadriceps to control impact forces. Individuals following ACLR often develop persistent neuromuscular adaptations, including increased muscle co‐contraction and modified motor unit recruitment patterns[27-28]. These adaptations are thought to enhance joint stability with altered proprioception or residual mechanical laxity. Consequently, the neuromuscular system of ACLR individuals may respond to fatigue differently from healthy individuals, relying on altered recruitment strategies rather than exhibiting a simple fatigue‐induced spectral shift. Similar compensatory activation patterns have been described in ACLR populations during dynamic tasks, reflecting attempts to maintain knee stability during demanding movements[29]. Methodological characteristics of EMG spectral analysis during dynamic gait should also be considered. Median frequency measured within short gait‐phase windows can be influenced by muscle length changes, contraction velocity, and the non‐stationary nature of EMG signals during locomotion[23]. These factors may reduce MF sensitivity to detect classical fatigue patterns compared with sustained isometric contractions. Therefore, the heterogeneous MF responses observed in the present study likely reflect a combination of physiological adaptations and task‐specific measurement properties.

4.2. Neuromuscular differences between graft types

Another important finding was graft‐specific neuromuscular differences, particularly during early stance. Participants in the allograft group exhibited significantly higher MF in the RF and VM compared with healthy individuals and the DB‐HT group. Elevated MF during dynamic tasks has been associated with recruitment of motor units with higher conduction velocities or increased firing rates. However, MF alone cannot directly determine muscle fiber type composition or neural drive[30]. Nevertheless, higher MF values may indicate a neuromuscular strategy characterized by increased activation of higher‐threshold motor units to enhance dynamic joint stiffness. In the context of ACLR, such a strategy may serve as a protective mechanism to compensate for residual deficits in proprioception or mechanical stability[29]. Enhanced quadriceps activation during early stance may help control knee flexion and absorb impact forces during running. However, reliance on higher‐threshold motor units may also increase metabolic cost and potentially reduce movement efficiency during prolonged activity.

Graft type may further influence neuromuscular control due to donor‐site morbidity and graft incorporation

characteristics. In hamstring autograft procedures, harvesting of the ST and gracilis tendons can lead to persistent weakness and altered hamstring activation patterns[31-32]. Previous studies have shown compensatory activation of synergistic muscles, such as the BF, following hamstring tendon harvest[33]. Although the present study did not observe large hamstringMF differences between groups during all phases, the graft‐related variations in quadriceps activity may reflect broader neuromuscular adaptations associated with surgical technique and rehabilitation history. In contrast, allografts avoid donor-site morbidity but may show slower biological incorporation and ligamentization than autografts[34]. The higher quadriceps MF observed in the allograft group during early stance may therefore represent a compensatory strategy to enhance dynamic knee stability during impact loading. Such adaptations may reflect subtle neuromuscular adjustments that persist even after rehabilitation is complete.

.4.3Phase‐specific neuromuscular behavior during running

The phase‐specific nature of the findings is also consistent with the biomechanics of running. Early stance represents the weight acceptance phase, during which the lower limb must rapidly attenuate ground reaction forces and stabilize the knee joint. This phase places high demands on the quadriceps and hip stabilizers, making it particularly sensitive to neuromuscular alterations following ACLR. In contrast, late stance primarily involves propulsion and coordinated activation of the plantar flexors and posterior chain muscles[35]. The observed decrease in TAMF during late stance following fatigue may reflect reduced dorsiflexor contribution as the neuromuscular system prioritizes propulsion and energy transfer. Conversely, the increase in GMMF during late stance suggests greater recruitment of hip abductors to maintain frontal plane pelvic stability during push‐off. The GM plays a crucial role in stabilizing the pelvis and controlling femoral alignment during running, and altered activation of this muscle has been linked to compensatory gait patterns and increased contralateral loading[36-37]. The increase in GMMF after fatigue may therefore indicate greater reliance on hip stabilizers to maintain running mechanics as lower‐limb muscles fatigue.

Fatigue interaction in the vastus medialis

A notable finding of the present study was the group‐by‐fatigue interaction observed for the VM during late stance, where only the DB‐HT group demonstrated a significant increase in MF after fatigue. The VM plays a key role in mediating knee extension torque and maintaining patellofemoral alignment. An increase in MF following fatigue may indicate recruitment of higher‐threshold motor units to sustain knee extensor function during the propulsion phase of running. One possible explanation relates to the specific characteristics of the DB‐HT reconstruction technique. Because this procedure involves harvesting both the ST and gracilis tendons, neuromuscular adaptations may occur in synergistic muscle groups to compensate for hamstring deficits[31-32]. Increased reliance on quadriceps activation during fatigue may therefore reflect a compensatory strategy to maintain knee stability and propulsion during running. Similar compensatory recruitment patterns have been reported in ACLR populations performing high‐demand tasks[38]. However, persistent reliance on such strategies may alter joint loading patterns and potentially contribute to long‐term degenerative changes if not addressed through targeted rehabilitation .

Clinical Implications

From a clinical perspective, the findings suggest that fatigue influences neuromuscular control during running in a muscle‐ and graft‐specific manner rather than producing a uniform fatigue response. Elevated MF values in certain muscles should not necessarily be interpreted as improved performance; instead, they may represent compensatory recruitment of higher‐threshold motor units aimed at stabilizing the knee joint. Rehabilitation programs following ACLR may therefore benefit from emphasizing neuromuscular coordination, endurance, and hip stabilization, rather than focusing solely on maximal strength recovery. Training approaches that challenge neuromuscular control under fatigue conditions such as prolonged running drills, perturbation training, or hip abductor endurance exercises may help restore more physiologic recruitment patterns[39]. Overall, these findings highlight that neuromuscular adaptations after ACLR persist during functional tasks such as running and are influenced by both fatigue and graft type. Understanding these adaptations may provide valuable insight for optimizing rehabilitation strategies and improving safe return‐to‐sport decision making in

athletes following ACLR.

Limitations

Several limitations should be considered when interpreting this study's findings. First, due to the cross-sectional design, causal relationships between graft type and the observed changes in EMGMF cannot be firmly established. Future longitudinal investigations tracking neuromuscular spectral parameters throughout rehabilitation and return-to-sport phases are warranted to clarify adaptive progression over time. Second, the treadmill protocol primarily induced systemic cardiovascular fatigue rather than localized muscular fatigue of the knee and hip stabilizers. Consequently, the absence of fatigue-related MF shifts may reflect insufficient peripheral fatigue in key muscles (e.g., VL, BF, ST). Future studies should employ localized fatigue protocols (such as repeated resisted squats or lunges until exhaustion) to represent task-specific neuromuscular fatigue better. Third, kinematic data (joint angles and movement patterns) were not collected simultaneously with EMG recordings, preventing integrated biomechanical analysis of motor control alterations. Combining synchronized EMG and motion capture (3D gait analysis) would provide more comprehensive insight into compensatory mechanisms and movement adaptations following ACLR .

The treadmill protocol primarily induced systemic cardiovascular fatigue rather than localized muscular fatigue of the knee and hip stabilizers. This may explain the absence of expected decreases in MF in several muscles. Future studies should employ localized fatigue protocols (e.g., repeated squatting to failure) to capture peripheral muscle fatigue better.

Clinical Implications

The findings suggest that fatigue during running produces muscle‐ and phase‐specific neuromuscular responses rather than a uniform reduction in muscle activation. Increases in EMGMF in muscles such as the RF and GM may reflect compensatory recruitment strategies to maintain knee stability as fatigue increases. The results also indicate that graft type may influence neuromuscular behavior after ACLR. Therefore, rehabilitation programs may benefit from being tailored to graft type, with particular attention to restoring quadriceps–hamstring coordination and improving fatigue resistance of key stabilizing muscles. Finally, the increased activity of the GM highlights the importance of hip stabilizers in maintaining pelvic and lower‐limb control during fatigued running. Incorporating hip abductor endurance training and assessing neuromuscular performance under fatigued conditions may improve return‐to‐sport decision-making.

## Conclusion

This study investigated the effects of fatigue on EMGMF of lower limb muscles during running in individuals after ACLR with different graft types. Fatigue did not produce a uniform decrease in MF; instead, muscle and phase specific changes were observed. RFMF increased during early stance after fatigue, whereas TA decreased and GM increased during late stance, suggesting adaptive recruitment strategies to maintain running stability. In addition, graft related differences were identified. The allograft group showed higher RF and VMMF during early stance, while only the DB HT group demonstrated a fatigue related increase in VM during late stance. These findings indicate that neuromuscular control during running after ACLR may vary according to graft type and fatigue state, highlighting the importance of task specific neuromuscular assessment in rehabilitation and return to sport decisions.

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## Conflict of Interest Disclosures

There are no conflicting interests listed by the authors.

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## Authors' Contributions

M. Habibi 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.1404.078). All participants provided written informed consent to participate in this study. 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

None.

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