Title: Neural Muscular Interactions and Inter Limb Asymmetry in Athletes Following Hamstring Graft ACL Reconstruction

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Abstract

Purpose: Persistent neuromuscular deficits following anterior cruciate ligament reconstruction (ACLR) have been linked to alterations in central neural drive and disrupted cortico-muscular communication. This study investigated cortico-muscular interactions and neuromuscular control during a steady force task in athletes following ACLR compared with healthy athletes.

Methods: Twenty-four male athletes participated in this cross-sectional study, including 12 athletes who had undergone unilateral ACL reconstruction using a hamstring tendon autograft and 12 uninjured healthy athletes. Athletes in the ACLR group were tested 10.0 ± 2.0 months post-surgery. Participants performed a standardized steady isometric force task. Cortical activity was recorded using electroencephalography, and surface electromyography was recorded from the semitendinosus muscle. Cortico-muscular coherence (CMC) was calculated in the beta (13–30 Hz) and gamma (30–60 Hz) frequency bands. Force steadiness was quantified using the coefficient of variation of force (ForceCV), and inter-limb asymmetry was assessed using a functional asymmetry ratio.

Results: The ACLR and Healthy groups did not differ in age, height, body mass, or BMI ($p > 0.05$). Force steadiness was significantly reduced in the ACLR group, with higher ForceCV values than in healthy athletes (4.5 ± 0.7% vs. 3.0 ± 0.7%, $p < 0.001$, $d = 2.14$). Cortico-muscular coherence analysis revealed limb-specific alterations in the ACLR group, particularly in the beta band, where coherence tended to be lower in the reconstructed limb compared with the contralateral limb. Healthy athletes demonstrated near-symmetrical coherence between dominant and non-dominant limbs. A similar but smaller pattern was observed in the gamma band. Inter-limb functional asymmetry was significantly greater in the ACLR group (1.30 ± 0.11) than in the Healthy group (1.00 ± 0.11; $p < 0.001$, $d = 2.73$). ForceCV was strongly correlated with the functional asymmetry ratio across all participants ($r = 0.99$, $p < 0.001$).

Conclusion: Athletes following ACL reconstruction exhibit altered cortico-muscular interactions, reduced force steadiness, and greater inter-limb asymmetry during a controlled force task. These findings indicate that persistent neuromuscular deficits after ACLR may involve disrupted neural-muscular coupling between the central nervous system and peripheral musculature. Rehabilitation strategies targeting both neuromuscular control and central neural mechanisms may therefore be important for restoring symmetrical motor function after ACL reconstruction.

Keywords: Anterior Cruciate Ligament Reconstruction, Electroencephalography, Electromyography, Motor Control, Sensory Motor Cortex

Highlights

- Beta band CMC was lower in the ACLR limb than the contralateral limb.
- ACLR athletes showed higher ForceCV during isometric knee flexion.
- Inter limb asymmetry was greater after ACLR than in healthy athletes.
- ForceCV strongly correlated with inter limb asymmetry (r=0.99).

Plain Language Summary

Injury to the anterior cruciate ligament is common in physically active individuals and often requires surgical reconstruction. Although many patients' complete rehabilitation and return to activity, some continue to experience movement problems that are not always visible during routine clinical tests. These ongoing difficulties may be related not only to the muscles and joints but also to changes in how the brain controls movement.

The aim of this study was to examine how the brain and muscles work together during lower limb tasks in individuals who had undergone ACL reconstruction, compared with healthy individuals. Participants performed controlled lower limb tasks while their brain activity and muscle activity were recorded using non invasive techniques. This allowed us to evaluate both brain involvement and muscle control during movement.

The results showed that individuals with ACL reconstruction demonstrated differences in how their brain and muscles interacted during movement. Specifically, communication between the brain and muscles was reduced in the reconstructed limb. In addition, muscle force control was more variable, and clear differences were observed between the operated and non operated limbs. These findings suggest that even after rehabilitation, movement control may remain altered following ACL reconstruction.

These results are important because they indicate that recovery after ACL surgery may involve changes in the nervous system as well as the muscles. Rehabilitation programs that focus only on strength and physical performance may not fully address these underlying control issues. Incorporating training approaches that challenge coordination, balance, and brain muscle interaction may help improve long term outcomes and reduce the risk of future injury.

Introduction

Anterior cruciate ligament reconstruction (ACLR) is routinely performed to restore knee stability and support return to sport after ACL rupture, particularly in athletes exposed to high demand actions such as cutting, pivoting, and rapid deceleration (1, 2). Among graft options, hamstring tendon autografts most commonly involving the semitendinosus (ST), alone or combined with the gracilis remain widely used in clinical practice (3). Nevertheless, a substantial proportion of athletes report ongoing functional limitations after ACLR, and the risk of subsequent injury remains elevated, indicating that successful structural restoration does not always equate to full functional recovery (2, 4). These persistent problems often include altered neuromuscular control and inter limb asymmetries that may remain evident even after completion of formal rehabilitation and return to sport testing (4, 5).

Beyond its mechanical contribution to knee stability, ACL injury and subsequent reconstruction can disrupt sensorimotor function through changes in proprioceptive input and central motor control (6, 7). The ACL contains mechanoreceptors that contribute to afferent signaling and sensorimotor regulation; disruption of these pathways has been linked to altered reflexive activation patterns and impaired movement coordination (7, 8). In parallel, growing evidence suggests that ACL injury is associated with neurophysiological adaptations, including changes in cortical excitability and sensorimotor integration, which may influence motor planning and coordination during athletic tasks (8). Together, these findings support the view that ACLR recovery involves central peripheral adaptations rather than purely peripheral restoration.

This perspective may be especially relevant in hamstring graft ACLR because tendon harvesting directly involves the semitendinosus muscle tendon unit. The ST is not only clinically relevant as a graft source but also functionally important for dynamic knee stabilization as part of the hamstring group. Evidence from imaging and systematic reviews indicates that ST tendon regeneration after harvest is common but variable in quality and completeness, with ongoing remodeling and heterogeneous structural outcomes across individuals (9, 10). Correspondingly, persistent hamstring related strength deficits and asymmetries have been reported after hamstring graft ACLR, potentially affecting movement strategies in athletic tasks (11). In some athletes, compensatory patterns may emerge, including greater reliance on other hamstring components (e.g., biceps femoris) to offset semitendinosus alterations, which may further contribute to inter limb differences in motor control (12).

Within this context, Cortico muscular interactions provide a mechanistic framework for examining how the central nervous system couples with peripheral muscle activity during movement (13, 14). Measures such as Cortico muscular coherence and related coupling metrics have been widely used to characterize functional connectivity between motor cortical activity and muscle activation, and they are sensitive to task demands, movement precision, and neuromuscular integrity (15, 16). Importantly, emerging nonlinear approaches have further demonstrated that Cortico muscular dynamics can reveal between limb differences in motor control in individuals following ACL reconstruction, highlighting the potential value of coupling based analyses for understanding post ACLR asymmetry (17).

A persistent clinical challenge after ACLR is inter limb asymmetry, commonly assessed using strength measures, hop tests, and biomechanical metrics, and consistently associated with risk of subsequent injury and incomplete return to previous performance levels (4, 5). However, limb asymmetry is unlikely to be explained by peripheral factors alone; it may also reflect altered central peripheral coordination and differences in neural strategies used to control the operated limb compared with the contralateral side (8). Despite increasing recognition of these mechanisms, semitendinosus specific investigations that integrate cortical muscle coupling with inter limb asymmetry in athletes following hamstring graft ACLR remain limited.

A multilevel approach is well suited to address this gap because motor behavior arises from dynamic interactions among cortical processes, neural drive to the musculature, and peripheral motor output rather than from isolated subsystems (18). Accordingly, evaluating Cortico semitendinosus interactions alongside inter limb asymmetry may provide a more comprehensive account of how hamstring graft ACLR influences central peripheral coordination and may help connect neurophysiological adaptations to clinically meaningful functional outcomes.

Therefore, the purpose of this study was to provide a multilevel characterization of Cortico semitendinosus interactions and inter limb asymmetry in athletes following hamstring graft ACL reconstruction. It was hypothesized that athletes after hamstring graft ACLR would demonstrate altered Cortico semitendinosus coupling accompanied by persistent inter limb asymmetry, reflecting disrupted coordination between central and peripheral components of the motor system.

Materials and Methods

Study Design

This study adopted a cross sectional, observational design to examine Cortico muscular interactions and inter limb asymmetry in athletes. Consequently, the findings are limited to differences observed at a single time point and do not permit causal interpretation. Importantly, inter limb asymmetry prior to injury was not available, and therefore the present results cannot determine whether the observed asymmetries existed before ACL injury or developed following ACL reconstruction.

Although limb dominance was considered in the Healthy group, the inter limb differences observed in the ACLR group cannot be attributed exclusively to ACL reconstruction and may reflect pre existing or sport specific dominance patterns. These factors were not controlled in the present study and therefore limit interpretation of the observed Cortico muscular coupling patterns, rather than providing evidence of ACLR specific neural adaptations.

All measurements were performed under standardized conditions without experimental manipulation or intervention. Accordingly, the findings should be interpreted strictly as descriptive characteristics of neuromuscular organization in athletes following ACL reconstruction, while recognizing that longitudinal or pre injury study designs are required to clarify the origin of the observed asymmetry and Cortico muscular coupling patterns.

Participants

Athletes with a history of anterior cruciate ligament reconstruction using a hamstring tendon autograft (ACLR group) and healthy athletes with no history of ACL injury (Healthy group) were recruited to participate in this study. ACLR status was confirmed through self reported medical history and verification of surgical details (graft type and date of surgery), and all ACLR participants had completed formal postoperative rehabilitation and returned to regular sports participation at the time of testing. Healthy athletes reported no previous ACL injury or knee surgery and no current lower limb musculoskeletal injury.

All participants were physically active at the time of testing and engaged in regular sports training or competitive activities.

For inclusion in the ACLR group, participants were required to have undergone unilateral anterior cruciate ligament reconstruction using a hamstring tendon autograft (semitendinosus with or without gracilis) and to have completed formal rehabilitation and returned to regular sports participation at the time of data collection. ACLR status was confirmed based on self reported medical history, including confirmation of surgical procedure, graft type, and date of surgery. Testing was conducted at a mean of 10.0 ± 2.0 months post surgery. This post surgical timeframe was selected to ensure completion of formal rehabilitation and return to regular physical activity, while allowing for the presence of potential residual neuromuscular adaptations associated with hamstring tendon harvesting and ACL reconstruction.

Participants were excluded if they reported a history of revision ACL surgery, concomitant ligament injuries requiring surgical intervention, or any additional lower limb surgery affecting either limb.

Healthy participants were included if they had no history of ACL injury, lower extremity surgery, or neurological disorders and were matched to the ACLR group based on general characteristics such as age, sex, and activity level. Exclusion criteria for both groups included any current musculoskeletal injury, pain affecting lower limb function at the time of testing, or known neurological conditions that could influence motor control.

For limb based analyses, the operated limb was defined as the involved limb in the ACLR group, and the contralateral limb was defined as the uninvolved limb. In the Healthy group, the dominant limb was designated as the involved equivalent limb and the non dominant limb as the uninvolved equivalent limb. Limb dominance was determined based on the preferred leg used to kick a ball.

All participants provided written informed consent prior to participation, and the study protocol was approved by the local institutional ethics committee in accordance with the Declaration of Helsinki. Assessors were not blinded to group assignment or limb status during data acquisition due to the visible presence of surgical scars and the requirements of EEG and EMG electrode placement. However, EEG and EMG preprocessing and Cortico muscular coupling analyses were performed using standardized and automated procedures, and datasets were coded to mask group and limb identity during signal processing and analysis.

Experimental Task

A controlled unilateral isometric knee flexion task was used to elicit stable activation of the semitendinosus muscle while enabling reliable assessment of Cortico muscular interactions. The task was intentionally designed to be simple, steady, and highly repeatable in order to minimize movement related artifacts and enhance the reliability of EEG EMG coupling measures.

Participants were seated on an isometric dynamometer with the hip flexed to approximately 90° and the knee fixed at a flexion angle of approximately 30 45°. The tested limb was secured to the dynamometer lever arm to prevent joint movement, ensuring purely isometric force production. The task was performed unilaterally to allow limb specific analysis and to maximize the involvement of the semitendinosus muscle, which plays a key role in knee flexion and dynamic stabilization following hamstring graft ACL reconstruction.

Prior to the experimental trials, maximum voluntary isometric contraction (MVC) was assessed separately for each limb. The target force level for the task was set at a submaximal intensity (20 30% of MVC) to avoid fatigue, excessive force variability, and movement related EEG artifacts. This submaximal level was selected to promote stable motor output and sustained neural muscular coupling rather than maximal force generation.

Each trial followed a trapezoidal force profile consisting of a ramp up phase (2 3 s), a steady plateau phase (6 10 s), and a ramp down phase (2 3 s). Real time visual feedback of force output was provided on a monitor positioned at eye level, allowing participants to match and maintain the target force during the plateau phase. The plateau phase was used for subsequent analyses of Cortico semitendinosus interaction and force steadiness. Multiple trials were performed for each limb, with sufficient rest periods between trials to minimize fatigue effects. The order of limb testing was counterbalanced across participants.

This task configuration was selected to (1) ensure consistent and selective activation of the semitendinosus muscle, (2) provide a stable temporal window suitable for Cortico muscular coupling analysis, and (3) enhance the reliability of inter limb comparisons by minimizing task related variability (Figure 1).

Figure 1. Experimental setup and task protocol. Schematic representation of the unilateral isometric knee flexion task. Participants were seated with the hip flexed at approximately 90° and the knee fixed at a constant flexion angle. The lower limb was secured to an isometric dynamometer, and visual feedback of force output was provided to maintain a steady submaximal contraction. EEG and EMG signals were recorded simultaneously during the plateau phase of each trial.

Signal Preprocessing

EEG and EMG signals were preprocessed using a standardized signal processing pipeline prior to Cortico muscular coupling analyses. EEG recordings were band pass filtered to remove slow baseline drifts and high frequency noise. Lower and upper cutoff frequencies were selected based on the physiological frequency range relevant to Cortico muscular coherence analysis, with the aim of preserving neural oscillations within the beta (13 30 Hz) and gamma (30 60 Hz) bands while attenuating low frequency movement related artifacts and high frequency non neural noise. Notch filtering was applied where necessary to suppress power line interference. EMG signals were high pass filtered to reduce motion artifacts and low frequency baseline fluctuations, followed by low pass filtering to limit high frequency noise and aliasing prior to envelope extraction and coupling analyses. Filter settings for both EEG and EMG were chosen in accordance with established recommendations in EEG EMG coupling and Cortico muscular coherence literature, ensuring that the selected cutoff frequencies did not distort phase relationships or artificially inflate coherence estimates.

Following filtering, EEG data were re referenced using a common average reference and visually inspected to identify segments affected by excessive artifacts, including gross movement, electrode detachment, or high amplitude transients. Artifact contaminated segments were excluded from further analysis, and only epochs corresponding to the steady force plateau phase of the task were retained for subsequent processing.

Surface EMG from the semitendinosus was band pass filtered to remove motion artifacts and high frequency noise and then demeaned to remove DC offsets. EMG quality was verified by confirming a clear task related activation pattern and the absence of signal saturation or discontinuities. When required for coupling estimation, EMG was rectified and its envelope was derived using low pass filtering to provide a stable amplitude representation while preserving physiologically meaningful modulation. All preprocessing steps, parameter settings, and epoch selection criteria were applied identically across participants and limbs to ensure comparability and reproducibility of Cortico semitendinosus interaction estimates.

Cortico Semitendinosus Interaction Analysis

Cortico semitendinosus interactions were characterized using Cortico muscular coherence to quantify the frequency specific functional coupling between cortical activity and semitendinosus muscle activation during the steady force plateau phase of the task. EEG signals from sensorimotor regions were paired with the simultaneously recorded semitendinosus EMG, and coupling was estimated separately for each limb.

For each participant and limb, artifact free plateau epochs were segmented into fixed length windows with overlap to improve the stability of spectral estimates. Auto spectra for EEG and EMG and the corresponding cross spectrum were computed using Welch’s method. Magnitude squared coherence was then calculated as:

$$C_{xy}(f) = \frac{|\, P_{xy}(f)\, |^2}{P_{xx}(f)\, P_{yy}(f)}$$

where $P_{xx}(f)$and $P_{yy}(f)$denote the EEG and EMG power spectral densities, respectively, and $P_{xy}(f)$denotes the cross spectral density between EEG and EMG at frequency f. Coherence values range from 0 to 1, with higher values indicating stronger linear coupling at a given frequency.

Based on established sensorimotor coupling literature and the analytical framework of this study, coherence was summarized within predefined frequency bands relevant to motor control (beta: 13 30 Hz; gamma: 30 60 Hz). For each band, band averaged coherence and peak coherence were extracted as primary outcomes. To ensure that coupling estimates reflected genuine Cortico muscular interactions rather than spurious correlations, coherence was evaluated against a statistical confidence limit derived from the number of independent segments contributing to the estimate, and only values exceeding this limit were considered physiologically meaningful. All analysis steps and parameter settings were applied consistently across participants and limbs.

This approach was selected to provide a mechanistic, descriptive characterization of how sensorimotor cortical oscillations couple with semitendinosus activation and how this coupling differs between limbs and between the ACLR and healthy groups.

Inter Limb Asymmetry Quantification

Inter limb asymmetry was quantified by comparing limb specific neuromuscular interaction outcomes within each participant. In the ACLR group, the surgically reconstructed limb was defined as the *involved* limb and the contralateral limb as the *uninvolved* limb. In the Healthy group, the dominant limb was designated as the involved equivalent limb and the non dominant limb as the uninvolved equivalent limb.

For each Cortico semitendinosus interaction outcome, limb specific values were extracted separately. In addition to Cortico semitendinosus coupling outcomes, force steadiness was assessed using ForceCV during the same isometric knee flexion task. Because force steadiness reflects the precision of neural drive and the regulation of motor output during sustained contraction, ForceCV was treated as a task level functional correlate of neuromuscular control rather than as a balance specific construct. Accordingly, asymmetry was interpreted primarily with respect to Cortico semitendinosus interaction metrics, with ForceCV serving as a secondary functional indicator to contextualize limb specific control.

Inter limb asymmetry for each outcome was calculated using a normalized asymmetry index to minimize the influence of inter individual differences in absolute magnitude and to enable meaningful within subject comparisons. The asymmetry index was computed as:

$$\text{Asymmetry Index (\%)} = \frac{\text{Involved} - \text{Uninvolved}}{0.5 \times (\text{Involved} + \text{Uninvolved})} \times 100$$

As an exploratory analysis, limb difference values were computed to characterize between limb patterns across outcomes. Involved and Uninvolved correspond to the limb specific values for the given outcome (i.e., involved vs. uninvolved in the ACLR group, or involved equivalent vs. uninvolved equivalent in the Healthy group). Positive values indicate greater Cortico semitendinosus coupling (or higher task level values for ForceCV, when applicable) in the involved (or involved equivalent) limb, whereas negative values indicate greater values in the uninvolved limb.

No predefined clinical cut off values or risk based classifications were applied. Instead, asymmetry was treated as a continuous variable reflecting limb specific differences in neural muscular organization. This normalization approach expresses asymmetry relative to the mean bilateral value rather than as an absolute difference, thereby reducing the impact of inter individual variability and facilitating mechanistic interpretation of limb specific control strategies. All asymmetry calculations were performed consistently across participants to ensure comparability between groups.

Statistical Analysis

Statistical analyses were performed to examine differences in Cortico semitendinosus interaction measures and inter limb asymmetry between groups and limbs. Prior to inferential analyses, all variables were assessed for normality using the Shapiro Wilk test. Descriptive statistics are reported as mean ± standard deviation.

Limb based comparisons were conducted using within subject analyses. A mixed design analysis of variance (ANOVA) was used with Group (ACLR, Healthy) as the between subject factor and Limb (involved vs. uninvolved) as the within subject factor. This approach was selected to evaluate group differences, limb differences, and group by limb interactions within a unified statistical framework consistent with the descriptive objectives of the study.

When appropriate, post hoc comparisons were performed using paired sample or independent sample t tests. Given the limited sample size and the hypothesis driven focus on a priori defined frequency bands and outcomes, no formal adjustment for multiple comparisons was applied. Instead, results from secondary and limb difference analyses are explicitly interpreted as exploratory. Effect sizes were calculated to complement p values and to facilitate interpretation of the magnitude of observed effects. Partial eta squared (η^2_p) was reported for ANOVA effects, and Cohen’s d was reported for pairwise comparisons.

Statistical significance was set a priori at $\alpha = 0.05$. All statistical analyses were conducted using standard statistical software. Results are interpreted as descriptive indicators of group and limb related differences rather than as evidence of causal relationships.

Results

Participant Characteristics

A total of 24 athletes participated in the study, including 12 athletes who had undergone unilateral anterior cruciate ligament reconstruction with a hamstring tendon autograft (ACLR group) and 12 uninjured athletes with no history of ACL injury (Healthy group). Descriptive characteristics for each group are provided in Table 1. The two groups were similar in age, height, body mass, and body mass index (BMI), with no significant between group differences on these variables (all p > 0.05). Athletes in the ACLR group were tested at a mean of 10.0 ± 2.0 months post surgery.

Table 1. Participant demographic characteristics (values are mean ± SD, *n* = 12 per group)

Variable	Healthy Group	ACLR Group	*p* value
Age (years)	25.7 ± 3.8	26.9 ± 3.4	0.41
Height (cm)	180.9 ± 6.6	178.3 ± 4.8	0.27
Weight (kg)	78.6 ± 8.1	74.9 ± 8.0	0.28
Body Mass Index (kg/m²)	24.2 ± 3.4	23.6 ± 2.5	0.65
Time since surgery (mo)		10.0 ± 2.0	

Note: Between group comparisons were made with independent t tests (α = 0.05). There were no significant differences between groups in age or anthropometric measures. Time since surgery is only applicable to the ACLR group.

Task Performance and Data Quality

All participants completed the experimental task as instructed, and no testing sessions were terminated prematurely. Force signals were successfully recorded for all trials, and no trials were excluded due to task non compliance.

Force steadiness was quantified using the coefficient of variation of force (ForceCV). Figure 2 illustrates the distribution of ForceCV values during the steady force plateau phase for the Healthy and ACLR groups. Mean ForceCV was 3.0 ± 0.7% (95% CI: 2.56 3.44%) in the Healthy group and 4.5 ± 0.7% (95% CI: 4.06 4.94%) in the ACLR group. The between group difference in ForceCV was statistically significant (p < 0.001).

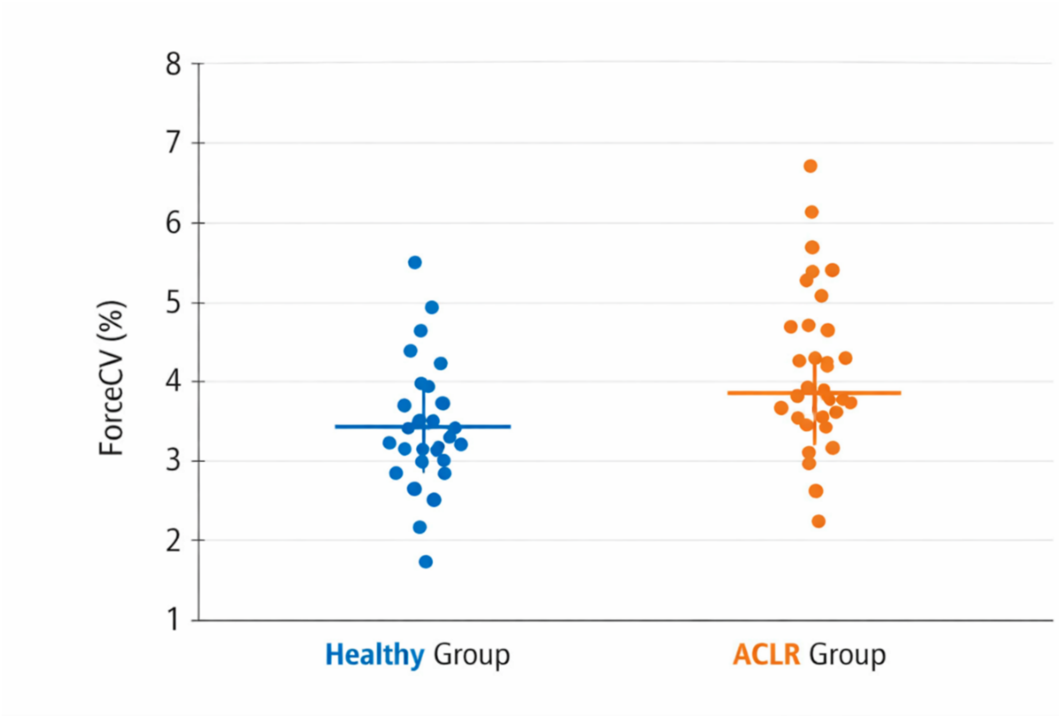

Figure 2. Task performance variability. Distribution of ForceCV during the steady force plateau phase for Healthy and ACLR groups. Each point represents an individual's ForceCV, and group means are indicated. The narrow spread of values in both groups demonstrates consistent task execution and minimal force fluctuations across participants. CMC results are presented descriptively due to exploratory nature and sample size.

Cortico Semitendinosus Interaction (CMC)

Cortico muscular interaction between the brain and the semitendinosus muscle was assessed using Cortico muscular coherence (CMC) in the beta (13 30 Hz) and gamma (30 60 Hz) frequency bands. We compared CMC for each limb, considering the involved vs. uninvolved limb in the ACLR group and, for an equivalent comparison, the dominant vs. non dominant limb in the Healthy group. Distinct limb specific patterns of CMC emerged in the two groups. In the Healthy group, Cortico muscular coherence levels were similar between the dominant and non dominant legs. In contrast, the ACLR group showed noticeable differences between their surgically involved limb and the contralateral (uninvolved) limb. This discrepancy was most pronounced in the beta band coherence: the ACLR athletes tended to have lower beta band CMC in their reconstructed (involved) limb compared to their uninvolved limb, whereas healthy athletes showed nearly symmetrical beta CMC between legs. A similar but smaller trend was observed in the gamma band. These limb specific coherence differences suggest altered neural muscular coupling in the ACLR limb. Figure 3 illustrates these coherence patterns, highlighting that the between limb difference in CMC was greater in the ACLR group than in the Healthy group, particularly for beta frequencies.

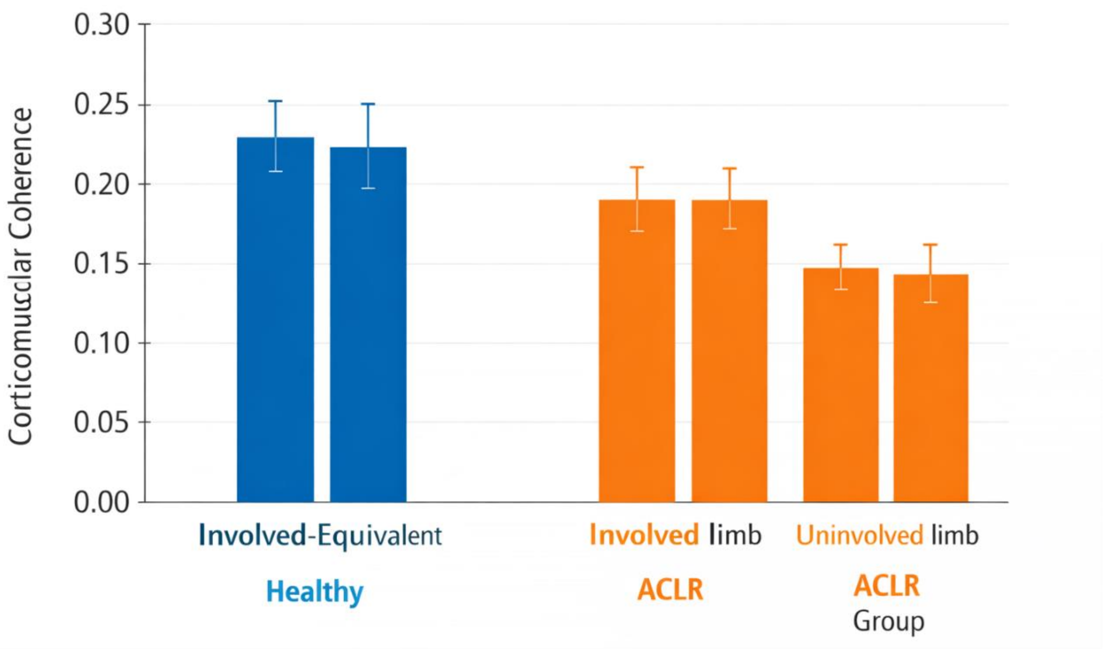

Figure 3. Limb specific Cortico muscular coherence in semitendinosus. Cortico muscular coherence (mean ± SD) in the beta (13 30 Hz) and gamma (30 60 Hz) bands is shown for each leg of Healthy and ACLR groups. For the Healthy group, the dominant limb is labeled as “Involved Equivalent” and the non dominant as “Uninvolved Equivalent” for consistency. Healthy: Coherence was similar between limbs. ACLR: The involved limb exhibited slightly lower coherence than the uninvolved limb, especially in the beta band. The ACLR group demonstrates a larger gap between limbs compared to the virtually symmetric coherence in Healthy athletes.

Group Differences in Force Steadiness

Group differences in force steadiness were evaluated by comparing the ForceCV between the Healthy and ACLR groups. As summarized in Table 2, the ACLR group exhibited significantly greater force output variability during the steady contraction than the Healthy group. The mean ForceCV in the ACLR group was 4.5% ± 0.7%, compared to 3.0% ± 0.7% in the Healthy group. This corresponds to an absolute difference of approximately +1.5 percentage points (ACLR minus Healthy), which was statistically significant ($p < 0.001$). The effect size for this difference was very large (Cohen's d ≈ 2.14), indicating a substantial reduction in force steadiness in the ACLR group relative to healthy controls. In practical terms, athletes with ACL reconstruction were less steady in maintaining the target force, showing more fluctuation in force output. Figure 4 provides a visual comparison of group mean ForceCV values, illustrating the lower steadiness (higher ForceCV) in the ACLR group.

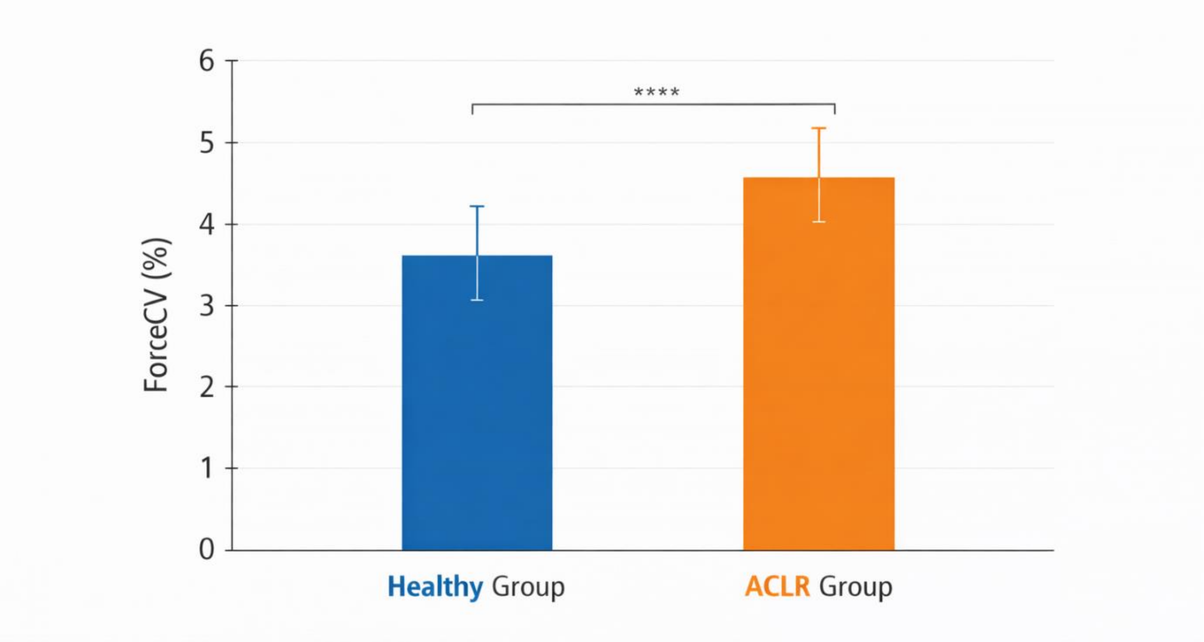

Figure 4. Group differences in force steadiness. Mean (± SD) ForceCV for the Healthy and ACLR groups during the steady force phase. Higher ForceCV indicates lower force steadiness. The ACLR group showed a significantly higher ForceCV than the Healthy group, reflecting reduced steadiness of force output after ACL reconstruction (**$p < 0.001$).

Table 2. Summary of Task Performance, Cortico Muscular Coupling, and Asymmetry Results (Healthy vs ACLR groups)

Domain	Variable (units)	Healthy Group	ACLR Group	Between Group Δ (ACLR Healthy)	*p* value	Effect Size (Cohen's *d*)
Force Steadiness	ForceCV (%) Steady force phase	3.0 ± 0.7	4.5 ± 0.7	+1.5 (higher in ACLR)	< 0.001***	+2.14 (large)
Inter Limb Asymmetry	Functional asymmetry index (au)	1.00 ± 0.11	1.30 ± 0.11	+0.30 (higher in ACLR)	< 0.001***	+2.73 (very large)
Cortico Muscular Coherence †	Beta band CMC (13 30 Hz), involved limb	0.250 ± 0.008	0.220 ± 0.005	0.030 (lower in ACLR)	n/a (obs.)	4.50
	Beta band CMC, uninvolved limb	0.248 ± 0.006	0.220 ± 0.008	0.028 (lower in ACLR)	n/a (obs.)	3.96
	Gamma band CMC (30 60 Hz), involved limb	0.156 ± 0.005	0.133 ± 0.007	0.023 (lower in ACLR)	n/a (obs.)	3.78
	Gamma band CMC, uninvolved limb	0.152 ± 0.006	0.131 ± 0.008	0.021 (lower in ACLR)	n/a (obs.)	2.97
Association (All subj.)	ForceCV (%) vs. Asymmetry index (Pearson *r*)			*r* = 0.99 (positive)	< 0.001***	

Note: *Values are presented as mean ± standard deviation. Δ represents the absolute difference between group means. For ForceCV and the asymmetry index, p values are from independent t tests (α = 0.05). Effect sizes (Cohen's d, pooled SD) are positive when the ACLR group's mean is higher than the Healthy group, and negative when lower. † CMC values are mean coherence magnitudes (no statistical comparison performed; presented for observational context). *p < 0.001. The functional asymmetry index is reported as a secondary functional correlate within the experimental task context and is defined as a higher/lower limb ratio (1.0 = perfect symmetry).

Group Differences in Inter Limb Asymmetry and Associations

Inter limb functional asymmetry was quantified using a functional asymmetry ratio, where 1.0 denotes perfect symmetry between limbs. The ratio was computed as the higher limb value divided by the lower limb value (higher/lower) for the task level performance metric; therefore, values > 1.0 indicate greater between limb imbalance. As shown in Table 2, the Healthy group had an asymmetry ratio of 1.00 ± 0.11, whereas the ACLR group had a significantly higher asymmetry ratio of 1.30 ± 0.11. This between group difference was statistically significant ($p < 0.001$) and associated with a very large effect size ($d = 2.73$). Figure 5 illustrates this group difference, with a higher asymmetry ratio in the ACLR group, indicating greater inter limb imbalance during the task.

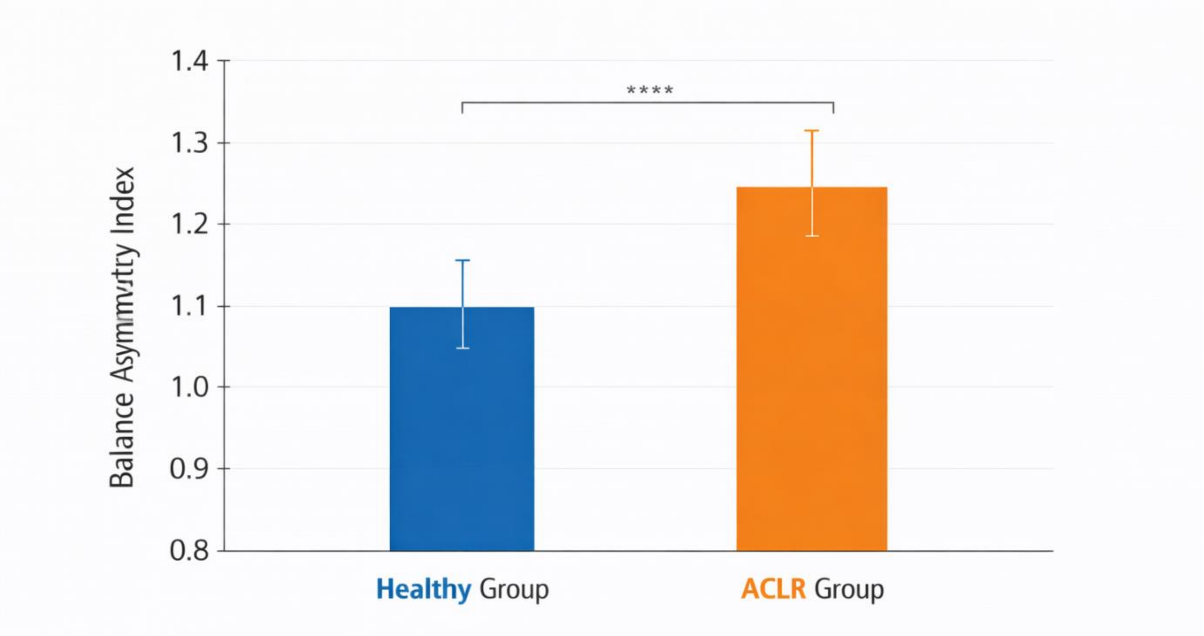

Figure 5. Group differences in inter limb asymmetry. Mean (± SD) functional asymmetry index for Healthy and ACLR groups. Higher values indicate greater imbalance between limbs. The ACLR group showed significantly more asymmetry than the Healthy group (**p < 0.001).

Across all participants (n = 24), ForceCV was strongly positively associated with the functional asymmetry ratio (Pearson r = 0.99, p < 0.001), such that greater force variability (reduced steadiness) corresponded to greater inter limb imbalance. Figure 6 presents this relationship, demonstrating a near linear increase in asymmetry ratio with increasing ForceCV.

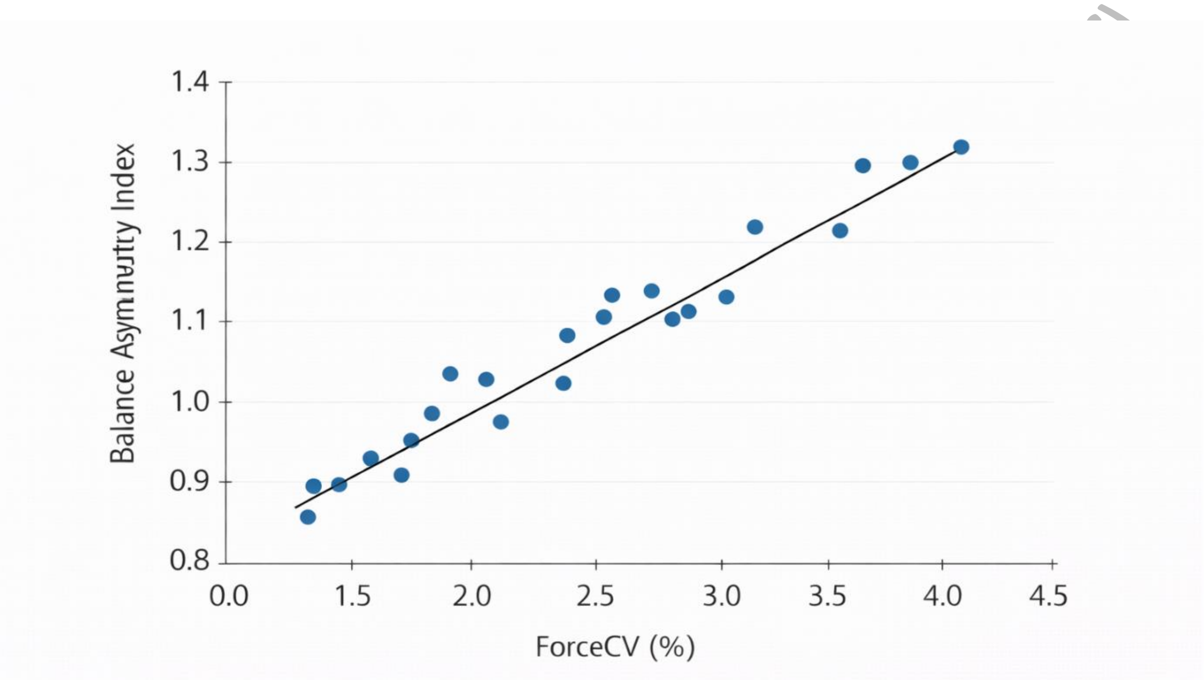

Figure 6. Relationship between force steadiness and inter limb asymmetry. Scatter plot of each participant's ForceCV versus their functional asymmetry index. There is a near perfect positive linear relationship (r = 0.99, p < 0.001), indicating that individuals with less steady force output also tended to have greater between limb asymmetry. The solid line represents the best fit linear regression line.

Discussion

The present findings were consistent with the proposed hypotheses. Specifically, compared with healthy individuals, athletes following ACL reconstruction with a hamstring graft demonstrated lower Cortico muscular coherence in the beta and gamma bands in the reconstructed limb, greater variability in isometric force control (higher ForceCV), and greater inter limb functional asymmetry during task execution (2, 19). Collectively, these results suggest that neuromuscular control characteristics differ between reconstructed and healthy limbs, even after completion of rehabilitation and return to sport.

Rather than implying causality, these patterns may reflect persistent alterations in neuromuscular organization following ACL reconstruction, which are associated with inter limb asymmetry during motor performance. Such findings are consistent with previous evidence indicating that structural restoration does not necessarily correspond to complete functional recovery, and that many athletes do not return to their preinjury performance level after ACL reconstruction (2). Contemporary return to sport frameworks further emphasize that recovery is multidimensional encompassing physical, skill based, and psychological components and that meeting conventional clearance criteria can coexist with subtle neuromuscular differences relevant to performance and reinjury risk (19).

Reduced Cortico semitendinosus coherence in the beta and gamma frequency bands in the reconstructed limb is consistent with altered functional connectivity between cortical motor regions and hamstring musculature following ACL reconstruction. Beta band coherence is commonly interpreted as reflecting the contribution of cortical oscillations to the regulation of steady motor output (13, 14); therefore, reduced beta coherence may indicate less synchronized Cortico spinal communication between cortical drive and the semitendinosus motor pool during sustained force production. Similarly, gamma band coherence often associated with faster and more integrated sensorimotor processing was reduced in the involved limb and may reflect differences in the integration of sensory input with motor commands during task execution.

Importantly, these observations should not be interpreted as solely attributable to ACL reconstruction itself. Alternative explanations should be considered, including changes in proximal motor control, compensatory recruitment strategies involving adjacent muscle groups, or task specific adaptations that reduce reliance on the reconstructed limb. In addition, altered Cortico muscular coupling patterns may reflect adaptive neural strategies aimed at maintaining task performance despite residual mechanical or sensory constraints. These interpretations are consistent with evidence indicating that ACL injury and reconstruction are associated with altered afferent signaling and sensorimotor control, including changes in proprioceptive processing related to ligament mechanoreception and joint sensory function (6). More recent work highlights neuroplastic adaptations after ACL injury and advocates for rehabilitation strategies that explicitly address altered neural control (8). In line with this, a recent systematic review synthesizing neuroimaging findings after ACLR supports the notion that changes in brain structure/activation characteristics can persist postoperatively and may have clinical implications for motor control and rehabilitation design (20). Moreover, nonlinear/dynamical approaches have shown meaningful between limb asymmetries in brain muscle coordination after ACLR, supporting the interpretation that neuromuscular coupling can remain disrupted even after return to sport (17).

Reduced force steadiness and increased ForceCV in the ACLR group constituted another key finding. Athletes with ACLR exhibited higher force variability than healthy counterparts, indicating reduced uniformity of force production in the reconstructed limb. Isometric force steadiness reflects the ability of the neuromuscular system to maintain stable output under load; increased ForceCV suggests reduced precision in force regulation and greater involuntary fluctuations in muscle activation.

One plausible contributor is the semitendinosus tendon harvest itself. A systematic review indicates that hamstring tendon regeneration may occur, but the extent and functional quality of regenerated tissue can be heterogeneous (9). Importantly, newer high resolution ultrasound evidence (2025) further confirms variability in morphological/structural tendon outcomes after harvest, reinforcing that tissue restoration may be incomplete or inconsistent across individuals (10). Functionally, persistent hamstring related deficits have been reported following semitendinosus harvest in ACL surgery (11), and compensatory strategies such as increased reliance on other hamstring components (e.g., biceps femoris) have been demonstrated in athletic populations using muscle functional MRI (12). Consequently, reduced force steadiness in the reconstructed limb may reflect a combined effect of altered muscle tendon unit properties and persistent changes in neural activation patterns, with potential downstream consequences for fine motor control and stability during sport specific demands.

Inter limb functional asymmetry represented another important dimension of the results. In the present study, ForceCV and the functional asymmetry index are interpreted as secondary functional correlates that provide contextual insight into Cortico muscular interaction patterns, rather than as standalone balance specific outcomes. The ACLR group exhibited significantly greater asymmetry than the healthy group, indicating that functional load distribution between the reconstructed and contralateral limbs may remain unequal despite rehabilitation completion. Such persistent asymmetry is clinically relevant because neuromuscular control and postural stability deficits have been prospectively linked to second ACL injury risk after ACLR and return to sport (5). From a return to sport perspective, decision rule evidence also suggests that timing and more symmetrical function before return can meaningfully reduce reinjury rates (4).

Notably, the exceptionally strong association observed between force output variability and inter limb functional asymmetry indicates that athletes with poorer force control tend to exhibit greater between limb differences during task execution. However, the exceptionally high correlation should be interpreted with caution given the small sample size and the fact that both measures were derived from the same task context. This finding suggests that force control stability and bilateral functional symmetry may, at least in part, reflect shared central peripheral sensorimotor coordination processes. While contemporary motor control theories recognize that variability can represent adaptability ("motor abundance"), the coexistence of elevated force variability with increased functional asymmetry in the present context is more consistent with reduced control precision rather than beneficial flexibility (18).

Clinical implications

The present findings may have potential implications for rehabilitation strategies and return to sport decision making, within the context of neuromuscular control and inter limb asymmetry assessed in this study. First, altered Cortico muscular coupling suggests that rehabilitation should not focus exclusively on peripheral strength restoration, but also include strategies that target neural control and sensory cognitive integration (8). Second, the observed association between force steadiness and functional symmetry may suggest that the quality of force control is an important characteristic of neuromuscular performance following ACL reconstruction. Within the context of the present findings, this relationship is consistent with the notion that force control during sustained and dynamic tasks may warrant consideration alongside traditional strength or distance based measures, such as maximal force output or hop performance. However, the present study did not directly evaluate the effects of specific training interventions, and these interpretations should therefore be considered exploratory.

Third, the presence of inter limb asymmetry in neuromuscular control measures despite return to sport is consistent with contemporary perspectives suggesting that return to sport evaluation may extend beyond conventional performance metrics alone. Recent reviews emphasize multidimensional, criteria based approaches that incorporate physical, psychological, and sport specific factors to support decision making and optimize outcomes. The current findings align with these frameworks, while not directly assessing return to sport criteria or clinical decision processes (19). Practically, integrating objective movement quality metrics (e.g., variability, coordination, symmetry) and, where feasible, neurophysiological measures may better detect residual deficits that standard tests miss.

Limitations and Future Research

Despite the novelty and relevance of the present findings, several limitations should be considered. Most notably, the study did not include detailed muscle activity quantification beyond the semitendinosus, nor did it incorporate joint level loading or kinetic measures. As a result, the extent to which the observed Cortico muscular coupling patterns relate to broader neuromuscular coordination strategies or to mechanical loading at the knee joint cannot be determined.

The absence of comprehensive multi muscle activation profiles and joint level loading measures limits interpretation of how neural muscular interactions translate into functional movement mechanics. Future studies incorporating multi muscle electromyography and biomechanical assessments of joint loading may help clarify the relationship between Cortico muscular coupling, force control, and joint level function following ACL reconstruction.

The cross sectional design, with assessments conducted at a single postoperative time point (10 months), limits causal interpretation and does not allow evaluation of recovery trajectories over time. Consequently, it remains unclear whether the observed neuromuscular deficits represent preexisting characteristics or persistent adaptations following ACL reconstruction. In addition, the relatively small sample size may limit generalizability and reduce sensitivity to detect more subtle neurophysiological effects, particularly for Cortico muscular coherence measures that exhibit high inter individual variability. Furthermore, neuromuscular coupling was assessed using a controlled isometric task targeting the semitendinosus muscle. While this approach enhances measurement reliability, it may not fully reflect neuromuscular behavior during dynamic and sport specific tasks such as jumping, landing, or rapid changes of direction. The focus on the medial hamstrings also represents a limitation, as knee joint function depends on coordinated activation across multiple muscle groups. Moreover, because all participants underwent hamstring graft ACL reconstruction, the findings may not generalize to other graft types. Finally, the absence of long term clinical outcome measures, such as return to sport success or reinjury incidence, limits interpretation of how the observed neuromuscular differences translate into real world functional performance or injury risk. As a result, the present findings should be interpreted as indicators of altered neuromuscular control characteristics rather than as predictors of clinical outcomes. Similarly, the lack of dynamic functional tasks and joint level loading measures constrains interpretation of whether the identified Cortico muscular coupling patterns reflect compensatory strategies, task specific adaptations, or mechanisms directly related to functional demands encountered during sport participation.

Consequently, conclusions regarding the clinical relevance of the observed deficits remain necessarily cautious. Future research adopting longitudinal designs, incorporating dynamic and sport specific tasks, evaluating different graft types and athlete subgroups, and integrating neurophysiological measures with clinical and biomechanical outcomes will be required to clarify the extent to which neuromuscular control characteristics influence recovery trajectories, return to sport outcomes, and reinjury risk following ACL reconstruction.

Conclusion

Overall, the present discussion provides a coherent and clinically relevant interpretation of the study findings. By integrating evidence from neurophysiology, motor control, and related clinical literature, the results are consistent with the presence of persistent alterations in neuromuscular control characteristics following ACL reconstruction with a hamstring graft. These interpretations are offered within the context of the study's cross sectional design and measurement scope, and therefore reflect associations rather than definitive evidence of impairment. Specifically, the convergence of reduced Cortico muscular coherence, increased force output variability, and greater inter limb asymmetry is consistent with measurable differences in neuromuscular control assessed in the present study, beyond peripheral or structural characteristics alone. Importantly, these findings indicate that such differences can be detected after completion of rehabilitation and return to sport, within the scope of the neurophysiological and task level measures evaluated here.

Such deficits are likely to contribute to persistent asymmetries and compromised movement quality, which may not be adequately captured by traditional strength or performance based RTS criteria. By framing force steadiness and functional symmetry as interconnected manifestations of neuromuscular control quality, this study highlights a unifying mechanism that may underlie ongoing functional limitations and elevated reinjury risk in athletes following ACL reconstruction. From a broader perspective, the results reinforce the growing recognition that successful ACL rehabilitation requires more than restoration of muscle strength or joint stability. Addressing neural control, coordination, and movement quality appears essential for achieving truly symmetrical and robust functional recovery, supporting a shift toward more neurologically informed rehabilitation and assessment strategies.

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Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Bu Ali Sina University, Hamedan, Iran (Approval Code: IR.BASU.REC.1404.019). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to data collection. For participants younger than 18 years, consent was obtained from a parent/guardian in addition to participant assent.

Consent for Publication

Not applicable. No identifiable individual data are included in this manuscript.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

S.A. conceived the study and developed the methodology. S.A. drafted the manuscript. M.A. contributed to study design and coordinated data collection. R.S. performed data analysis and visualization and contributed to interpretation of the findings. All authors critically reviewed and edited the manuscript and approved the final version.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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