

Frequency-Dependent Characteristics of Intermuscular Functional Networks During Continuous Single-Leg Hopping

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Abstract

Objective

Continuous single-leg hopping requires the lower limb to repeatedly absorb impact, maintain postural stability, and generate propulsive force during successive landing–push-off cycles. However, conventional electromyographic measures primarily characterize the activation of individual muscles and provide limited information regarding the overall functional organization among multiple muscles. This study constructed intermuscular functional networks based on surface electromyography (sEMG) to investigate the frequency-dependent characteristics of lower-limb intermuscular connectivity during the stance phase of continuous single-leg hopping and to compare network organization between the dominant and non-dominant limbs.

Methods

Twenty-seven healthy male participants performed a continuous single-leg vertical hopping task. Bilateral sEMG signals were recorded from eight lower-limb muscles. Signals were extracted within the alpha (8–13 Hz), beta (14–30 Hz), and gamma (31–60 Hz) frequency bands. Eight-node weighted intermuscular functional networks were constructed using bias-corrected normalized mutual information (NMI). Mean NMI, weighted global efficiency, and weighted clustering coefficient were calculated, and network characteristics were compared across frequency bands and between the dominant and non-dominant limbs.

Results

Overall intermuscular connectivity was relatively greater in the alpha band, with particularly strong connections observed within the quadriceps, including RF–VM, RF–VL, and VM–VL. Mean NMI, weighted global efficiency, and weighted clustering coefficient generally decreased with increasing frequency. All three network metrics showed significant frequency effects in the dominant limb ($P < 0.001$), whereas only weighted global efficiency showed a significant frequency effect in the non-dominant limb ($P = 0.004$). No significant differences in network metrics were detected between the dominant and non-dominant limbs within the alpha, beta, or gamma frequency bands ($P > 0.05$).

Conclusion

Multi-muscle coordination during the stance phase of continuous single-leg hopping exhibited clear frequency-dependent organization. Lower-frequency activity was characterized by stronger overall intermuscular statistical dependence and greater network integration, while relatively strong intermuscular connections were mainly concentrated among the quadriceps muscles involved in knee

extension and landing–push-off control. In the present sample of healthy males, Intermuscular network organization exhibited clear frequency-dependent characteristics, whereas no significant between-limb differences were detected within the examined frequency bands. NMI-based intermuscular functional network analysis provides information on multi-muscle statistical dependence and network-level organization beyond conventional single-muscle activation measures, and may provide a healthy reference for future comparisons of neuromuscular coordination during continuous hopping across injury, rehabilitation, and training conditions.

1 Introduction

Single-leg hopping is widely involved in running, jumping, and change-of-direction tasks and is commonly used as a functional test to assess lower-limb function, dynamic stability, and athletic performance [1] [2]. Because it can reflect functional recovery of the lower extremity, single-leg hop testing has been widely applied in rehabilitation assessment and return-to-sport decision-making following anterior cruciate ligament injury [3]. However, conventional assessments primarily focus on external performance outcomes, such as hopping distance, completion time, and limb symmetry, and therefore provide limited insight into the underlying functional organization of muscles during movement [4][5]. Compared with a single hop, continuous single-leg hopping requires the lower limb to repeatedly absorb impact, maintain stability, and generate propulsion during successive landing–push-off cycles. This process involves coordinated control of the hip, knee, and ankle joints as well as rapid transitions between eccentric and concentric muscle actions, thereby imposing substantial demands on dynamic neuromuscular control [6][7]. Therefore, investigating the functional organization of multiple muscles during continuous single-leg hopping may provide further insight into the neuromuscular control of dynamic movement.

Surface electromyography (sEMG) is an important noninvasive technique for investigating muscle activity during human movement [8]. Previous sEMG-based studies have primarily used EMG amplitude measures and muscle synergy analysis to characterize muscle recruitment patterns [9]. EMG amplitude reflects the activation level of individual muscles, whereas muscle synergy analysis describes coordinated patterns across multiple muscles by extracting common activation modules. However, these approaches mainly characterize the magnitude of muscle activation or common activation patterns and provide limited information regarding the strength of functional interactions between individual muscles and the network-level organization of the multi-muscle system. In recent years, intermuscular functional network analysis has emerged as a complementary approach for investigating multi-muscle coordination during complex motor tasks [10]. In this framework, individual muscles are represented as network nodes, and functional connections are constructed on the basis of statistical dependencies between EMG signals, allowing both local intermuscular interactions and global network topology to be characterized. In contrast to conventional intermuscular coherence analysis, which primarily quantifies linear relationships in the frequency domain, information-theoretic mutual information (MI) can capture both linear and nonlinear statistical dependencies between signals and may therefore be suitable for characterizing functional interactions among muscles during complex

dynamic movements [11]. Previous studies have used MI to construct intermuscular functional networks and have demonstrated task-dependent differences in network connectivity and topology, suggesting that intermuscular functional interactions are dynamically modulated by task demands and physiological state [12]. Nevertheless, existing studies have mainly focused on relatively stable motor tasks, such as walking, standing, and squatting. The connectivity patterns and topological organization of intermuscular functional networks during continuous single-leg hopping, a highly dynamic task characterized by rapid stretch–shortening actions, repetitive impact loading, and pronounced periodicity, remain poorly understood. Moreover, whether network-level differences exist between the dominant and non-dominant limbs during such dynamic tasks remains unclear [13].

Accordingly, the present study recorded bilateral lower-limb sEMG activity from multiple muscles in healthy adults during continuous single-leg hopping and constructed intermuscular functional networks in the alpha (8–13 Hz), beta (14–30 Hz), and gamma (31–60 Hz) frequency bands using normalized mutual information (NMI). Complex network analysis was then applied to systematically compare intermuscular connectivity and network topology across frequency bands and between the dominant and non-dominant limbs. The aim of this study was to characterize the frequency-dependent organization of the multi-muscle system during continuous single-leg hopping and to determine whether intermuscular network characteristics differed between limbs, thereby providing a network-based perspective for the assessment of dynamic motor function and neuromuscular coordination.

2 Subjects and Methods

2.1 Subjects

Thirty-two male university students were initially recruited for this study. Five participants were excluded because of missing sEMG data, leaving 27 participants for the final analysis. The inclusion criteria were as follows: (1) no history of chronic disease and normal neuromuscular function; (2) no strenuous exercise within 24 h before testing and no symptoms of muscle fatigue; and (3) no significant lower-limb injury within the previous 6 months. All participants were fully informed of the experimental procedures before testing and provided written informed consent. The study protocol was approved by the Ethics Committee of Peking University Third Hospital Qinhuangdao Hospital (approval no. 2026Y012).

2.2 Experimental Procedure

The experimental setup consisted of four Kistler 9260AAG force plates (sampling frequency: 2000 Hz), eight Qualisys Argus infrared motion-capture cameras (sampling frequency: 200 Hz), and a Delsys surface electromyography system (sampling frequency: 2000 Hz). Signals from all devices were integrated through a central analog-to-digital acquisition unit, and precise temporal synchronization across acquisition systems was achieved using a 5-V TTL synchronization pulse.

Before electrode placement, the skin was prepared by removing superficial keratinized tissue and cleaning the recording sites with 75% medical alcohol. The skin was allowed to air-dry to reduce skin–

electrode impedance. sEMG signals were recorded from eight lower-limb muscles: the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF), gluteus maximus (GMAX), tibialis anterior (TA), medial gastrocnemius (GM), and lateral gastrocnemius (GL). Electrode placement is illustrated in Fig. 1. Electrode locations were determined according to the anatomical placement recommendations of SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles). Electrodes were positioned over the muscle belly and aligned with the direction of the muscle fibers, while avoiding tendons, muscle borders, and areas susceptible to obvious movement artifacts.

Before formal testing, limb dominance was determined using a kicking test. Participants were not instructed which limb to use and were asked to perform three kicks in their habitual manner. The limb used to kick the ball in the majority of trials was defined as the dominant limb, and this classification was further confirmed by the participant's self-reported habitual kicking preference. All participants in the present study were right-limb dominant; therefore, the right limb was defined as the dominant limb and the left limb as the non-dominant limb.

After determination of limb dominance, participants completed a standardized 5-min warm-up followed by 18 practice repetitions of continuous single-leg hopping under the supervision of the investigators to familiarize themselves with the task and experimental environment. During the formal test, participants placed their hands on their hips to minimize the influence of arm swing on hopping strategy. Following a verbal start command, participants performed 18 consecutive single-leg vertical hops in place. They were instructed to maintain a relatively consistent hopping rhythm and movement pattern throughout the trial and to perform take-off and landing within the effective area of the same force plate whenever possible.

The testing order of the two limbs was randomized to minimize potential learning and fatigue effects associated with a fixed testing sequence. After completing the test with one limb, participants rested for 3–5 min before testing the contralateral limb, provided that they subjectively reported no obvious fatigue. If obvious loss of movement control, interruption of continuous hopping, contact of the non-testing foot with the ground, or marked fatigue or discomfort was observed or reported, the trial was terminated and repeated after sufficient rest.

To reduce the influence of movement adjustment at the beginning of the trial and anticipatory changes near the end, the first two and the final hop were excluded from analysis. Consequently, the 3rd through 17th hops, comprising 15 complete consecutive hops, were retained for subsequent analysis.

2.3 Phase Definition

The stance phase was defined as the interval from initial foot contact to toe-off. Initial contact and toe-off were identified as the instants at which the vertical ground reaction force (vGRF) first exceeded and subsequently fell below the 10-N threshold, respectively. Signals recorded during this phase were extracted for subsequent intermuscular functional network analysis.

2.4 Data Processing

Raw sEMG signals were sampled at 2000 Hz. The continuous sEMG recordings were first demeaned and linearly detrended, followed by band-pass filtering between 5 and 500 Hz using a fourth-order Butterworth infinite impulse response (IIR) filter. A 49–51 Hz band-stop filter was additionally applied to suppress 50 Hz power-line interference. All filtering procedures were performed using bidirectional zero-phase filtering (filtfilt) to avoid phase distortion. Subsequently, fourth-order Butterworth band-pass filters were used to extract the alpha (8–13 Hz), beta (14–30 Hz), and gamma (31–60 Hz) frequency bands. No full-wave rectification, envelope extraction, resampling, or temporal normalization was performed. Because the gamma band (31–60 Hz) includes the 50 Hz power-line frequency, a narrow 49–51 Hz band-stop filter was applied before frequency-band extraction to reduce line-noise contamination. Consequently, frequency components around 49–51 Hz within the gamma band were attenuated.

Each frequency-specific network comprised eight lower-limb muscles: the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), tibialis anterior (TA), biceps femoris (BF), gluteus maximus (Gmax), medial gastrocnemius (GM), and lateral gastrocnemius (GL). For each participant, limb, and frequency band, an eight-node intermuscular functional network was constructed. Each node represented one muscle, and edge weights reflected the statistical dependence between the activities of paired muscles within the corresponding frequency band.

For each participant, limb, and frequency band, sEMG samples from the 15 stance phases were vertically concatenated according to their original sample points and used collectively to estimate a single intermuscular functional network. Therefore, stance phases of longer duration contained more samples and consequently contributed relatively more to the network estimate. However, circular-shift surrogate procedures used to construct the null distribution of mutual information were strictly confined within individual stance phases to prevent boundaries between successive stance phases from being treated as continuous temporal data.

Mutual information (MI) was used to quantify the statistical dependence between two sEMG signals and can capture both linear and nonlinear dependencies [14]. For any pair of muscle signals, (X) and (Y), the filtered sEMG time series were first discretized using equal-frequency rank binning, with the number of bins set to ($Q = 16$) [15]. The mutual information between the resulting discrete variables $x \in \{1, \dots, Q\}$ and $y \in \{1, \dots, Q\}$ was defined as:

$$I(X; Y) = \sum_{a=1}^Q \sum_{b=1}^Q p(a, b) \ln \left(\frac{p(a, b)}{p(a) p(b)} \right)$$

where $p(a, b)$ denotes the joint probability, and $p(a)$ and $p(b)$ denote the corresponding marginal probabilities, all of which were estimated from the observed frequencies. The Shannon entropies, $H(X)$ and $H(Y)$, were also calculated for subsequent normalization.

Because MI estimates are positively biased under finite-sample conditions, a circular-shift surrogate procedure was used to construct a null distribution and correct for estimation bias [16]. For each muscle channel, an independent random circular shift was performed within each stance phase, with the shift amount randomly sampled from $[1, L_s - 1]$, where L_s denotes the number of samples in the corresponding stance phase. This procedure disrupted the statistical dependence between muscle signals while preserving the marginal distribution and temporal structure of each individual signal. For each of the 28 possible intermuscular connections, the surrogate procedure was repeated ($S = 2000$) times to generate the corresponding null distribution, $I_{\text{null},s}$. The bias-corrected mutual information was then defined as:

$$I_{\text{corr}} = \max \left(I_{\text{obs}} - \left(\frac{1}{S} \right) \sum_{s=1}^S I_{\text{null},s}, 0 \right)$$

The bias-corrected mutual information was subsequently symmetrically normalized to obtain normalized mutual information (NMI), which was used as the network edge weight:

$$NMI = \frac{I_{\text{corr}}}{\sqrt{H(X) H(Y)}}$$

To ensure that the edge weights remained within the interval $([0,1])$, the calculated NMI values were constrained to this range. Each intermuscular connection was assigned a single NMI weight, and all edge weights were assembled into an 8×8 symmetric weighted adjacency matrix, (W), with the main diagonal set to zero.

Based on the weighted adjacency matrix (W), the following network topological metrics were calculated. Mean NMI was defined as the average NMI weight across all 28 possible edges among the eight nodes, including edges with zero weight, and was used to represent the overall strength of intermuscular connectivity. Weighted global efficiency [17] was calculated by first converting NMI weights into connection distances according to $d_{ij} = 1/W_{ij}$. When ($W_{ij} = 0$, the corresponding distance was set to $d_{ij} = \infty$). The shortest path length, l_{ij} , between each ordered pair of nodes was then determined using the Floyd–Warshall algorithm, and weighted global efficiency was defined as:

$$E_{\text{glob}} = \frac{1}{N(N-1)} \sum_{i \neq j} \frac{1}{l_{ij}}$$

where ($N = 8$) represents the number of network nodes.

The weighted clustering coefficient was calculated for each node i using the Rubinov–Sporns/Brain Connectivity Toolbox (BCT) formulation [18]:

$$C_i = \frac{\sum_{j,k} (W_{ij} W_{jk} W_{ki})^{\frac{1}{3}}}{k_i (k_i - 1)}$$

where k_i denotes the degree of node i , defined as the number of edges with $W_{ij} > 0$. When $k_i < 2$, the node cannot participate in a triangular connection; therefore, its weighted clustering coefficient was defined as $C_i = 0$. The network-level weighted clustering coefficient was calculated as the mean C_i across all nodes.

To evaluate the sensitivity of network topology to the choice of connection density, a density-based sensitivity analysis was additionally performed using the complete bias-corrected NMI weighted adjacency matrix. Binary networks were constructed across target densities ranging from 0.30 to 0.60 in increments of 0.05. At each target density, the 28 possible edges among the eight nodes were ranked in descending order according to their NMI weights, and the strongest positive connections were preferentially retained such that the number of retained edges was as close as possible to that corresponding to the target density. If the number of positive-weight edges was smaller than that required for a given target density, all available positive-weight edges were retained. Binary global efficiency and binary clustering coefficient were then calculated at each density to assess the stability of network topology and its variation across different connection-density thresholds. This analysis was performed solely as a sensitivity analysis and was not used in the calculation of the primary weighted network metrics.

For visualization, only the top 40% of positive connections ranked by mean NMI weight were displayed in each network, with line width proportional to the corresponding mean NMI value. This threshold was used exclusively for graphical presentation and did not affect network construction or network-metric calculations. Mean NMI, weighted global efficiency, and weighted clustering coefficient were all calculated from the complete bias-corrected NMI weighted adjacency matrix. In addition, the 28 intermuscular connections were ranked according to their mean NMI values after pooling the dominant and non-dominant limbs, and the 10 strongest connections within each frequency band were selected for visual comparison. This ranking was used only for descriptive and visualization purposes and was not applied as a threshold for network construction, edge selection, or network-metric calculation.

2.5 Statistical Analysis

The participant was treated as the statistical unit. For each participant, network metrics were calculated separately for the dominant and non-dominant limbs within the alpha, beta, and gamma frequency bands, including mean NMI, weighted global efficiency, and weighted clustering coefficient. Frequency-band differences in each network metric were examined separately within the dominant and non-dominant limbs. Normality of the paired differences between frequency bands was first assessed. When the normality assumption was satisfied, a one-way repeated-measures analysis of variance (ANOVA) was performed; otherwise, the Friedman test was used. For post hoc pairwise comparisons between frequency bands, paired-samples t-tests or Wilcoxon signed-rank tests were applied according to the normality of the paired differences, with Holm correction used to adjust for multiple comparisons. Within each frequency band, network metrics were compared between the dominant and non-dominant limbs using paired analyses. The normality of the paired between-limb differences was first assessed. A paired-samples t-test was used when the normality assumption was satisfied; otherwise, the Wilcoxon

signed-rank test was applied. In addition, NMI values for each of the 28 possible intermuscular connections among the eight muscles were compared between the dominant and non-dominant limbs within each frequency band. Paired-samples t-tests were performed for individual connections, and the Benjamini–Hochberg procedure was applied within each frequency band to control the false discovery rate (FDR) across the 28 comparisons. Statistical significance was set at ($P < 0.05$).

3 Results

3.1 Intermuscular Functional Network Analysis

As shown in Figs. 2 and 3, the intermuscular functional network of the non-dominant limb exhibited relatively high overall connection weights in the alpha band, with a particularly strong RF–VL connection. In the beta band, relatively strong connections were observed for VM–VL, RF–VM, and VL–GMAX. In the gamma band, VM–VL remained one of the stronger connections, although the overall connectivity level was lower than that observed in the alpha and beta bands.

The dominant limb similarly showed relatively high overall intermuscular connectivity in the alpha band, with prominent connections among the quadriceps muscles RF, VM, and VL. In the beta band, VM–VL and RF–VM showed relatively strong connection weights. In the gamma band, VM–VL remained among the relatively stronger intermuscular connections.

3.2 Complex Network Analysis

To quantify the frequency-dependent characteristics of bilateral lower-limb intermuscular functional networks, mean NMI, weighted global efficiency, and weighted clustering coefficient were compared across the alpha, beta, and gamma frequency bands for the non-dominant and dominant limbs (Fig. 4). For the non-dominant limb, a significant frequency-band effect was observed only for weighted global efficiency ($(P = 0.004)$). Holm-corrected post hoc comparisons showed that weighted global efficiency was significantly higher in the alpha band than in the beta and gamma bands ($(P = 0.011)$ and $(P = 0.003)$, respectively), and significantly higher in the beta band than in the gamma band ($(P = 0.007)$). No significant frequency-band effects were detected for mean NMI ($(P = 0.104)$) or weighted clustering coefficient ($(P = 0.163)$). For the dominant limb, significant frequency-band effects were observed for all three network metrics (all $(P < 0.001)$). Post hoc comparisons consistently showed the pattern $\alpha > \beta > \gamma$, indicating progressive decreases in overall connection strength, global efficiency, and local clustering as frequency increased. Paired comparisons between the dominant and non-dominant limbs within each frequency band revealed no significant differences in any of the network metrics (all $(P > 0.05)$; Fig. 5). The 10 intermuscular connections with the highest mean NMI values within each frequency band were further examined (Fig. 6). In the alpha band, strong connections were predominantly observed within the quadriceps, including RF–VM, RF–VL, and VM–VL. In the beta band, VM–VL, VL–GMAX, and RF–VM ranked among the strongest connections. Although the overall connectivity level decreased in the gamma band, VM–VL, VL–GMAX, and GM–GL remained among the

highest-ranked connections. These rankings reflect only the relative magnitude of connection weights and should not be interpreted as measures of network centrality or as evidence of core-node properties. The network-density sensitivity analysis (Fig. 7) showed that binary global efficiency and binary clustering coefficient increased with network density across the 0.30–0.60 density range in all three frequency bands. Similar trends were observed for the dominant and non-dominant limbs, suggesting that the overall network patterns exhibited a certain degree of stability across different density thresholds.

4 Discussion

The present study compared bilateral lower-limb intermuscular functional connectivity and network topological characteristics across the alpha, beta, and gamma frequency bands during continuous single-leg hopping. The results showed that overall intermuscular connectivity was relatively high in the alpha band, with the strongest connections predominantly involving RF, VM, and VL. As frequency increased, mean NMI, weighted global efficiency, and weighted clustering coefficient generally decreased. Within the same frequency band, no significant between-limb differences were detected in these network metrics. Overall, these findings indicate that intermuscular functional networks during the stance phase of continuous single-leg hopping exhibit clear frequency-dependent characteristics, whereas no distinct limb-related differences were observed.

The relatively high connection weights observed among RF, VM, and VL in the alpha band are functionally consistent with the coordinated role of the quadriceps in repeated landing absorption and push-off during continuous hopping. Continuous single-leg hopping consists of repeated landing–push-off cycles and represents a typical stretch–shortening cycle (SSC) task, requiring the knee extensors to rapidly absorb mechanical energy eccentrically and subsequently generate concentric propulsive force [19]. The RF crosses both the hip and knee joints, whereas the VM and VL primarily contribute to knee extension and jointly participate in dynamic stabilization of the patellofemoral joint [20]. Previous studies have also reported a certain degree of functional coupling among RF, VM, and VL [21, 22]. Evidence suggests that neural activity in the alpha frequency range may be associated with the temporal organization of muscle coordination and common neural drive [23, 24]. However, NMI in the present study quantified statistical dependence between sEMG signals and is inherently non-directional. It therefore cannot distinguish among common central input, spinal modulation, peripheral mechanical coupling, or other sources of shared modulation. Accordingly, the relatively high alpha-band intermuscular connectivity observed here should be interpreted as a frequency-specific organizational feature of continuous single-leg hopping rather than as direct evidence of a specific neural drive. The underlying physiological mechanisms require further investigation using approaches such as electroencephalography (EEG), corticomuscular coherence, motor-unit analysis, or spinal reflex measurements.

Further analysis showed that mean NMI, weighted global efficiency, and weighted clustering coefficient generally decreased as frequency increased, suggesting that, under the present experimental conditions, lower-frequency activity exhibited stronger overall intermuscular statistical dependence and greater

network integration, whereas overall connectivity was reduced at higher frequencies. Cui et al. [11], who constructed lower-limb muscle functional networks using mutual information, similarly reported that global efficiency and clustering coefficient decreased with increasing frequency at the same walking speed. Boonstra et al. [26] also found that lower-frequency muscle networks generally exhibited greater connection strength, global efficiency, and clustering. However, unlike the present findings, the alpha band did not show the highest connection strength during walking in the study by Cui et al. This discrepancy may be related to differences in task type and contraction mode. Walking is a relatively stable cyclic task, whereas continuous single-leg hopping is a high-impact, highly rhythmic dynamic task characterized by rapid muscle lengthening and shortening. The repeated landing–push-off cycles and SSC demands of continuous hopping may promote more stable low-frequency statistical dependencies within the quadriceps. These findings suggest that the frequency organization of muscle functional networks should be interpreted in the context of the specific task and contraction mode, and that the predominance of the alpha band observed here should be considered a task-specific feature of continuous single-leg hopping.

The lower network metrics observed in the beta and gamma bands should not be interpreted as indicating that high-frequency coupling is inherently unimportant; rather, they may reflect the specific neuromuscular control strategy adopted during continuous single-leg hopping under the present experimental conditions. Jensen et al. [29] reported that visually guided walking enhanced beta- and gamma-band common drive among ankle-related muscles. Kenville et al. [30] found greater beta- and gamma-band intermuscular coherence during the eccentric phase of squatting than during isometric and concentric phases. Gwin and Ferris [31] further demonstrated that beta- and gamma-band components of lower-limb corticomuscular coupling vary between isometric and dynamic contraction conditions. Collectively, these findings suggest that high-frequency coupling is not universally weaker, but may become enhanced during visually guided control, rapid sensory feedback, eccentric control, or specific phases of muscle contraction.

No significant between-limb differences in network metrics were detected within any frequency band. This finding may be related to the fact that both limbs performed the same hopping task and were exposed to similar landing and push-off demands. McPherson et al. [33] reported no significant between-limb differences in major sagittal-plane kinematic variables during single-leg landing. In contrast, Edwards et al. [34] showed that complete bilateral symmetry in movement patterns cannot be assumed during a stop-jump task. These findings suggest that limb-related effects may depend on task characteristics, outcome measures, and movement phase. Importantly, the absence of a statistically significant difference should not be interpreted as evidence that the two limbs are identical. Failure to reject the null hypothesis may be influenced by sample size, effect magnitude, and interindividual variability. Future studies could incorporate equivalence testing or Bayesian analysis to determine whether bilateral network metrics are practically equivalent [35]. Therefore, the present between-limb findings should be described as “no significant difference detected” rather than as evidence of “no difference” or “complete symmetry.”

From a practical perspective, the relatively prominent alpha-band connections among RF, VM, and VL observed in the present study provide a potential analytical feature for evaluating multi-muscle coordination during continuous hopping using intermuscular functional networks. However, only healthy male participants were included, and injury status, rehabilitation, and training interventions were not directly evaluated. Therefore, the clinical discriminatory ability and training-monitoring value of these network characteristics remain uncertain. Future studies should further examine their reliability, sensitivity, and practical applicability in populations undergoing anterior cruciate ligament rehabilitation, individuals with patellofemoral pain, and participants with different training backgrounds.

Several limitations should be acknowledged. First, NMI reflects statistical dependence between sEMG signals and can capture both linear and nonlinear components, but it is non-directional and therefore cannot determine the direction of information transfer or identify a specific source of neural drive. Second, because RF, VM, and VL are anatomically close to one another, surface EMG recordings may be influenced by volume conduction and crosstalk. Therefore, the high connectivity observed within the quadriceps may reflect genuine functional coordination but may also contain contributions from shared signal contamination. Third, although a network-density sensitivity analysis was performed, weighted network metrics may still be influenced by overall connection strength. Future studies should incorporate additional approaches, such as weight normalization or more explicit strength-matched analyses, to further distinguish changes in connection strength from changes in network topology. Fourth, only healthy male participants were included; therefore, caution is warranted when generalizing the present findings to females, individuals with different training backgrounds, or populations with lower-limb injuries. Fifth, the present study did not include EEG, magnetoencephalography, corticomuscular coherence, or spinal reflex measurements, and therefore could not directly verify the central or peripheral origins of connectivity within different frequency bands. These limitations primarily constrain interpretation of the origin and functional significance of the observed connections but do not alter the main observations regarding frequency-dependent network characteristics and the absence of statistically significant between-limb differences.

In summary, intermuscular functional networks during the stance phase of continuous single-leg hopping exhibited clear frequency-dependent characteristics. The alpha band showed relatively high overall intermuscular connectivity, with the strongest connections predominantly involving RF, VM, and VL, suggesting relatively strong statistical dependence within the quadriceps under this task condition. As frequency increased, mean NMI and the corresponding weighted network metrics generally decreased, indicating a relative reduction in overall intermuscular statistical dependence at higher frequencies.

5 Conclusion

The present study constructed lower-limb intermuscular functional networks during the stance phase of continuous single-leg hopping using sEMG signals and normalized mutual information. The results demonstrated clear frequency-dependent patterns in intermuscular functional connectivity. Descriptively,

overall connectivity was relatively greater in the alpha band, with the strongest intermuscular connections predominantly involving RF, VM, and VL. As frequency increased, mean NMI, weighted global efficiency, and weighted clustering coefficient generally decreased, indicating clear frequency-dependent characteristics of intermuscular statistical dependence during continuous hopping.

No significant between-limb differences in network metrics were detected within any frequency band, indicating that no distinct limb-related differences were observed in the present sample of healthy males under the current task conditions. By characterizing intermuscular statistical dependence and weighted network organization, this study provides a new analytical perspective for understanding multi-muscle coordination during continuous hopping. Future studies integrating kinematic, kinetic, and corticomuscular coupling measures are warranted to further investigate the physiological origins of frequency-specific intermuscular connectivity and to evaluate its potential applications in populations with injury and during rehabilitation.

Declarations

Conflict of Interest

The authors declare no commercial or financial conflicts of interest related to this study.

Author Contribution

Conceptualization, Y.F. and Y.G.; Methodology, Y.F. and Y.G.; Software, Y.F.; Validation, M.J. and W.G.; Formal analysis, J.L.; Investigation, L.D.; Writing—original draft preparation, Y.F.; All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The raw data supporting the conclusions of this article contain sensitive biomechanical and personal information that could compromise participant privacy, and thus cannot be shared openly. De-identified summary data may be provided by the corresponding author upon reasonable request, pending institutional ethics committee approval.

References

1. Logerstedt D et al (2012) Single-legged hop tests as predictors of self-reported knee function after anterior cruciate ligament reconstruction: the Delaware-Oslo ACL cohort study. *Am J Sports Med* 40(10):2348–2356

2. Weber M et al (2024) Evaluation of hop test movement quality to enhance return to sport testing. A cross-sectional study. *Front Sports Act Living* 6:1305817
3. Grindem H et al (2016) Simple decision rules can reduce reinjury risk by 84% after ACL reconstruction: the Delaware-Oslo ACL cohort study. *Br J Sports Med* 50(13):804–808
4. Kotsifaki A et al (2022) Single leg hop for distance symmetry masks lower limb biomechanics: time to discuss hop distance as decision criterion for return to sport after ACL reconstruction? *Br J Sports Med* 56(5):249–256
5. Wellsandt E, Failla MJ, Lynn Snyder-Mackler (2017) Limb symmetry indexes can overestimate knee function after anterior cruciate ligament injury. *J Orthop Sports Phys Therapy* 47(5):334–338
6. Taube W et al (2012) The drop height determines neuromuscular adaptations and changes in jump performance in stretch-shortening cycle training. *Scand J Med Sci Sports* 22(5):671–683
7. Krosshaug T et al (2007) Mechanisms of anterior cruciate ligament injury in basketball: video analysis of 39 cases. *Am J Sports Med* 35(3):359–367
8. Disselhorst-Klug C, Schmitz-Rode T, Günter R (2009) Surface electromyography and muscle force: Limits in sEMG–force relationship and new approaches for applications. *Clin Biomech Elsevier Ltd* 24(3):225–235
9. Hug François (2011) Can muscle coordination be precisely studied by surface electromyography? *J Electromyogr Kinesiol* 21(1):1–12
10. Boonstra TW et al (2015) Muscle networks: Connectivity analysis of EMG activity during postural control. *Sci Rep* 5(1):17830
11. Caihong CUI et al (2023) Analysis of muscle synergy and muscle functional network at different walking speeds based on surface electromyographic signal. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. J Biomed Eng* 40(5):938
12. Kerkman JN et al (2020) Muscle synergies and coherence networks reflect different modes of coordination during walking. *Front Physiol* 11:751
13. Krzysztofik M et al (2021) A comparison of muscle activity of the dominant and non-dominant side of the body during low versus high loaded bench press exercise performed to muscular failure. *J Electromyogr Kinesiol* 56:102513
14. Steuer R et al (2002) The mutual information: detecting and evaluating dependencies between variables. *Bioinformatics* 18:S231–S240
15. Kraskov A, Stögbauer H, Peter Grassberger (2004) Estimating mutual Inform *Phys Rev E—Statistical Nonlinear Soft Matter Phys* 69(6):066138
16. Theiler J et al (1992) Testing for nonlinearity in time series: the method of surrogate data. *Physica D* 58(1–4):77–94
17. Latora V, Marchiori M (2001) Efficient behavior of small-world networks. *Phys Rev Lett* 87(19):198701

18. Rubinov M, Sporns O (2010) Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52.3 : 1059–1069
19. Komi PV (2000) Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. *J Biomech* 33(10):1197–1206
20. Waligora AC, Norman A, Johanson, Bruce Elliot Hirsch (2009) Clinical anatomy of the quadriceps femoris and extensor apparatus of the knee. *Clin Orthop Relat Research®* 467(12):3297–3306
21. Chang Y-J et al (2012) Increases of quadriceps inter-muscular cross-correlation and coherence during exhausting stepping exercise. *Sensors* 12.12 : 16353–16367
22. Maudrich T et al (2022) Motor control strategies differ between monoarticular and biarticular quadriceps muscles during bipedal squats. *Scand J Med Sci Sports* 32(11):1569–1580
23. Laine CM, Brian A, Cohn, Francisco J, Valero-Cuevas (2021) Temporal control of muscle synergies is linked with alpha-band neural drive. *J Physiol* 599(13):3385–3402
24. Boonstra TW et al (2009) Corticomuscular and bilateral EMG coherence reflect distinct aspects of neural synchronization. *Neurosci Lett* 463(1):17–21
25. De Luca, Carlo J et al (2010) Filtering the surface EMG signal: Movement artifact and baseline noise contamination. *J Biomech* 43(8):1573–1579
26. Boonstra TW et al (2015) Muscle networks: Connectivity analysis of EMG activity during postural control. *Sci Rep* 5(1):17830
27. Rubinov M, Sporns O (2010) Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52.3 : 1059–1069
28. Kerkman JN et al (2018) Network structure of the human musculoskeletal system shapes neural interactions on multiple time scales. *Sci Adv* 4(6):eaat0497
29. Jensen P et al (2018) Increased central common drive to ankle plantar flexor and dorsiflexor muscles during visually guided gait. *Physiological Rep* 6(3):e13598
30. Kenville R et al (2020) Intermuscular coherence between homologous muscles during dynamic and static movement periods of bipedal squatting. *J Neurophysiol* 124(4):1045–1055
31. Gwin JT, Daniel P (2012) Ferris. Beta-and gamma-range human lower limb corticomuscular coherence. *Front Hum Neurosci* 6:258
32. Gelman A (2006) The difference between significant and not significant is not itself statistically significant. *Am Stat* 60(4):328–331
33. McPherson AL et al (2016) Sagittal plane kinematic differences between dominant and non-dominant legs in unilateral and bilateral jump landings. *Phys Ther Sport* 22:54–60
34. Edwards S et al (2012) Lower limb movement symmetry cannot be assumed when investigating the stop-jump landing. *Med Sci Sports Exerc* 44(6):1123–1130
35. Lakens Daniël (2017) Equivalence tests: A practical primer for t tests, correlations, and meta-analyses. *Social Psychol personality Sci* 8(4):355–362

36. Farina D et al (2002) Surface EMG crosstalk between knee extensor muscles: experimental and model results. Muscle Nerve: Official J Am Association Electrodiagn Med 26(5):681–695
37. Generative AI statement
38. The authors declare that no Gen AI was used in the creation of this manuscript

Figures

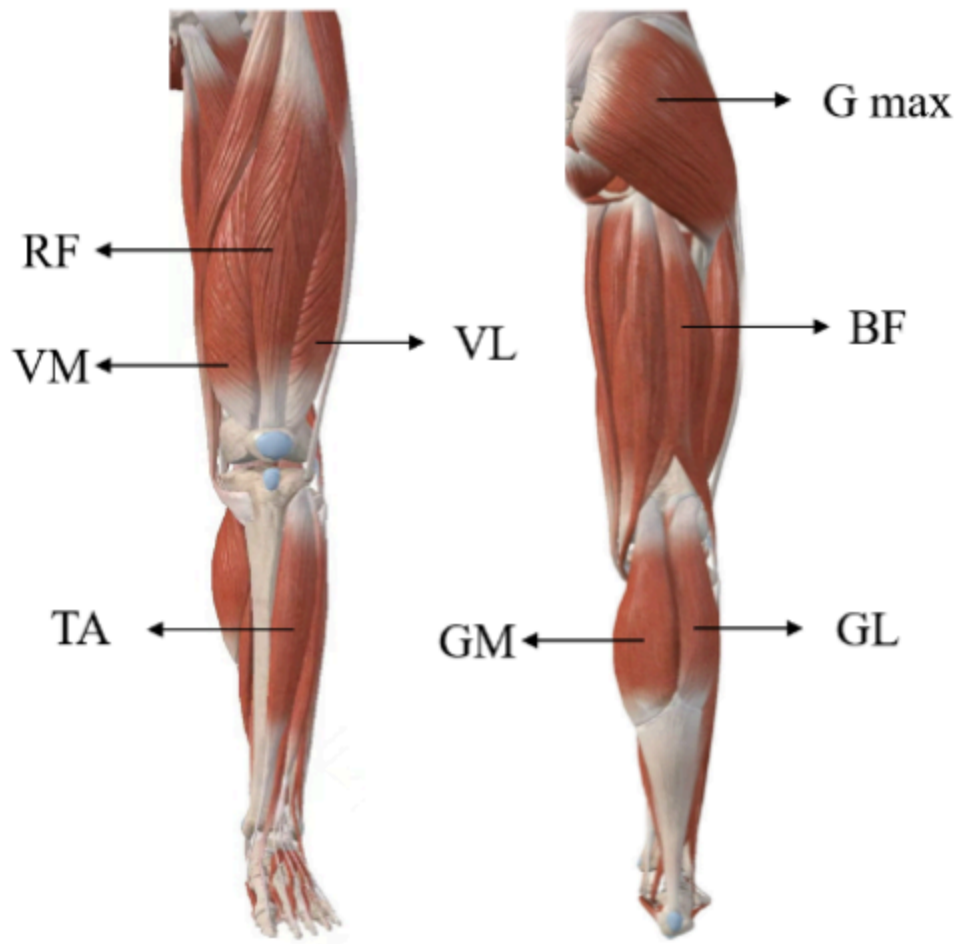


Figure 1

Schematic illustration of surface electromyography electrode placement.

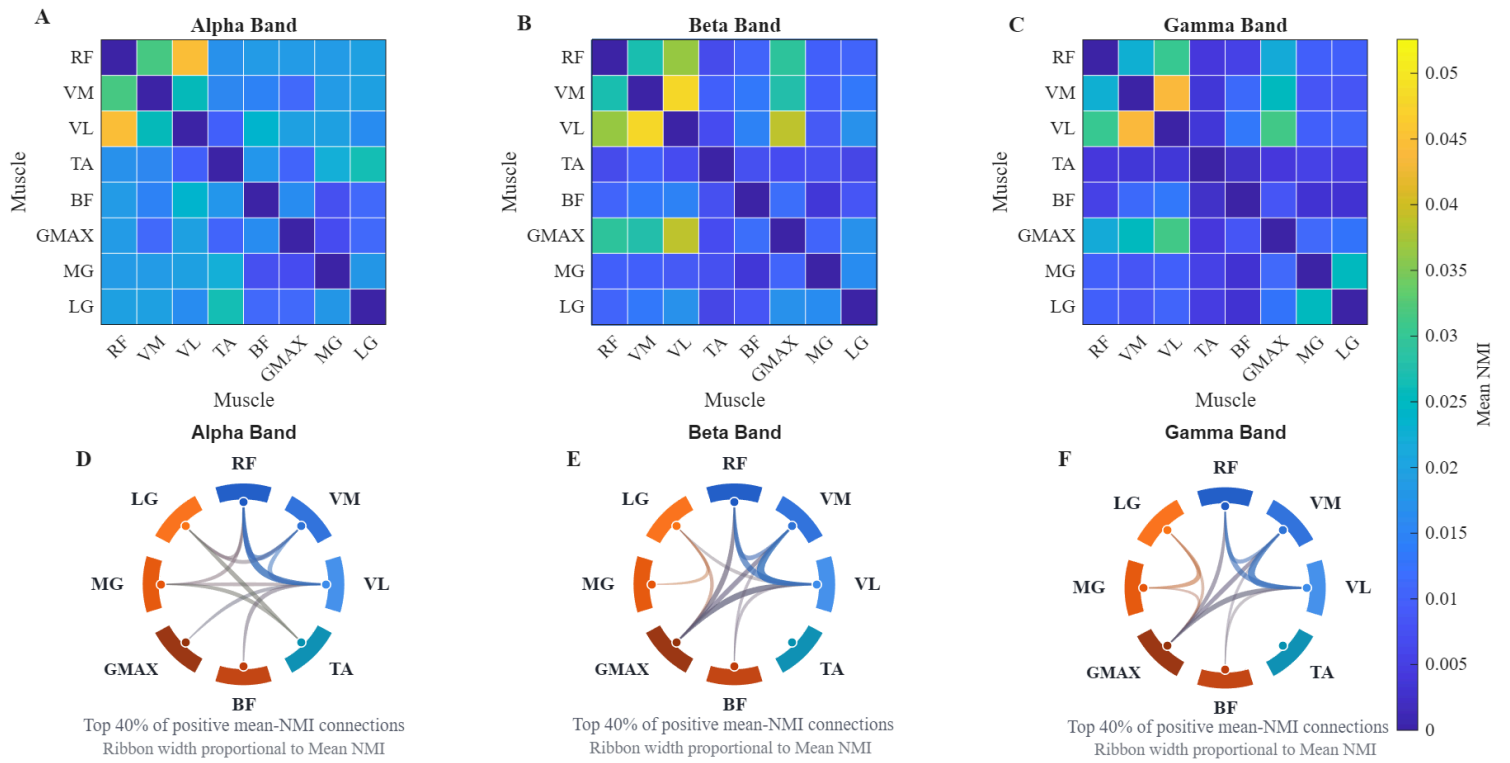


Figure 2

left leg intermuscular networks across frequency bands

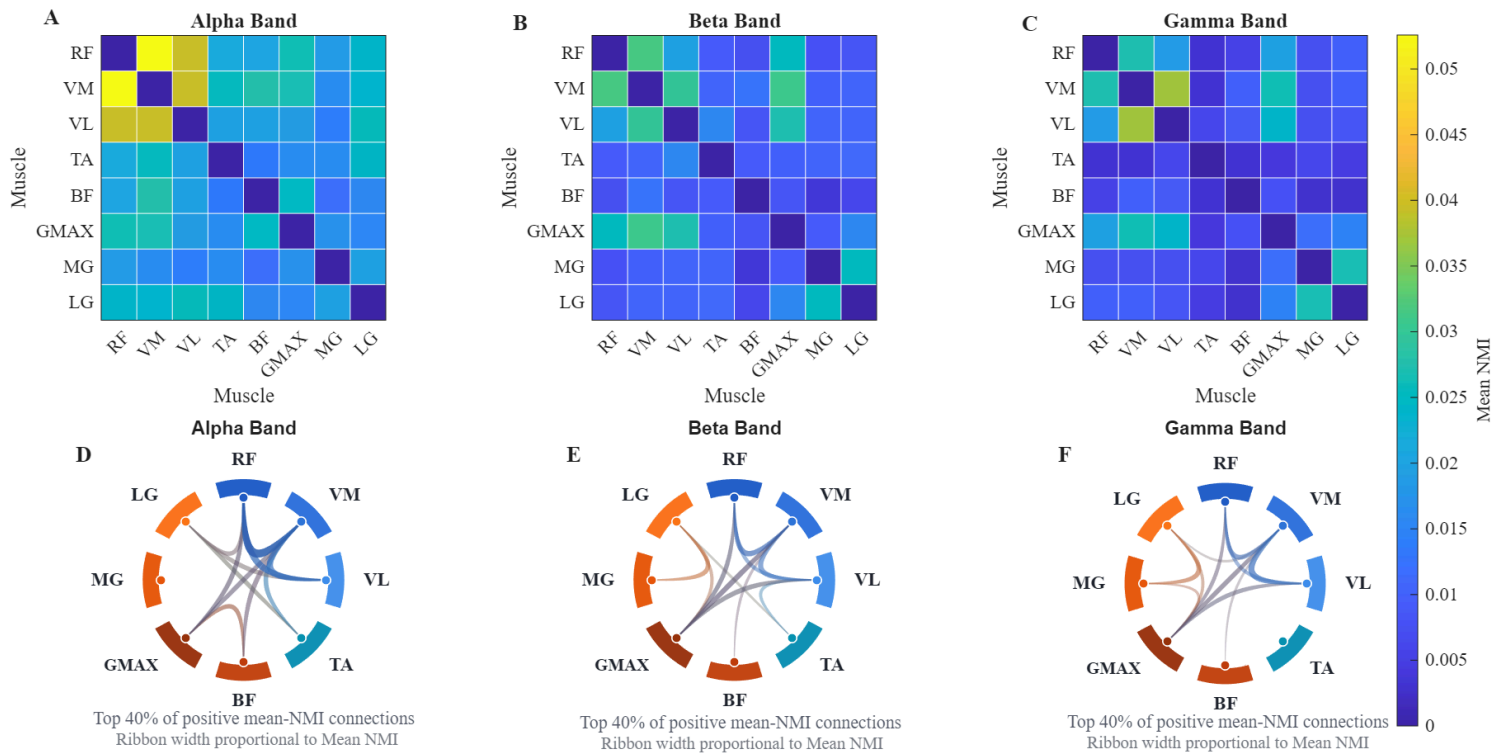


Figure 3

Right leg intermuscular networks across frequency bands

Note: For visualization, only the top 40% of positive connections ranked by mean NMI weight are displayed in each network. Line width is proportional to the corresponding mean NMI value.

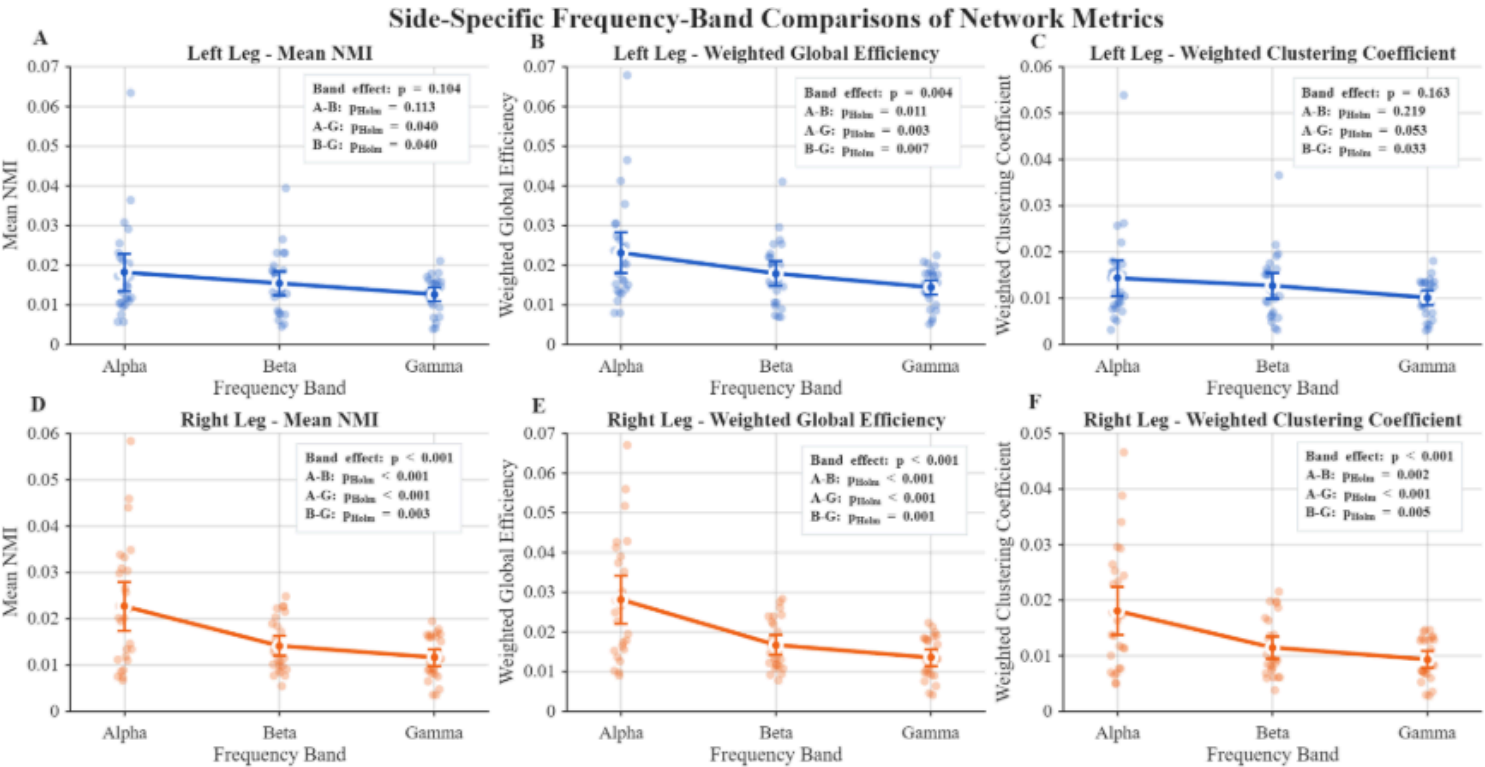


Figure 4
Side-specific frequency-band comparisons of network metrics

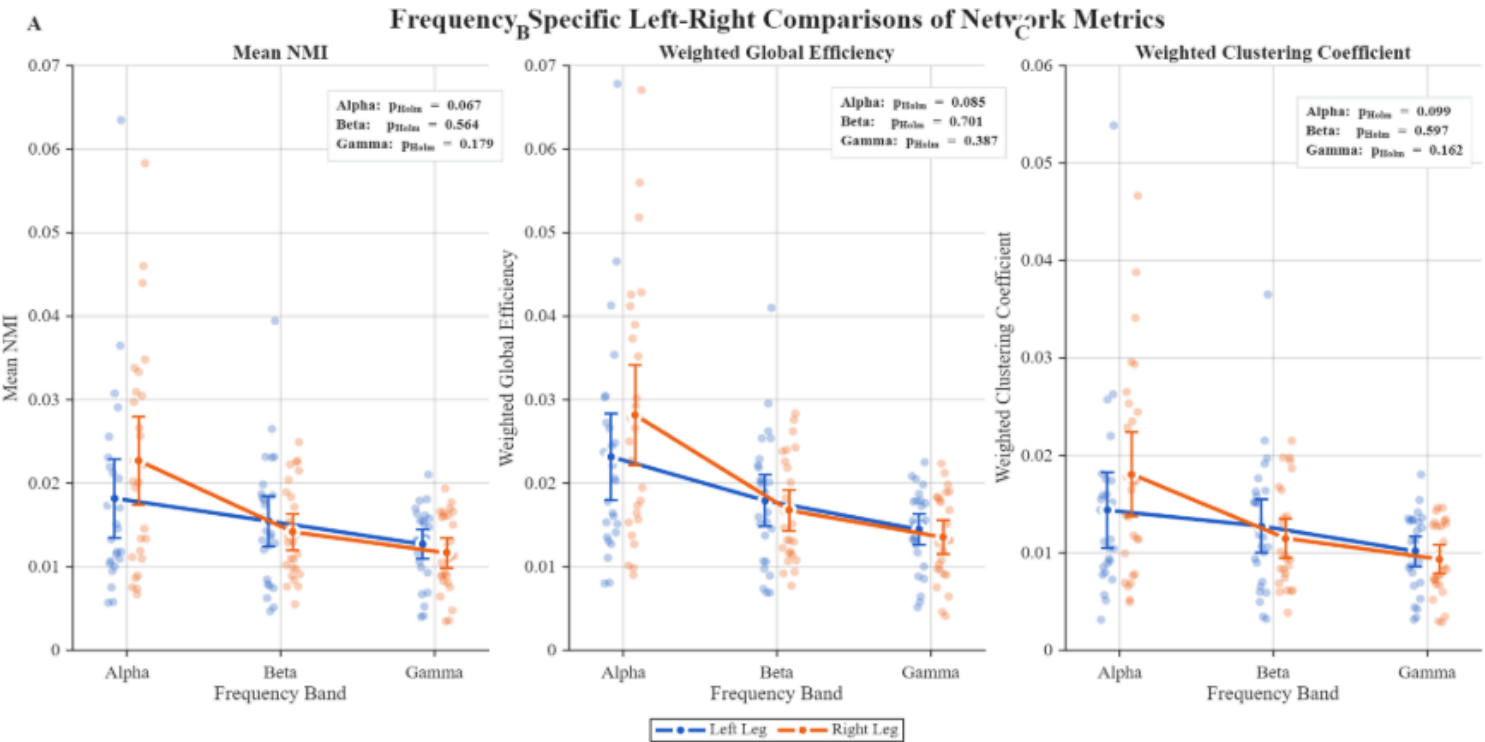


Figure 5
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Frequency specific left-right comparisons of network metrics

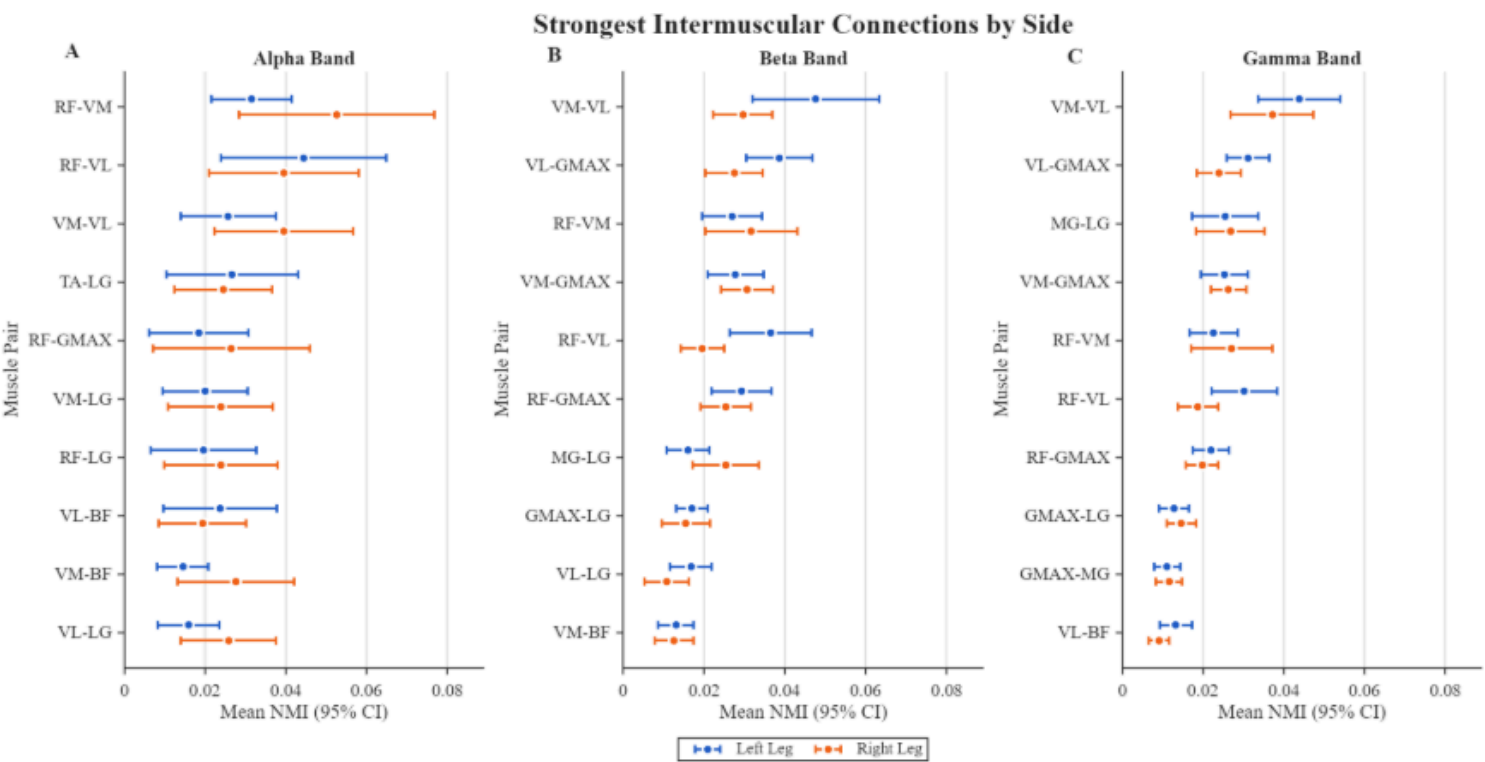


Figure 6

Top 10 intermuscular connections ranked by mean NMI across frequency bands

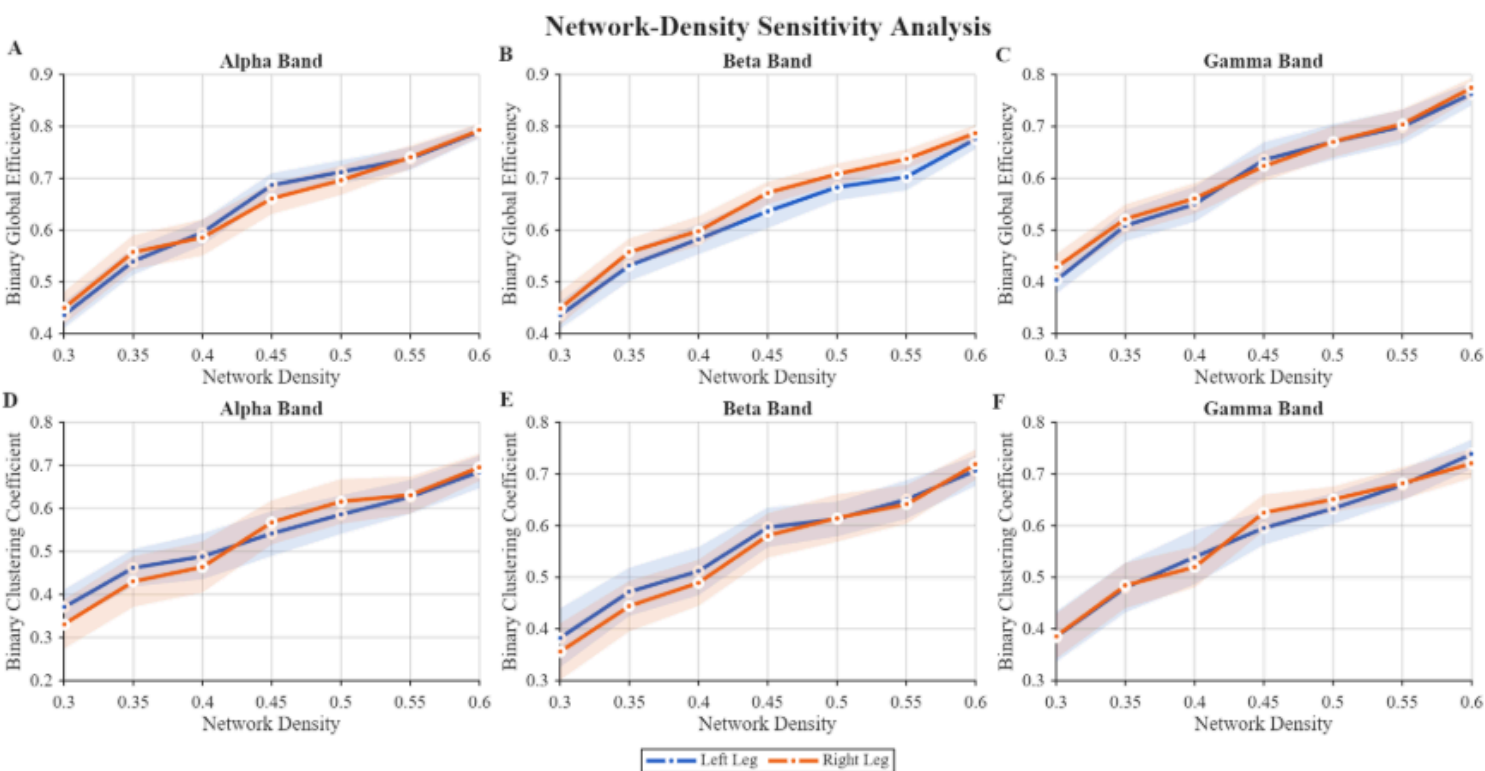


Figure 7

Sensitivity analysis of bilateral intermuscular functional network metrics across different network densities