JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

DOI: https://doi.org/10.61919/gqnqcq41

Original Article

Muscle-Specific Neuromuscular Recruitment as a Candidate Mediator of Functional Recovery in Robot-Assisted Gait Training Combined with Transcutaneous Spinal Cord Stimulation: A Secondary Analysis in Incomplete Spinal Cord Injury

Abdul Rashad[1], Asma Shahid[2], Shakeel Ahmad[3], Muhammad Talha Arsorail[4], Kiran Iqbal[5], Minab Faisal[6]

[1] Assistant Professor, Department of Rehabilitation and Health Sciences, Nazeer Hussain University
[2] Junior Lecturer, Department of Rehabilitation and Health Sciences, Nazeer Hussain University
[3] Lecturer, Jouf University, Sakaka, Saudi Arabia
[4] Junior Lecturer, Department of Rehabilitation and Health Sciences, Nazeer Hussain University
[5] Assistant Professor, University of Sialkot, Punjab, Pakistan
[6] Physical Therapist, University of Sialkot, Punjab, Pakistan

*Corresponding author: Abdul Rashad, rashadabdul66@gmail.com

"Cite this Article" **Received**: 13 January 2026; **Accepted**: 23 June 2026; **Published**: 16 July 2026
Author Contributions: Concept: AR, AS, MTA; Design: AR, AS, MTA; Data Collection: MTA, KI, MF; Analysis: AR, MTA; Drafting: AR, AS, SA. **Ethical Approval**: Nazeer Hussain University, Karachi, Pakistan. **Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest. **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: NA

ABSTRACT

Background: Robot-assisted gait training combined with transcutaneous spinal cord stimulation may improve mobility after incomplete spinal cord injury, but the muscle-specific neuromuscular pattern underlying these effects remains uncertain. Objective: To examine whether muscle-specific intramuscular electromyographic recruitment and progressive body-weight-support reduction provided a plausible mechanistic explanation for the functional advantage of combined robotic gait training and spinal stimulation. Methods: This hypothesis-generating secondary analysis used data from a double-blind randomized trial of 20 adults with intermediate-phase incomplete spinal cord injury allocated to RAGT+tSCS (n=13) or CPT+tSCS (n=7). Participants completed 40 sessions over 8 weeks. Group × Time effects were examined for iEMG amplitudes of the gastrocnemius medialis, vastus lateralis, tibialis anterior, and biceps femoris and compared with functional outcomes. Associations between treatment assignment and body-weight-support requirement were evaluated using Pearson's correlation. Results: Large interactions were observed for the gastrocnemius medialis (partial η^2=0.924), vastus lateralis (η^2=0.880), and tibialis anterior (η^2=0.859), but not the biceps femoris (η^2=0.061; p=0.293). Functional effects were largest for the Timed Up and Go test (η^2=0.844) and Berg Balance Scale (η^2=0.702), whereas the 10-Meter Walk Test showed no between-group interaction (η^2=0.008; p=0.708). Treatment assignment correlated with body-weight-support requirement at sessions 1 (r=−0.886) and 20 (r=−0.867), both p<0.001. Conclusion: RAGT+tSCS was associated with selective recruitment of ankle and knee stabilizing muscles corresponding with balance and transitional-mobility gains. Progressive unloading represented a plausible candidate mechanism but was not formally established as a mediator. Keywords: Incomplete spinal cord injury; robot-assisted gait training; transcutaneous spinal cord stimulation; intramuscular electromyography; body-weight support; neuromuscular recruitment; balance; functional mobility.

INTRODUCTION

Incomplete spinal cord injury (iSCI) disrupts descending motor control while preserving variable degrees of supraspinal, propriospinal, and segmental connectivity that may support activity-dependent neurological recovery (1,2). Spinal cord injury remains an important cause of long-term motor disability worldwide, and its burden has increased substantially across several regions, including China, during the past three decades (3,4). Restoration of walking after iSCI depends not only on global neurological recovery but also on the coordinated re-engagement of lower-limb muscles responsible for stance stability, weight transfer, limb advancement, and propulsion. Electromyography can therefore provide information about neuromuscular recovery that may not be fully captured by clinical balance and mobility scales alone (5,6).

Robot-assisted gait training (RAGT) provides repetitive, task-specific stepping practice with controlled kinematics, adjustable body-weight support, and high-intensity sensory input. These features are intended to promote use-dependent reorganization of residual neural pathways and improve locomotor control after spinal cord injury (7). Transcutaneous spinal cord stimulation (tSCS) is a non-invasive neuromodulatory approach that activates posterior-root afferents and influences the excitability of spinal interneuronal and motor networks involved in locomotion (8,9). Rather than directly generating functional movement, tSCS is considered to modulate the physiological state of spinal circuits so that residual descending commands and task-related afferent inputs can produce more effective motor responses during rehabilitation (9,10). Its clinical effects should therefore be reflected in measurable changes in muscle activation during locomotor practice.

Previous studies and systematic reviews have examined the effects of RAGT, wearable robotic devices, body-weight-supported gait training, and spinal neuromodulation on walking ability, balance, motor scores, and mobility after SCI (7,11,12). However, most investigations have concentrated on overall clinical efficacy and have evaluated individual functional outcomes separately from the underlying neuromuscular response. Consequently, it remains uncertain whether functional improvement following combined RAGT and tSCS is accompanied by a generalized increase in lower-limb activation or by a selective pattern involving muscles with specific biomechanical roles. Intramuscular electromyography (iEMG) offers greater spatial specificity than surface recordings and reduces interference from adjacent muscle groups, making it particularly suitable for identifying muscle-specific recruitment patterns during rehabilitation (13).

The parent randomized trial compared RAGT combined with tSCS against dose-matched conventional physical therapy (CPT) combined with tSCS in adults with intermediate-phase iSCI. That trial collected iEMG signals from the vastus lateralis, tibialis anterior, gastrocnemius medialis, and biceps femoris together with measures of balance, mobility, neurological status, and body-weight-support requirements, and reported greater clinical improvement in the robotic-training group (14). The primary trial addressed the comparative effectiveness of the two rehabilitation strategies but did not specifically examine whether the distribution of recruitment gains across the sampled muscles was biomechanically consistent with the functional domains that improved most strongly.

A muscle-selective response would provide a more specific mechanistic hypothesis than a generalized training effect. The vastus lateralis contributes to knee extension and stance stability, the gastrocnemius medialis contributes to postural control and plantarflexion during late stance, and the tibialis anterior assists foot clearance and controlled ankle positioning. By contrast, the biceps femoris has an important role in knee flexion and limb advancement during the swing phase. Progressive reduction of external body-weight support during robotic training may increase active loading demands on the ankle and knee musculature and thereby preferentially influence muscles involved in weight acceptance, postural stability, and controlled progression.

This secondary, hypothesis-generating analysis therefore examined whether the previously observed functional advantage of RAGT combined with tSCS was accompanied by a coherent muscle-specific pattern of neuromuscular recruitment. It was hypothesized that Group × Time effects would be larger for the gastrocnemius medialis, vastus lateralis, and tibialis anterior than for the biceps femoris, and that the body-weight-support trajectory would align with treatment-group assignment in a manner consistent with progressive unloading as a candidate shared driver of neuromuscular and functional improvement.

The analysis was intended to generate a testable mechanistic and mediation hypothesis rather than to establish a formal causal pathway.

MATERIAL AND METHODS

This study was a post-hoc, hypothesis-generating secondary analysis of data obtained from a prospective, double-blind, parallel-arm randomized controlled trial registered with the Chinese Clinical Trial Registry under registration number ChiCTR2300074090. The parent trial was conducted at Xi'an International Medical Center Hospital and received approval from the institutional Medical Ethics Committee (approval no. 202348). All participants provided informed consent for trial participation and for the use of their de-identified data in analyses related to the trial objectives. The complete trial design, participant characteristics, intervention procedures, and primary clinical findings have been reported separately (14). The present analysis addressed a distinct mechanistic question by jointly examining muscle-specific iEMG effects, body-weight-support requirements, and selected functional outcomes from the existing trial dataset.

The parent trial included 20 adults with intermediate-phase iSCI who were classified as American Spinal Injury Association Impairment Scale grades B–D and were between 3 and 6 months post-injury. Participants were randomized to receive either RAGT combined with tSCS or CPT combined with tSCS. Thirteen participants were allocated to the RAGT+tSCS group and seven to the CPT+tSCS group. Randomization, allocation procedures, baseline comparability, and blinding arrangements were inherited from the parent trial and were not modified for this secondary analysis.

Both groups completed 40 rehabilitation sessions over 8 weeks, corresponding to five sessions per week. Before each rehabilitation session, participants received an identical 20-minute tSCS protocol consisting of a biphasic square-wave current delivered at 30 Hz with a pulse duration of 1 ms. The cathode was positioned over the T11–T12 spinal level, and symmetrical anodes were placed in the peri-umbilical region. The stimulation protocol was held constant across groups so that the principal between-group difference was the form of locomotor rehabilitation received after stimulation.

Participants assigned to the RAGT+tSCS group trained using a lower-limb rehabilitation robotic system with harness-supported body-weight unloading. Initial body-weight support was set at 50% and was progressively reduced according to participant tolerance and training performance. This progression was intended to increase active lower-limb loading as participants acquired greater postural and locomotor control. Participants assigned to the CPT+tSCS group received duration-matched conventional physiotherapy comprising range-of-motion exercises, lower-limb strengthening, balance activities, and assisted gait training. Treatment exposure was therefore matched by session frequency and duration, while the task structure, robotic guidance, and systematic body-weight-support progression differed between groups.

The present analysis used outcomes collected at baseline and after 8 weeks of intervention. Neuromuscular recruitment was assessed using iEMG recordings from the vastus lateralis, tibialis anterior, gastrocnemius medialis, and biceps femoris. Signals were acquired using needle electrodes at a sampling frequency of 10,240 Hz and were bandpass filtered between 100 and 4,400 Hz. These four muscles were examined because they represent distinct contributions to knee extension, ankle control, plantarflexion, stance stability, and swing-phase movement. The principal neuromuscular outcome was the change in iEMG amplitude, expressed in microvolts, for each sampled muscle.

Body-weight-support requirement was recorded as the percentage of external support used during robotic training. Values from sessions 1 and 20 were included to characterize the progression of unloading during the intervention. Functional outcomes used as clinical anchors were the Berg Balance Scale and Timed Up and Go test because they reflect static and dynamic balance, postural transitions, turning, and short-distance functional mobility. The 10-Meter Walk Test and Walking Index for Spinal

Cord Injury II were used where relevant to compare the muscle-selective pattern with walking-related outcomes, while the ASIA Motor Score was retained as a global indicator of neurological motor function.

The analysis did not repeat the primary efficacy testing of the parent randomized trial. Instead, the four sampled muscles were ranked according to the magnitude of their previously calculated Group × Time interaction effects. Partial eta-squared values derived from the repeated-measures general linear models used in the parent trial were examined to determine whether neuromuscular improvement was distributed uniformly across muscles or concentrated in muscles with particular biomechanical roles. Corresponding F statistics and p-values were retained as originally calculated in the parent analysis.

Previously calculated Pearson correlation coefficients between treatment-group assignment and body-weight-support requirement at sessions 1 and 20 were examined as indicators of the relationship between robotic-training exposure and progressive unloading. These coefficients were interpreted descriptively as protocol-related associations rather than as evidence that group assignment itself constituted a continuous biological determinant. The direction and magnitude of these associations were considered together with the muscle-specific effect sizes and functional anchor outcomes to assess whether the findings formed a coherent biomechanical pattern.

The analysis was exploratory and did not constitute a formal mediation analysis. No bootstrapped indirect effects, structural equation models, causal mediation models, or additional inferential tests were performed. No multiplicity-adjusted secondary hypothesis testing was undertaken because the analysis reorganized and jointly interpreted statistics already generated in the parent trial rather than conducting a new family of significance tests. Accordingly, partial eta-squared values and correlation coefficients were interpreted as indicators of the magnitude and coherence of the observed pattern, not as proof of a causal pathway.

Potential confounding was considered by examining whether baseline demographic characteristics differed between the randomized groups in the parent trial and by assessing the reported relationship of age with body-weight-support measures. Age was associated with support requirements but did not differ meaningfully between treatment groups, reducing concern that the observed association between robotic-training assignment and unloading was attributable solely to baseline age imbalance. Nevertheless, the small and unequally allocated sample limited precision and precluded reliable multivariable mediation modelling.

All statistical estimates used in this secondary analysis were reproduced from the parent trial dataset and statistical output. The openly archived dataset provides an additional basis for reproducibility and independent verification (15). Statistical findings were reported using exact p-values where available, with $p<0.001$ retained for values below that threshold. Partial eta-squared was used to express Group × Time interaction effect magnitude, and Pearson's r was used to describe the strength and direction of the reported associations involving body-weight support. Interpretation emphasized effect patterns, biomechanical consistency, and hypothesis generation rather than definitive causal inference.

RESULTS

All 20 participants included in the parent randomized trial contributed to this secondary analysis, comprising 13 participants in the RAGT+tSCS group and seven in the CPT+tSCS group. The analysis examined whether the magnitude of the Group × Time interaction differed across the four sampled lower-limb muscles and whether this pattern corresponded with the functional outcomes and body-weight-support trajectory reported in the parent trial.

The neuromuscular response was strongly muscle-specific rather than uniformly distributed across the lower limb. Gastrocnemius medialis demonstrated the largest Group × Time interaction, with $F(1,18)=219.969$, $p<0.001$, and partial $\eta^2=0.924$. Vastus lateralis showed the next-largest interaction, $F(1,18)=131.387$, $p<0.001$, partial $\eta^2=0.880$, followed by tibialis anterior, $F(1,18)=109.845$, $p<0.001$, partial

η^2=0.859. In contrast, the biceps femoris showed no statistically significant between-group interaction, $F(1,18)=1.176$, $p=0.293$, partial η^2=0.061. The marked separation between the first three muscles and the biceps femoris indicates that the treatment-related iEMG response was concentrated in muscles contributing to ankle control, plantarflexion, knee extension, and stance stability.

The absolute change values reported in the parent analysis were consistent with this effect-size pattern. Tibialis anterior iEMG amplitude increased by 333.1 μV in the RAGT+tSCS group compared with 127.1 μV in the CPT+tSCS group. By contrast, biceps femoris gains were similar between groups, increasing by 184.6 μV and 167.1 μV, respectively. These values support the interpretation that the between-group neuromuscular difference was not attributable to a generalized increase in activation across all sampled muscles.

The functional outcomes showed a similarly selective pattern. The Timed Up and Go test demonstrated a large Group × Time interaction with partial η^2=0.844 and $p<0.001$, while the Berg Balance Scale showed partial η^2=0.702 and $p<0.001$. The Walking Index for Spinal Cord Injury II demonstrated a smaller but statistically significant interaction, with partial η^2=0.321 and $p=0.009$. In contrast, the 10-Meter Walk Test showed minimal between-group separation, with partial η^2=0.008 and $p=0.708$. Thus, the largest functional effects occurred in balance and transitional mobility rather than over-ground walking speed.

The concordance between the muscle-specific and functional outcomes was biomechanically coherent. The largest iEMG effects occurred in the gastrocnemius medialis, vastus lateralis, and tibialis anterior, while the largest clinical effects occurred in the Timed Up and Go test and Berg Balance Scale. Conversely, the biceps femoris showed no meaningful between-group interaction, and the 10-Meter Walk Test similarly showed no evidence of differential improvement. This pattern is compatible with preferential improvement in postural control, weight acceptance, controlled stance, and transitional mobility rather than a generalized enhancement of all components of gait.

Treatment-group assignment was strongly associated with body-weight-support requirement at both examined training points. The correlation was $r=-0.886$ at session 1 and $r=-0.867$ at session 20, with $p<0.001$ for both associations. These coefficients reflect the structured unloading profile of the robotic-training intervention and its progressive reduction of external support. They do not independently demonstrate that reduction in body-weight support mediated the observed functional changes.

Age was associated with body-weight-support measures in the expected direction, with older participants requiring greater external support. However, age did not differ meaningfully between the randomized groups in the parent trial. The observed relationship between treatment-group assignment and body-weight-support requirement was therefore not attributable to an apparent baseline age imbalance. Given the small and unequally allocated sample, these findings remain exploratory and were interpreted according to the magnitude, direction, and biomechanical coherence of the reported effects rather than as confirmation of a causal mediating pathway.

Table 1. Group × Time Interaction Effects for Muscle-Specific iEMG and Functional Outcomes

Outcome	F statistic	Partial η²	p-value
Gastrocnemius medialis iEMG, μV	219.969	0.924	<0.001
Vastus lateralis iEMG, μV	131.387	0.880	<0.001
Tibialis anterior iEMG, μV	109.845	0.859	<0.001
Timed Up and Go test	—	0.844	<0.001
Berg Balance Scale	—	0.702	<0.001
Walking Index for Spinal Cord Injury II	—	0.321	0.009
Biceps femoris iEMG, μV	1.176	0.061	0.293
10-Meter Walk Test	—	0.008	0.708

Values were derived from repeated-measures general linear models in the parent randomized trial and are ranked by effect magnitude within the muscle and functional domains. F statistics were included only where explicitly reported. iEMG, intramuscular electromyography; µV, microvolts. RAGT+tSCS group, n=13; CPT+tSCS group, n=7.

Table 2. Association Between Treatment-Group Assignment and Body-Weight-Support Requirement

Training session	Pearson's r	p-value
Session 1	−0.886	<0.001
Session 20	−0.867	<0.001

Treatment-group assignment was coded according to the parent trial analysis. Negative coefficients indicate that assignment to the RAGT+tSCS group was associated with the structured body-weight-support profile used in the robotic-training protocol. These protocol-related associations should not be interpreted as evidence of causal mediation. RAGT, robot-assisted gait training; tSCS, transcutaneous spinal cord stimulation.

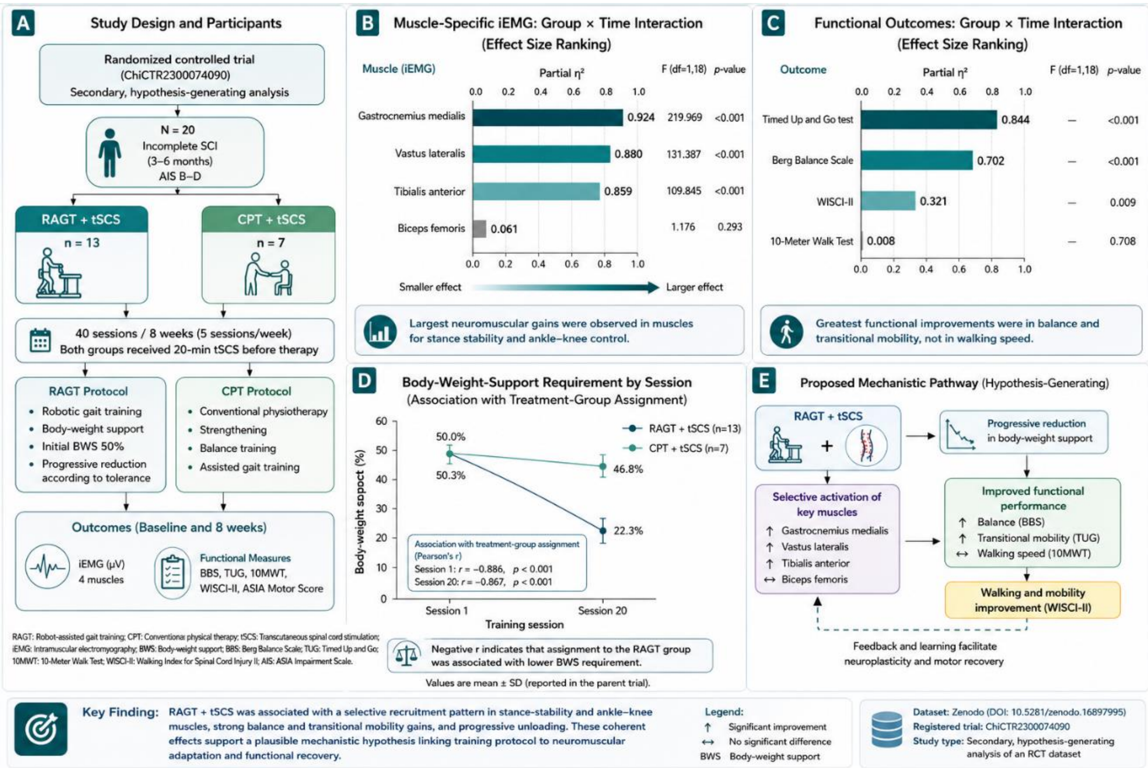

Figure 1 summarizes the secondary analysis of 20 participants with incomplete spinal cord injury allocated to RAGT+tSCS (n=13) or CPT+tSCS (n=7). The largest Group × Time effects were observed for gastrocnemius medialis, vastus lateralis, and tibialis anterior iEMG, alongside substantial improvements in Timed Up and Go and Berg Balance Scale scores, whereas biceps femoris recruitment and 10-Meter Walk Test performance showed minimal between-group differences. Progressive reduction in body-weight support was strongly associated with RAGT assignment, supporting a hypothesis-generating pathway linking selective ankle–knee muscle recruitment with improved balance and transitional mobility.

DISCUSSION

This secondary analysis indicated that the neuromuscular response to RAGT combined with tSCS was muscle-selective rather than uniformly distributed across the lower limb. The largest Group × Time effects occurred in the gastrocnemius medialis, vastus lateralis, and tibialis anterior, whereas the biceps femoris showed little evidence of differential recruitment between the treatment groups. This pattern corresponded with the larger treatment effects observed for balance and transitional mobility and with the absence of a between-group difference in walking speed. Collectively, the findings supported a biomechanically coherent, hypothesis-generating pathway in which progressive reduction of body-weight support during robotic training may have increased active loading demands on muscles involved in ankle control, knee extension, stance stability, and weight transfer.

The selective response of the gastrocnemius medialis, vastus lateralis, and tibialis anterior was consistent with the task-specific characteristics of robotic gait rehabilitation. RAGT provides repetitive stepping, standardized lower-limb trajectories, controlled loading, and intensive afferent input, all of which may facilitate activity-dependent reorganization within spared locomotor pathways after iSCI (7). The gastrocnemius contributes to plantarflexion, postural control, and forward progression during late stance, while the vastus lateralis supports knee extension and stability during weight acceptance. The tibialis anterior contributes to controlled ankle positioning and foot clearance. Preferential recruitment of these muscles was therefore compatible with the balance and mobility demands imposed by progressive body-weight-supported robotic stepping.

The potential contribution of tSCS should be interpreted as neuromodulatory rather than independently restorative. Transcutaneous stimulation is considered to increase the responsiveness of posterior-root afferents, interneuronal networks, and motor pools to residual descending and task-related sensory inputs (8,9). Exercise performed while spinal networks are maintained in a more excitable physiological state may promote repeated activation of spared circuits and facilitate adaptive plasticity (10). In the present dataset, the three muscles most closely related to ankle–knee control and stance stability demonstrated partial η^2 values ranging from 0.859 to 0.924. Although these effects were unusually large, their interpretation should account for the small sample, repeated-measures design, structured differences between the interventions, and potential instability of effect estimates in an unequally allocated cohort.

The large effects for the Berg Balance Scale and Timed Up and Go test were consistent with the muscle-specific iEMG findings. Both outcomes depend on postural stability, controlled weight transfer, turning, and transitional movement. Improved recruitment of the vastus lateralis, gastrocnemius medialis, and tibialis anterior could plausibly support these functional tasks through enhanced knee stabilization, ankle control, and stance-phase loading. Previous evidence has suggested that robotic and body-weight-supported gait training may improve walking and balance after motor-incomplete spinal cord injury, although effects vary according to injury severity, device characteristics, intervention dose, and outcome selection (11,12,19). The present analysis extended this literature by identifying a candidate muscle-level pattern that corresponded with the clinical domains showing the clearest treatment-related improvement.

The relationship between treatment-group assignment and body-weight-support requirement provided further contextual support for this interpretation. Assignment to the RAGT+tSCS group was strongly associated with the body-weight-support profile at session 1 and session 20. This association primarily reflected the design of the robotic intervention, in which external support was progressively reduced according to tolerance. As support decreased, participants were required to accept a greater proportion of their body weight through the lower extremities. This may have increased the repeated activation demands placed on the knee extensors and ankle musculature. Previous randomized evidence has similarly suggested that combining tSCS with robotic-assisted, body-weight-supported treadmill training may enhance motor and gait-related recovery in people with iSCI (16).

Nevertheless, the reported correlations did not establish mediation. Treatment-group assignment was a binary protocol indicator rather than a biological exposure measured on a continuous scale, and body-weight support was an intervention parameter rather than an independently randomized mediator. The analysis also lacked repeated temporal measurements capable of demonstrating that reductions in body-weight support preceded changes in iEMG and that these neuromuscular changes subsequently preceded functional improvement. Consequently, progressive unloading should be regarded as a candidate shared driver requiring prospective evaluation rather than a confirmed mechanism.

The lack of a between-group difference in the 10-Meter Walk Test was not necessarily inconsistent with the substantial effects observed for muscle activation, balance, and transitional mobility. Over-ground walking speed depends on several components that were not comprehensively represented by the four sampled muscles, including hip extension, limb advancement, intersegmental coordination, cardiovascular capacity, and propulsive force generation. The biceps femoris showed similar gains in both groups and no significant Group × Time interaction, suggesting that swing-related recruitment may not have been preferentially enhanced by the robotic protocol. Harness-supported treadmill practice may initially emphasize stability, controlled loading, and repetitive stepping more strongly than rapid over-ground propulsion. Improvements in walking speed may therefore require additional task-specific over-ground and speed-oriented training.

The modest but significant effect observed for WISCI-II may indicate that participants achieved clinically relevant changes in walking assistance or device requirements even when measured walking speed did not differ between groups. Functional walking scales and timed speed tests assess related but distinct aspects of mobility. WISCI-II reflects the level of physical assistance, braces, and walking devices required, whereas the 10-Meter Walk Test primarily quantifies speed over a short distance. This distinction reinforces the need to evaluate rehabilitation outcomes across complementary domains rather than assuming that improvements in postural control, independence, and speed occur simultaneously.

The use of iEMG was an important strength because it provided greater anatomical specificity and reduced the risk of cross-talk from adjacent muscles compared with surface recordings (13,21). The randomized parent-trial design, standardized tSCS exposure, matched treatment frequency, and collection of both neuromuscular and clinical outcomes further strengthened the internal coherence of the analysis. The combination of muscle-level measurements with balance, mobility, and protocol-related data enabled a more mechanistically informative interpretation than would have been possible from clinical scales alone. Previous research on wearable robotic systems has also supported their feasibility in SCI rehabilitation, although safety, device tolerance, and individualized progression remain important considerations (22).

Several limitations require emphasis. The analysis was post-hoc and was not prespecified as a causal mediation analysis in the original trial protocol. The total sample was small and unequally distributed between groups, limiting statistical precision and increasing the likelihood that effect estimates were inflated. Only four muscles were sampled, and the analysis did not include important hip extensors, hip flexors, trunk stabilizers, or additional muscles contributing to propulsion and swing. iEMG normalization and longitudinal recruitment trajectories could not be further examined using the statistics presented in the manuscript. The body-weight-support correlations largely reflected the intervention structure and should not be interpreted as independent evidence of a biological mechanism. No formal indirect effects, multiplicity-adjusted analyses, or adjusted multivariable models were performed. Generalizability was also limited by the single-centre setting, intermediate post-injury period, restricted AIS range, specific robotic device, and fixed stimulation protocol.

Future trials should prespecify mechanistic analyses and collect body-weight-support, iEMG, and functional measurements at multiple intermediate time points. Formal mediation models with bootstrapped confidence intervals could then assess whether changes in body-weight support precede muscle-specific recruitment gains and whether these gains statistically account for subsequent functional improvement. Broader EMG sampling should include hip extensors, hip flexors, trunk musculature, and additional swing- and propulsion-related muscles. Combining iEMG with motor-evoked potentials or other measures of corticospinal excitability may also help distinguish spinal, supraspinal, and peripheral contributions to recovery. Double-blind studies have shown that tSCS can modify quadriceps motor-evoked responses, supporting the inclusion of neurophysiological measures alongside functional outcomes (17). Trials integrating neuromodulation with structured robotic and over-ground exercise may further clarify how training specificity influences motor and autonomic recovery (18).

Clinically, the findings suggested that the earliest observable benefits of RAGT combined with tSCS may occur in postural control, weight transfer, and transitional mobility rather than walking speed. Progressive body-weight-support reduction may therefore be an important treatment parameter to document and individualize rather than a fixed technical setting. However, unloading should be advanced according to participant safety, movement quality, fatigue, and neurological capacity. The findings supported continued investigation of muscle-specific recruitment as a potential biomarker of functional recovery but did not establish that the identified muscles or unloading trajectory causally mediated the treatment effect.

CONCLUSION

This secondary analysis showed that RAGT combined with tSCS was associated with selective recruitment gains in the gastrocnemius medialis, vastus lateralis, and tibialis anterior, corresponding with larger improvements in balance and transitional mobility but not walking speed. The alignment of these neuromuscular effects with progressive body-weight-support reduction supported a biomechanically plausible, hypothesis-generating pathway linking robotic training demands with functional recovery. These findings did not establish causal mediation and require confirmation in adequately powered trials that prospectively measure unloading, muscle-specific activation, and functional outcomes over time.

REFERENCES

1. Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, et al. Spinal cord injury: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 2023;8(1):245.

2. Bennett J, Das JM, Emmady PD. Spinal cord injuries. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.

3. Safdarian M, Trinka E, Rahimi-Movaghar V, Thomschewski A, Aali A, Abady GG, et al. Global, regional, and national burden of spinal cord injury, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol. 2023;22:1026–47.

4. Liu C, Xu T, Xia W, Xu S, Zhu Z, Zhou M, et al. Incidence, prevalence, and causes of spinal injuries in China, 1990–2019: findings from the Global Burden of Disease Study 2019. Chin Med J. 2024;137(6):704–10.

5. Zhang H, Liu Y, Zhou K, Wei W, Liu Y. Restoring sensorimotor function through neuromodulation after spinal cord injury: progress and remaining challenges. Front Neurosci. 2021;15:749465.

6. Li X, Lu Z, Wang I, Li L, Stampas A, Zhou P. Assessing redistribution of muscle innervation zones after spinal cord injuries. J Electromyogr Kinesiol. 2021;59:102550.

7. Bin L, Wang X, Jiatong H, Donghua F, Qiang W, Yingchao S, et al. The effect of robot-assisted gait training for patients with spinal cord injury: a systematic review and meta-analysis. Front Neurosci. 2023;17:1252651.

8. Taylor C, McHugh C, Mockler D, Minogue C, Reilly RB, Fleming N. Transcutaneous spinal cord stimulation and motor responses in individuals with spinal cord injury: a methodological review. PLoS One. 2021;16(11):e0260166.

9. Hofstoetter US, Freundl B, Binder H, Minassian K. Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: elicitation of posterior root-muscle reflexes. PLoS One. 2018;13(1):e0192013.

10. Bilchak JN, Caron G, Côté MP. Exercise-induced plasticity in signaling pathways involved in motor recovery after spinal cord injury. Int J Mol Sci. 2021;22(9):4858.

11. Nam KY, Kim HJ, Kwon BS, et al. Robot-assisted gait training using Lokomat improves walking function and activity in people with spinal cord injury: a systematic review. J Neuroeng Rehabil. 2017;14:24.

12. Liu S, Chen F, Yin J, Wang G, Yang L. Comparative efficacy of robotic exoskeleton and conventional gait training in patients with spinal cord injury: a meta-analysis of randomized controlled trials. J Neuroeng Rehabil. 2025;22(1):121.

13. Al-Ayyad M, Owida HA, De Fazio R, Al-Naami B, Visconti P. Electromyography monitoring systems in rehabilitation: a review of clinical applications, wearable devices and signal acquisition methodologies. Electronics. 2023;12(7):1520.

14. Rashad A, He X, Wang H, Han X, Xiao Z, Jing Y, et al. Transcutaneous spinal cord stimulation combined with robot-assisted gait training to improve mobility and balance in spinal cord injury: intramuscular electromyography as a biomarker for functional recovery. Primary trial report.

15. Rashad A. SPSS dataset: intramuscular electromyography as a biomarker of functional recovery [dataset]. Zenodo. 2025. Available from: https://doi.org/10.5281/zenodo.17424997

16. Comino-Suárez N, Moreno JC, Megía-García Á, Del-Ama AJ, Serrano-Muñoz D, Avendaño-Coy J, et al. Transcutaneous spinal cord stimulation combined with robotic-assisted body weight-supported treadmill training enhances motor score and gait recovery in incomplete spinal cord injury: a double-blind randomized controlled clinical trial. J Neuroeng Rehabil. 2025;22(1):15.

17. Megía-García Á, Serrano-Muñoz D, Taylor J, Avendaño-Coy J, Comino-Suárez N, Gómez-Soriano J. Transcutaneous spinal cord stimulation enhances quadriceps motor-evoked potential in healthy participants: a double-blind randomized controlled study. J Clin Med. 2020;9(10):3275.

18. Shackleton C, Samejima S, Williams AM, Malik RN, Balthazaar SJ, Alrashidi A, et al. Machine training: motor and autonomic concomitant health improvements with neuromodulation and exercise training: a randomised controlled trial in individuals with spinal cord injury. BMJ Open. 2023;13(7):e070544.

19. Arroyo-Fernández R, Menchero-Sánchez R, Pozuelo-Carrascosa DP, Romay-Barrero H, Fernández-Maestra A, Martínez-Galán I. Effectiveness of body weight-supported gait training on gait and balance for motor-incomplete spinal cord injuries: a systematic review with meta-analysis. J Clin Med. 2024;13(4):1105.

20. Hutson TH, Di Giovanni S. The translational landscape in spinal cord injury: focus on neuroplasticity and regeneration. Nat Rev Neurol. 2019;15(12):732–45.

21. Rodrigues C, Fernández M, Megía Á, Comino N, Del-Ama A, Gil-Agudo Á, et al. Comparison of intramuscular and surface electromyography recordings towards the control of wearable robots for incomplete spinal cord injury rehabilitation. In: Proceedings of the 2020 8th IEEE RAS/EMBS International Conference for Biomedical Robotics and Biomechatronics; 2020.

22. Park GM, Cho SH, Hong JT, Kim DH, Shin JC. Effects and safety of wearable exoskeleton for robot-assisted gait training: a retrospective preliminary study. J Pers Med. 2023;13(4):676.