

Functional Electrical Stimulation of the Hamstring during gait: Effects on knee stability and voluntary movement control



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by

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Abstract – Background: Anterior cruciate ligament (ACL) injuries commonly reduce knee stability and increase joint loading, often leading to compensatory gait alterations that may increase injury risk. Functional electrical stimulation (FES) of the biceps femoris long head (BFLH) during the gait stance may improve knee stability by reducing harmful joint loading, but the effects on voluntary muscle control remain unclear.

Research question: This study examined whether FES of the BFLH during the stance phase of the gait reduces ACL-relevant knee joint loading in healthy adults and whether it alters voluntary muscle control. Additionally, the use of gluteus maximus (GLMAX) sEMG as a proxy for BFLH activation was assessed.

Method: Nine healthy participants walked on a treadmill under control and FES-assisted conditions. Kinematic, kinetic, and sEMG data were analyzed using statistical parametric mapping and linear mixed-effects models.

Results: FES of the BFLH significantly reduced internal knee rotation moment (KRM) with 9.37% during 42–48% of the gait cycle ($p = 0.0002$; $d = 0.42$). Knee adduction moment (KAM) showed non-significant reductions in both legs (non-stimulated: $p = 0.0317$, $d = 0.18$; stimulated: $p = 0.0492$, $d = 0.37$). Knee abduction angle (KAA) and knee rotation angle (KRA) showed no significant changes ($p > 0.05$). In sEMG analysis, inconsistent timing between GLMAX and BFLH activation indicated GLMAX is not a reliable surrogate for estimating BFLH activity. Regarding voluntary control, only peak KAM increased slightly over strides during FES-assisted walking ($p = 0.006$), possibly due to muscle fatigue. No significant retention or after-effects were observed.

Conclusion: Targeted FES of the BFLH can reduce ACL-relevant knee loading without impairing voluntary motor control. sEMG results highlight the need for direct BFLH monitoring, as GLMAX is an unreliable proxy. These findings support further exploration of FES strategies for ACL injury prevention and rehabilitation.

Keywords: Functional electrical stimulation; Biceps Femoris Long Head; Anterior Cruciate Ligament; Motor adaptation; Gait biomechanics

I INTRODUCTION

Injuries to the anterior cruciate ligament (ACL) are common and impactful in daily life, with approximately 400,000 ACL reconstruction surgeries performed annually in the United States alone [1]. The ACL plays a crucial role in maintaining dynamic knee stability by stabilizing anterior tibial translation (ATT) and resisting internal rotation of the tibia compared to the femur [2]. When the ACL is compromised, this stability and resistance to internal rotation are reduced, leading to altered knee mechanics and function.

After an ACL injury, individuals often develop persistent gait asymmetries and neuromuscular deficits. These include reduced knee flexion during stance, lower quadriceps activation, and decreased loading of the injured leg

[3]–[6]. These changes are believed to result from muscle weakness, reduced proprioceptive input, and altered motor control strategies aimed at protecting the joint [7], [8]. Although these adaptations may help reduce feelings of instability, they can also lead to changed movement patterns [9], a higher risk of second ACL injuries [10], and the early onset of knee osteoarthritis [11]–[13].

Addressing these gait alterations requires rehabilitation strategies that can both strengthen the surrounding musculature and promote more natural movement patterns during functional activities. Traditional rehabilitation after ACL injury typically follows one of two pathways: surgical reconstruction or conservative treatment through physiotherapy and neuromuscular training [14]. These conventional approaches focus on strengthening the muscles, improving proprioception, and restoring stability; However, some individuals continue to exhibit protective movement strategies even after undergoing these conventional rehabilitations [15]. This limitation has prompted the exploration of functional electrical stimulation (FES) as a novel therapeutic approach that can provide targeted muscular activation during walking to reduce compensatory strategies and support more normalized gait mechanics.

FES involves applying electrical currents to muscles to evoke contractions in a controlled and functional manner. FES may help reduce compensatory movement strategies by providing targeted muscular activation to increase knee stability during walking. Specifically, stimulation of the biceps femoris long head (BFLH) during the early stance phase increases posteriorly directed force on the tibia, which helps counteract ATT, a primary mechanism of ACL loading (see Figure 1). Additionally, BFLH activation contributes to controlling internal tibial rotation, another key factor associated with ACL strain [16], [17] (see Figure 1). By modulating these joint forces, FES may not directly alter gait kinematics but can reduce the mechanical demands placed on the ACL. This reduction in ACL loading may lessen the need for protective movement strategies and support a more natural gait pattern.

The BioStim short, developed by Biomex Ltd, builds on this concept by delivering FES to the BFLH during the early stance phase of gait. This wearable assistive device uses embedded inertial measurement units (IMUs) to predict gait events in real-time and apply closed-loop stimulation just before heel strike, aiming to enhance knee stability. While this targeted stimulation should offer promising support, it may also reduce voluntary motor control during gait. Such a decrease could negatively affect the rehabilitation process by limiting motor engagement and neuromuscular adaptation [18]. Consequently, this raises important questions about whether reliance on devices like the BioStim short might inter-

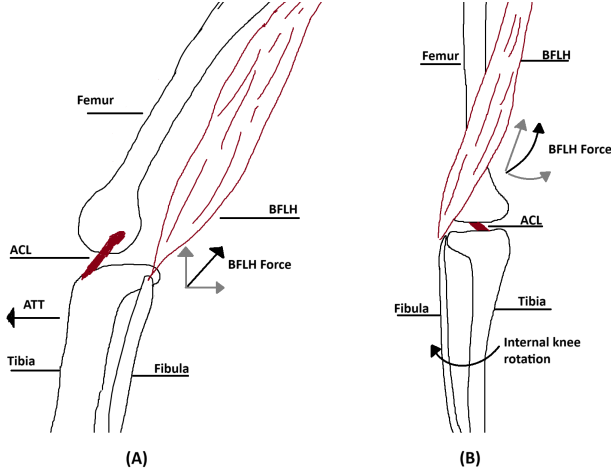


Figure 1: Biomechanical illustration of the BFLH of the left leg, highlighting its stabilizing effect on knee mechanics. (A) Sagittal view showing ATT, with the BFLH force counteracting this movement. (B) Posterior view showing internal tibial rotation, where the BFLH generates an external rotation moment.

interfere with the development of long-term voluntary motor control. Motor adaptation processes, including gradual adjustment over time during exposure, retention, and after-effects, are crucial indicators of whether an intervention, such as FES, leads to lasting changes in voluntary muscle control [19]. Additionally, changes in muscle excitation patterns can be measured by recording the electromyography (EMG) of the BFLH, which would be ideal for detecting alterations in motor unit recruitment. But stimulation artifacts caused by electrical stimulation make direct BFLH recordings during FES unreliable [20]. While GLMAX and BFLH activation patterns are correlated during normal voluntary gait [21], this relationship may not hold during FES-assisted gait where BFLH is activated artificially.

The objective of this study is to evaluate whether FES applied to the BFLH during the early stance phase can reduce biomechanical factors associated with increased ACL loading during gait in healthy individuals and whether this targeted FES influences voluntary muscle activation. Additionally, the study aims to investigate whether GLMAX sEMG patterns can serve as a surrogate marker for BFLH activation timing during FES-assisted gait, given the challenges of recording reliable BFLH signals during FES.

We hypothesize that applying FES to the BFLH during the early stance phase will (1) decrease biomechanical factors associated with ACL loading, and (2) maintain voluntary muscle activation patterns as observed before stimulation. Additionally, we expect that GLMAX sEMG will correlate positively with BFLH activation

and serve as a reliable indirect measure during FES-assisted gait.

To achieve this, FES will be applied to the BFLH of the dominant leg during treadmill walking while measuring knee biomechanics and muscle activation using motion analysis and electromyography. Healthy participants are used to establish the baseline biomechanical and neuromuscular effects of FES without the confounding factors present in ACL patients. The results from this study will provide insight into the mechanisms by which FES may improve gait stability, informing future rehabilitation strategies for individuals recovering from ACL injury.

II METHODS

A Methods and Materials

A.1 Participants

This study was approved by the Human Research Ethics Committee (HREC) of Delft University of Technology, and all procedures conformed to the principles of the Declaration of Helsinki. Before participation, all subjects received a verbal and written explanation of the study protocol and provided written informed consent. Ten healthy adults (5 male, 5 female; age: 24.40 ± 1.08 years; height: 1.75 ± 0.10 m; BMI: 21.66 ± 2.67 kg/m²; further details reported in Appendix A) were recruited. Inclusion criteria required participants to be free from any lower limb injuries, such as sprains, strains, fractures, or joint conditions involving the hip, knee, ankle, or foot, that could affect normal walking behavior. Participants were also required to have no known neurological disorders that could influence motor control or sensory function. In addition, they needed to be capable of walking continuously for at least 20 minutes without assistance, to ensure sufficient physical endurance. Individuals with prior experience using functional electrical stimulation (FES) for assisted walking were excluded to avoid any potential influence of habituation or neuromuscular adaptation to electrical stimulation.

Due to data loss during the experiment with Participant 3, accurate kinematic measurements and gait event detection could not be established. Consequently, this participant's data were excluded from the kinematic and sEMG analyses. Therefore, the final sample included nine participants whose data were used for the analyses.

A.2 Experimental Protocol

Each participant completed two treadmill walking trials in a single session at the Arthrose Research Lab (MOBI Lab) at Erasmus MC (see Figure 2). Each trial consisted of three consecutive walking periods: a 10-minute control

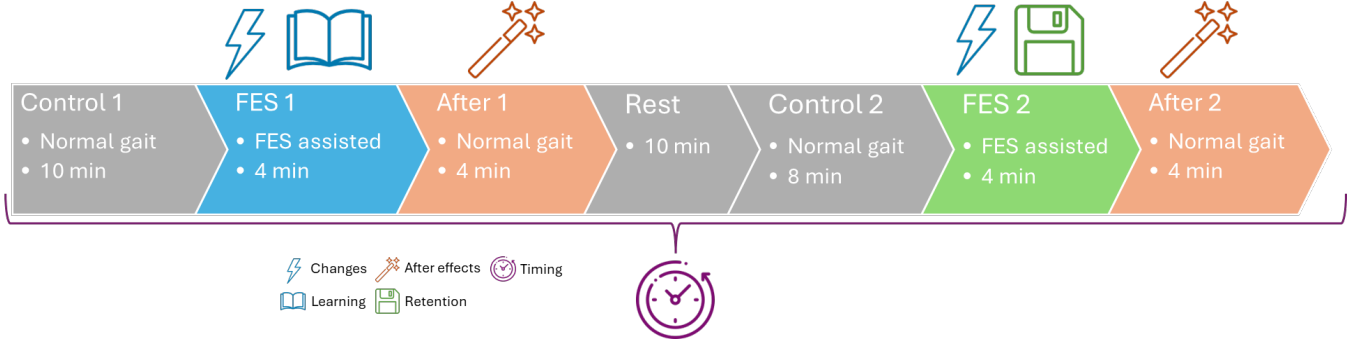


Figure 2: Overview of the experimental phase timing, illustrating the sequence, type of gait performed, and duration of each phase. Horizontal bars represent time intervals (in minutes) for different gait conditions—Normal Walking, FES-Assisted Walking, and Rest—presented in chronological order. Phase labels indicate the name and duration of each segment. Symbols positioned above each phase indicate the specific research questions or effects (e.g., baseline, learning (gradual adjustment over time during exposure), after-effect, retention) that the phase was designed to address.

period (including 6 minutes of acclimatization [22] and 4 minutes of normal walking), a 4-minute FES-assisted gait period where electrical stimulation was applied to the BFLH, and a 4-minute after-period to assess residual effects once stimulation ceased. Participants walked continuously without stopping as the treadmill conditions transitioned between periods, with a 10-minute rest between the two trials to minimize fatigue.

The control period establishes baseline gait mechanics and muscle activity before stimulation. The FES-assisted gait period assesses the immediate effects of electrical stimulation on knee biomechanics and muscle activation, while also allowing observation of any gradual adaptations that occur during exposure. The after-period assesses any residual effects once stimulation stops. Comparing the two trials further enables investigation of retention across repeated FES exposures.

Before the treadmill walking trials, a total of fifty-four retroreflective markers were attached to the anatomical bony landmarks of the participants following the CGM 2.5 protocol [23] for motion capture. An electrical stimulator was secured to the hip of the participant and connected to two transcutaneous electrodes placed over the BFLH of the dominant leg. A footswitch, positioned inside the shoe of the same leg, was used to trigger stimulation. Leg dominance was determined by asking which leg the participant would use to kick a ball [24]. Additionally, two surface electromyography (sEMG) electrodes were placed over the GLMAX and BFLH of the non-dominant leg to record muscle activity without interference from the stimulation signal (see Figure 3).

Following the placement of all markers, electrodes, and stimulation equipment, a proxy measure of participants’ fitness was obtained using a handgrip strength test with a Jamar dynamometer [25]. Furthermore, maximum voluntary contractions (MVCs) of the GLMAX and BFLH

were performed to normalize the sEMG signals. Walking trials were conducted on a treadmill equipped with integrated force plates (MotekForce Link). Speed was individualized by increasing in 0.1 m/s increments from 1.0 m/s until participants reported an uncomfortably fast pace, then reduced to their last comfortable speed, which was used throughout the trials.

A.3 Electrical stimulation

The FES-assisted gait was achieved by stimulating the BFLH of the dominant leg for a duration of 300ms at the heel strike. This stimulation duration covered approximately 50% of the stance phase [26]. Stimulation was triggered by a footswitch mounted under the dominant heel and connected to the stimulator via a lead. An additional device was used to create the connection between the footswitch and the stimulator, shown in Appendix C, ensuring precise activation upon heel strike.

FES was delivered using two surface electrodes (ON-EVER, B0CYPGFL6K 50x50mm), placed over the proximal and distal motor points of the BFLH on the dominant leg. One electrode was positioned at the midpoint between the ischial tuberosity and the lateral epicondyle of the tibia, and the second electrode was placed 7 cm distal to the first [16] (see Figure 3). Before electrode placement, the skin was cleaned with 70 percent isopropyl alcohol and shaved if necessary. Both the electrode and footswitch leads were attached to the participant’s leg to avoid accidental pulls on the leads that could interrupt the stimulation or cause tripping.

The electrical stimulator (Micromed ENERGY, Micromed S.p.A., Italy, or Odstock ODFS Pace, Odstock Medical Ltd., UK) delivered an asymmetrical pulse waveform with the following parameters: 350 μ s pulse duration, 40 Hz stimulation frequency, and intensity

ranging from 10-100 mA. No ramping (rising or falling) was applied. These parameters were based on previous research by Martijn Kok [27]. The stimulation current was individually adjusted to elicit a visible contraction of the BFLH, confirming correct electrode placement. The absence of visible contractions in surrounding muscles further verified the specificity and accuracy of the electrode positioning. After confirming correct placement, the current was increased to the maximum comfortably tolerated intensity. This decision was based on preliminary testing of lower intensities, which showed that lower intensities had minimal effects on kinematics (see Appendix D for details).

B Data acquisition

B.1 Kinematics and kinetics

Marker trajectories during all walking trials were recorded via motion capture at 100 Hz in Nexus using a system of 11 VICON cameras (Vicon Vero V1.3). Before testing each participant, the motion capture system and camera positions were calibrated. Ground reaction forces were recorded at 1000 Hz using two integrated force plates in the treadmill (Motek-Force Link), one for the left leg and one for the right leg. The force plates were zeroed before each participant to ensure accurate kinetic measurements.

B.2 Surface Electromyography

Muscle activation of the GLMAX and BFLH of the non-stimulated leg was recorded at 2000 Hz via surface electromyography (sEMG) (COMETA WaveX MiniX) during the entire experiment. Electrodes were placed following the SEMIAM guidelines [28]. A set of bipolar electrodes was placed halfway between the sacral vertebrae and the greater trochanter for the GLMAX, and another set midway between the ischial tuberosity and the lateral epicondyle of the tibia for the BFLH [21] (see Figure 3). Disposable ECG electrodes (Kendall H124SG) were attached to the designated locations in the direction of the muscle fibers with a 4cm distance between the centers of the electrodes. Skin preparation before placement included shaving if needed and cleaning with 70% isopropyl alcohol.

C Data analysis

Data analysis was performed via Python (version 3.11, Python Software Foundation <https://www.python.org/>)

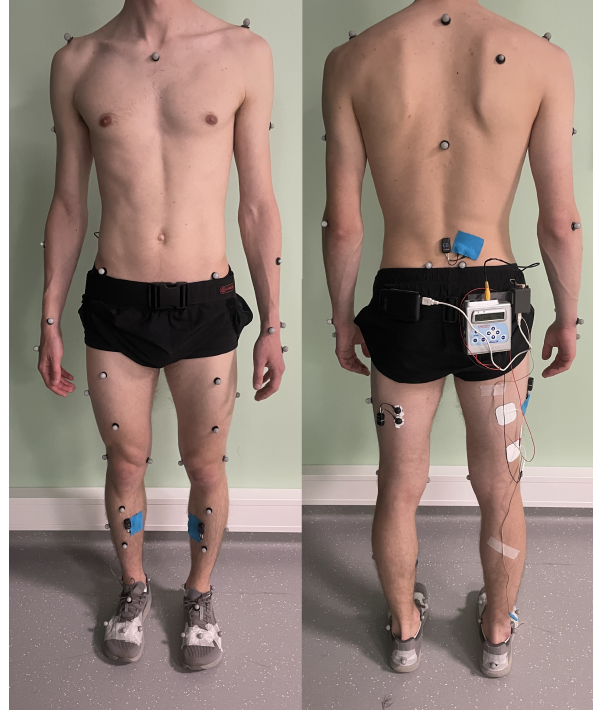


Figure 3: Location of the retro-reflective markers for motion capture, electrodes for electrical stimulation, and sEMG sensors for muscle timing measurements

C.1 Kinematics and Kinetics (Inverse dynamics)

Motion capture marker trajectories and ground reaction forces were filtered using a 4th-order low-pass Butterworth filter with a cut-off frequency of 10 Hz to remove high-frequency noise and non-human movement artifacts. To synchronize kinetic and kinematic data sampled at different rates (force plates at 1000 Hz, motion capture at 100 Hz), force plate signals were downsampled to 100 Hz following filtering. This ensured alignment of ground reaction force data with marker trajectories for accurate inverse dynamics analyses.

The 54 retro-reflective markers placed on anatomical landmarks were used to define local coordinate systems for the lower limb segments according to the Conventional Gait Model 2.5 (CGM2.5) protocol [23]. Participant-specific anthropometric measurements such as body mass, segment lengths, and joint widths were used to estimate segment masses, centers of mass, and moments of inertia. These values enabled individualized, segment-based inverse dynamics calculations.

Joint angles of the limbs were computed using the py-CGM2.5 toolbox, which internally applies the OpenSim inverse kinematics solver. This solver minimizes the squared differences between experimental and model marker positions to estimate time-varying joint angles.

The resulting angles were decomposed according to the Grood and Suntay joint coordinate system [29], defining flexion/extension, abduction/adduction, and internal/external rotation for each joint for each leg.

Net joint moments were then calculated via segment-based inverse dynamics by combining the joint kinematics with ground reaction force data recorded from the treadmill’s integrated force plates. These inverse dynamics calculations, based on Newton-Euler equations, incorporated the participant-specific anthropometric parameters.

The primary outcome measures derived from the kinematic and kinetic analyses were the parameters that have the most effect on the load of the ACL during gait: knee rotation angle (KRA), knee rotational moment (KRM), knee adduction angle (KAA), and knee adduction moment (KAM) [30]. Gait data was first segmented into individual strides per leg using heel strike events. Each stride was time-normalized and interpolated to 101 data points, representing 0–100% of the gait cycle in 1% increments, allowing for direct stride-to-stride comparisons for both stimulated and non-stimulated legs.

Gradual adjustments, retention, and after-effects over time were assessed by grouping normalized strides into non-overlapping bins of 15 consecutive strides, resulting in approximately 12 bins per condition per participant. This bin size was chosen to provide a balance between reducing variability from individual strides and maintaining enough temporal resolution to capture meaningful changes in motor adaptation. Within each bin, peak values were calculated for the KRM and KAM, while the excursion during the stance phase was calculated for the KRA and KAA. This binning approach enabled a structured assessment of motor adaptation across the various conditions of the experiment. The gradual adjustment over time, retention, and after-effects were only analyzed for the stimulated leg.

C.2 Surface Electromyography

Surface electromyography (sEMG) signals were preprocessed using a 4th-order Butterworth band-pass filter with a passband of 15 to 450 Hz to attenuate motion artifacts, DC offset, and high-frequency noise. Following this, a Fast Fourier Transform (FFT) spectrum analysis was performed, and additional notch filters were applied to remove any disturbance caused by the environment. The filtered signals were then full-wave rectified and normalized to the maximum activation obtained during maximum voluntary contraction (MVC) trials for each muscle.

Muscle activation timing was quantified by first grouping the gait cycles into bins of 15 strides each. This bin size was selected to balance minimizing variability in

the sEMG signals while preserving the temporal detail needed to observe changes in muscle activation patterns. For each bin, the mean sEMG curves for GLMAX and BFLH muscles were computed. To identify peak muscle activity, peak detection was applied to the averaged sEMG curves within a time window of $\pm 20\%$ centered on the heel strike event. Finally, the timing difference between the detected peaks in the GLMAX and BFLH signals was calculated by subtracting the GLMAX peak timing from the BFLH peak timing, providing a measure of relative muscle activation timing with reduced cycle-to-cycle variability.

C.3 Synchronization

The treadmill-embedded force plates, motion capture system, and surface electromyography (sEMG) recordings were synchronized within Vicon Nexus using a common trigger signal to ensure timing alignment across all data streams. Photographs were taken to document the placement of reflective markers, sEMG electrodes, and other sensors for quality control and reproducibility of the setup.

D Statistics

For the statistical analysis of the relationship between FES-assisted gait and the four outcome measures, a paired one-dimensional Statistical Parametric Mapping (SPM1D) model was employed to analyze the time-series data. SPM1D is a technique that allows for statistical testing across the entire gait cycle waveform rather than at discrete points, enabling identification of specific phases where significant differences occur. Before this, the Shapiro–Wilk test was conducted to assess the normality of the data distribution. Since normality was confirmed for the majority of the data, a paired parametric SPM1D t-test was applied to identify significant differences over the gait cycle.

Each of the four outcome measures for both legs (a total of eight tests) was compared between the Control and FES-assisted conditions using separate paired SPM1D t-tests. For each SPM1D test, the significance level (α) was initially set at 0.05, which accounts for multiple comparisons across the entire gait cycle (i.e., across all time points) within that single test. To control for multiple comparisons across the eight tests, a Bonferroni correction was applied, adjusting the overall significance level to:

$$\alpha = \frac{0.05}{8} = 0.00625.$$

Clusters with p-values below this corrected threshold were considered statistically significant. Additionally, Cohen’s d was calculated to estimate effect sizes within significant regions. For kinematic and kinetic variables,

data from both trials were averaged within each condition per participant before analysis.

When parametric SPM1D results showed clusters with uncorrected p -values below the initial threshold of $\alpha = 0.05$ but above the Bonferroni-corrected threshold ($0.00625 < p < 0.05$), corresponding non-parametric SPM1D t -tests were conducted to further evaluate these findings. These non-parametric tests were performed using the same uncorrected $\alpha = 0.05$ for initial cluster identification, and clusters were then assessed against the Bonferroni-corrected threshold ($\alpha = 0.00625$) in a confirmatory manner. This two-stage approach ensured consistency in inference while allowing potentially meaningful trends observed in parametric analyses to be further examined using distribution-free methods, which are more robust to violations of normality.

A linear mixed-effects model (LMM) was used to assess the relationship between stride count and the outcome measures for the stimulated leg only, focusing on the main effect of Condition and the Bin $\times$ Condition interaction. For all comparisons (Control vs. FES, FES1 vs. FES2, and Control1 vs. After1), both the main effect of Condition and the Bin $\times$ Condition interaction were examined. The Control versus FES comparison focused on adaptation over time, with the Bin $\times$ Condition interaction assessing gradual changes between conditions, and the main effect of Condition supporting the SPM{t} results by detecting any overall difference. The FES1 versus FES2 comparison assessed retention by testing whether the overall condition effect and the rate of change over bins differed between conditions. Finally, the Control1 versus After1 comparison aimed to identify after-effects, with the main effect of Condition evaluating overall change, and the Bin $\times$ Condition interaction investigating potential slopes indicative of relearning over time. All models included subject-level random intercepts to account for repeated measures and inter-subject variability. Since one LMM was fit per outcome measure, a Bonferroni correction was applied across the four outcome models, adjusting the significance threshold to $\alpha = 0.0125$ to control for the family-wise error rate.

To confirm that binning stride data did not mask transient effects, a sensitivity analysis was conducted comparing bin sizes of 5 versus 15 strides per data point. The results showed no meaningful differences in effect detection, supporting the robustness of the 15-stride binning approach.

The sEMG data were analyzed using LMMs to test condition effects on the timing differences between GLMAX and BFLH activations to see if the timing remains consistent between conditions and time. In the model, subject-level random intercepts were included to account for repeated measures and between-subject variability. Statistical significance was set at $\alpha = 0.05$ as only one

outcome measure was used.

III RESULTS

A Kinematic/Kinetic changes

The parametric SPM{t} analysis revealed a significant reduction in KRM for the stimulated leg during 42-48% of the gait cycle (cluster $p = 0.0002$), exceeding the Bonferroni-corrected significance threshold ($\alpha = 0.00625$). The mean difference was -0.0163 ± 0.0082 Nm/kg (95% CI: $[-0.0226, -0.0101]$; Cohen's $d = -0.418$). No significant clusters were found for the non-stimulated leg (cluster $p > 0.05$) (see Figure 4 and Figure 5).

KAM in the non-stimulated leg showed a reduction during 20-22% of the gait cycle in the parametric SPM{t} analysis (cluster $p = 0.0317$), with a mean difference of -0.0175 ± 0.0098 Nm/kg (95% CI: $[-0.0251, -0.0100]$). The mean Cohen's d in this interval was -0.181 (95% CI: $[-0.508, -0.115]$). However, this result did not meet the Bonferroni-corrected significance threshold ($\alpha = 0.00625$). The corresponding non-parametric SPM{t} test identified a partially overlapping cluster during 19-24% of the gait cycle (cluster $p = 0.0078$), with a mean difference of -0.0171 ± 0.0101 Nm/kg (95% CI: $[-0.0249, -0.0093]$). This result also did not meet the Bonferroni-corrected threshold (see Figure 6 and Figure 7).

KAM in the stimulated leg showed a reduction during 18-20% of the gait cycle in the parametric SPM{t} analysis (cluster $p = 0.0492$), with a mean difference of -0.0462 ± 0.0270 Nm/kg (95% CI: $[-0.0670, -0.0255]$). The mean Cohen's d in this interval was -0.371 (95% CI: $[-1.270, -0.246]$). This result did not reach the Bonferroni-corrected significance threshold. The corresponding non-parametric SPM{t} test identified a matching cluster during 18-20% of the gait cycle (cluster $p = 0.0391$), with the same mean difference. This result also did not pass the Bonferroni-corrected threshold (see Figure 6 and Figure 7).

No significant differences in KRA were observed between conditions for either leg. The parametric SPM{t} analysis did not identify any clusters with $p < 0.05$, and therefore, no non-parametric tests were performed (see Figure 8).

KAA in the non-stimulated leg showed a reduction during 19-21% of the gait cycle in the parametric SPM{t} analysis (cluster $p = 0.0494$), with a mean difference of -0.3261 ± 0.2095 Nm/kg (95% CI: $[-0.4872, -0.1651]$). The mean Cohen's d in this interval was -0.110 (95% CI: $[-0.234, -0.069]$). This result did not meet the Bonferroni-corrected significance threshold ($\alpha = 0.00625$). The corresponding non-parametric SPM{t} test identified a matching cluster during 16-23% of the gait cycle (cluster $p = 0.0039$), which did meet the

Bonferroni-corrected threshold. The mean difference in this interval was -0.3246 ± 0.2111 Nm/kg (95% CI: $[-0.4868, -0.1623]$). No significant differences in KAA were observed between conditions for the stimulated leg (cluster $p > 0.05$) (see Figure 9).

B Gradual adjustment, retention and after-effects

Results from the linear mixed-effects models (LMMs) are summarized in Table I, and changes in KRA stance excursion are illustrated in Figure 10. Significant main effects of Condition were observed in the comparison between control and FES-assisted gait for several outcomes: peak KRM ($p = 0.010$; $\beta = -0.026$ Nm/kg; 95% CI $[-0.045, -0.006]$), KRA stance excursion ($p < 0.001$; $\beta = -0.731^\circ$; 95% CI $[-1.108, -0.317]$), peak KAM ($p < 0.001$; $\beta = -0.045$ Nm/kg; 95% CI $[-0.058, -0.031]$), and KAA stance excursion ($p = 0.004$; $\beta = 0.234^\circ$; 95% CI $[0.080, 0.406]$), indicating overall differences between control and FES-assisted gait in both kinematic and kinetic measures. A significant Bin $\times$ Condition interaction was also found for peak KAM ($p = 0.006$; $\beta = 0.002$ Nm/kg; 95% CI $[0.001, 0.003]$), suggesting that during FES-assisted gait, peak KAM increased gradually over time by 0.002 Nm/kg per bin.

In the retention comparison between FES1 and FES2, significant Condition effects were observed for peak KAM ($p = 0.007$; $\beta = 0.018$ Nm/kg; 95% CI $[0.005, 0.031]$) and KRA stance excursion ($p < 0.001$; $\beta = 0.821^\circ$; 95% CI $[0.373, 1.257]$). In the after-effects comparison between control and post-stimulation, no significant main effects of Condition or Bin $\times$ Condition interactions were detected ($p > 0.0125$).

C GLMAX vs BLFH timing

The average timing lag between GLMAX and BFLH peak activations in the baseline condition (Control1) was approximately 86.8 ms (SE = 25.2 ms; 95% CI $[37.5, 136.2]$), which was significantly different from zero ($p = 0.001$). Compared to Control1, Control2 showed a non-significant increase in lag of 3.4 ms (SE = 2.3 ms; $p = 0.135$). FES1 was associated with a significant decrease in lag by 10.1 ms compared to Control1 (SE = 2.3 ms; 95% CI $[-14.6, -5.6]$; $p < 0.001$), indicating earlier GLMAX activation relative to BFLH. FES2 also showed a significant reduction in lag by 6.6 ms (SE = 2.5 ms; 95% CI $[-11.4, -1.8]$; $p = 0.008$). After1 and After2 did not differ significantly from Control1, with lag changes of -1.7 ms (SE = 2.3 ms; $p = 0.446$) and 0.5 ms (SE = 2.3 ms; $p = 0.836$), respectively.

Table I: Linear mixed-effects model (LMM) results for the four outcome measures: KAA, KAM, KRA, and KRM. Shown are the estimated fixed effects (β) for the main effect of Condition and the Bin $\times$ Condition interaction across comparisons assessing gradual adjustment (Control1 vs. FES1), retention (FES1 vs. FES2), and after-effects (Control1 vs. After1). Units: β for KAA and KRA in degrees ($^{\circ}$), and for KAM and KRM in Nm/kg.

	Control vs FES		FES1 vs FES2		Control vs After	
	Condition	Bin x Condition	Condition	Bin x Condition	Condition	Bin x condition
	P-value/ <i>Coef</i>	P-value/ <i>Coef</i>	P-value/ <i>Coef</i>	P-value/ <i>Coef</i>	P-value/ <i>Coef</i>	P-value/ <i>Coef</i>
KAA	0.004/	0.270/	0.094/	0.857/	0.084/	0.401/
stance excursion	0.243	-0.012	-0.172	-0.002	0.144	-0.009
KAM	0.000/	0.006/	0.007/	0.678/	0.745/	0.401/
peak	-0.045	0.002	0.018	-0.000	0.002	0.001
KRA	0.000/	0.797/	0.000/	0.540/	0.291/	0.360/
stance excursion	-0.731	0.007	0.815	0.017	0.212	0.023
KRM	0.010/	0.070/	0.429/	0.846/	0.088/	0.031/
peak	-0.026	0.002	0.005	-0.000	-0.013	0.002

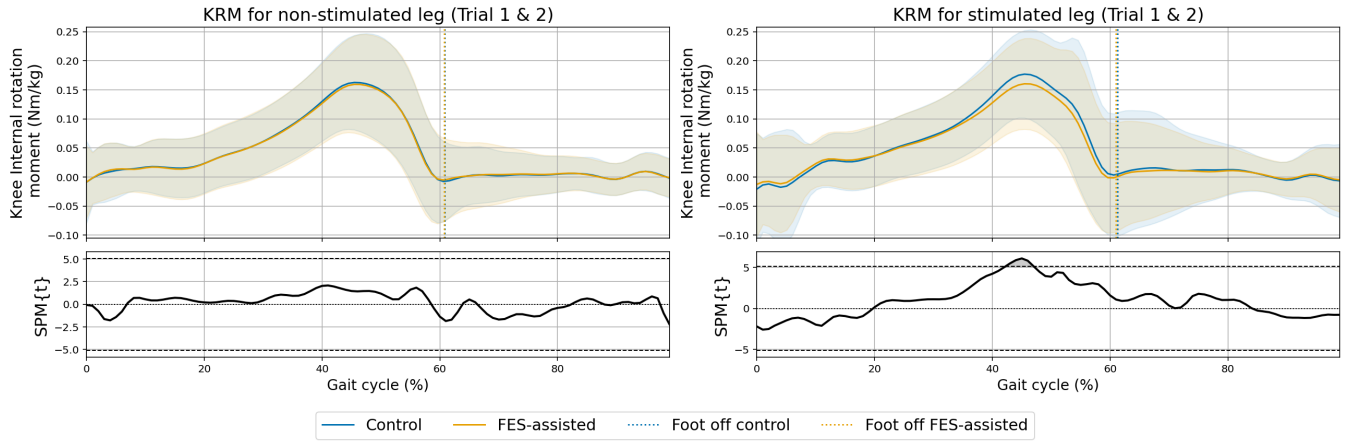


Figure 4: Mean KRM across the gait cycle for all participants during control (blue) and FES-assisted (orange) walking. Data are shown for the non-stimulated and stimulated legs, normalized to 0–100% of the gait cycle. Solid lines show group means; shaded areas represent ± 1 SD.

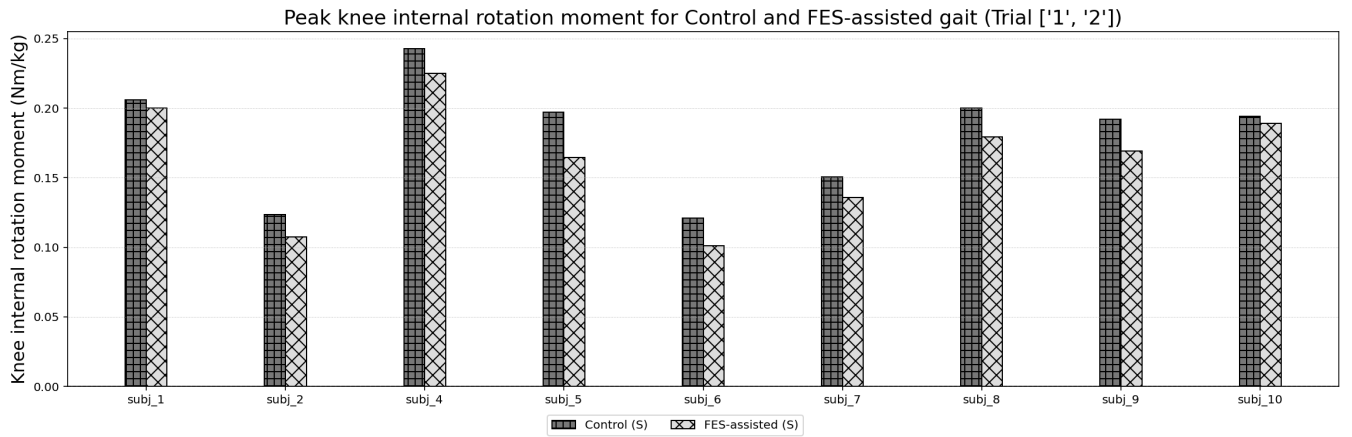


Figure 5: Mean Peak KRM across the entire gait cycle per participant during control and FES-assisted walking. Data are shown for the stimulated legs (S).

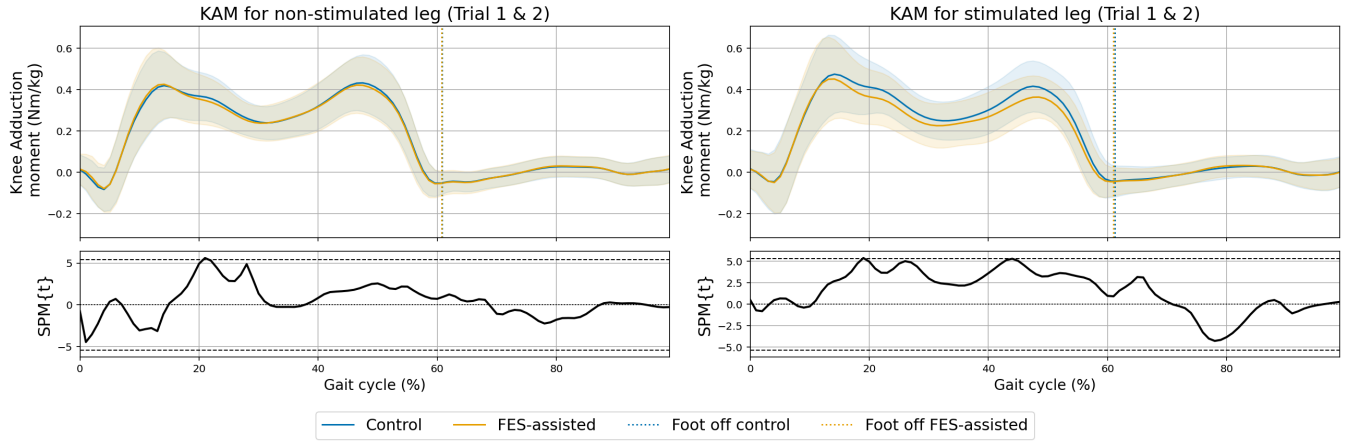


Figure 6: Mean KAM across the gait cycle for all participants during control (blue) and FES-assisted (orange) walking. Data are shown for the non-stimulated and stimulated legs, normalized to 0–100% of the gait cycle. Solid lines show group means; shaded areas represent ± 1 SD.

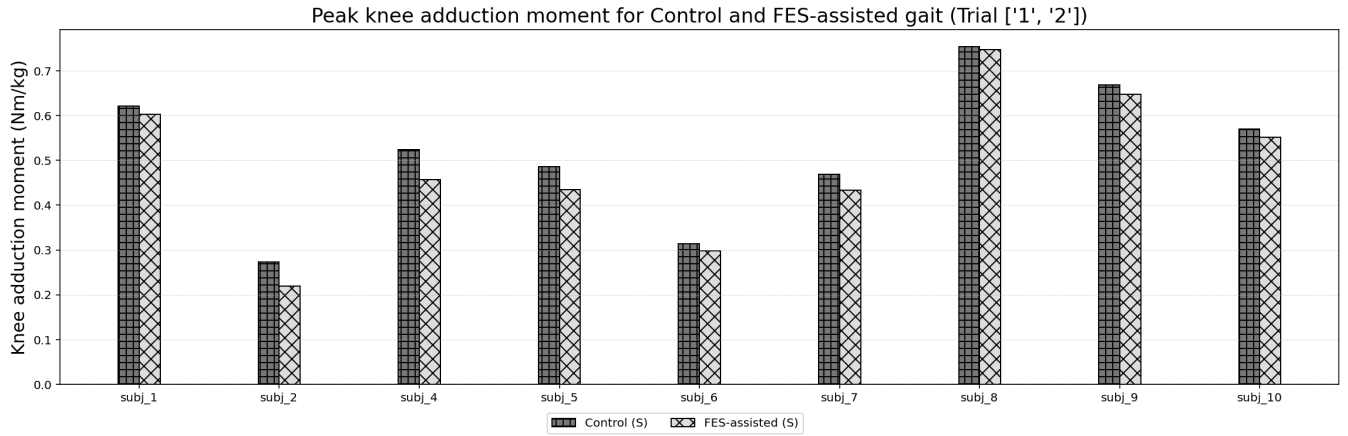


Figure 7: Mean Peak KAM for second peak in gait cycle (approx. 46% gait) per participant during control and FES-assisted walking. Data are shown for the stimulated legs (S).

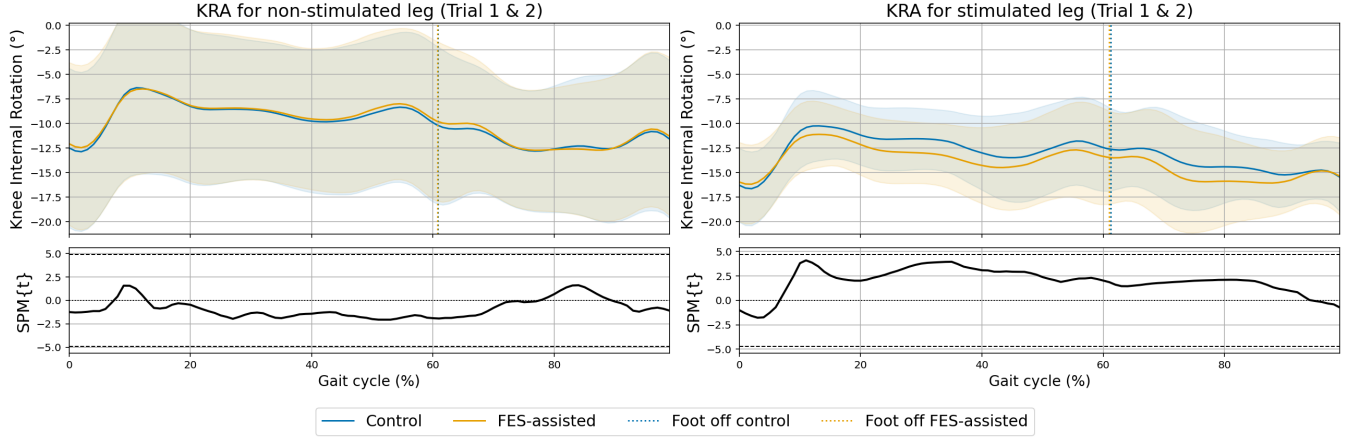


Figure 8: Mean KRA across the gait cycle for all participants during control (blue) and FES-assisted (orange) walking. Data are shown for the non-stimulated and stimulated legs, normalized to 0–100% of the gait cycle. Solid lines show group means; shaded areas represent ± 1 SD.

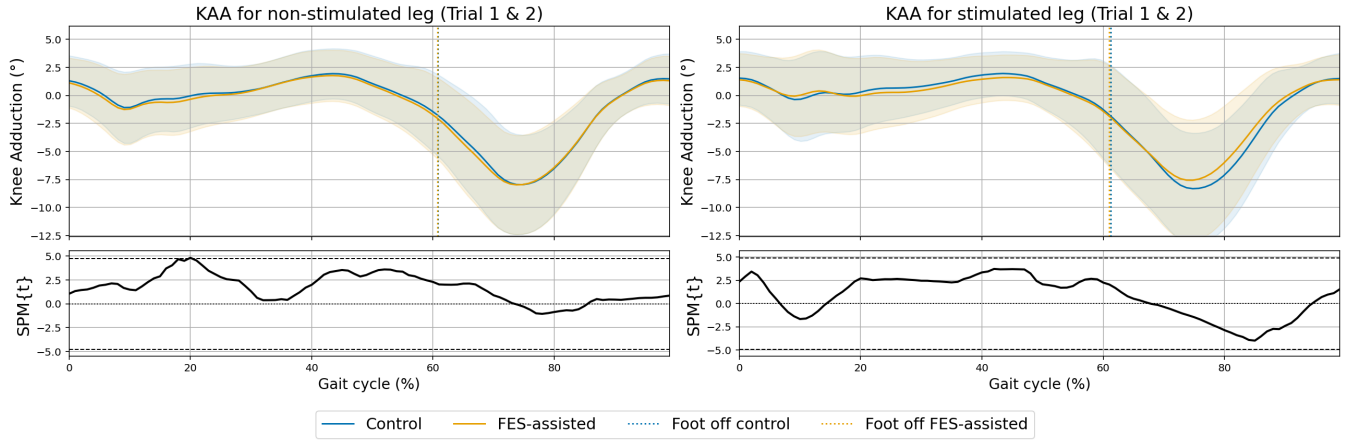


Figure 9: Mean KAA across the gait cycle for all participants during control (blue) and FES-assisted (orange) walking. Data are shown for the non-stimulated and stimulated legs, normalized to 0–100% of the gait cycle. Solid lines show group means; shaded areas represent ± 1 SD.

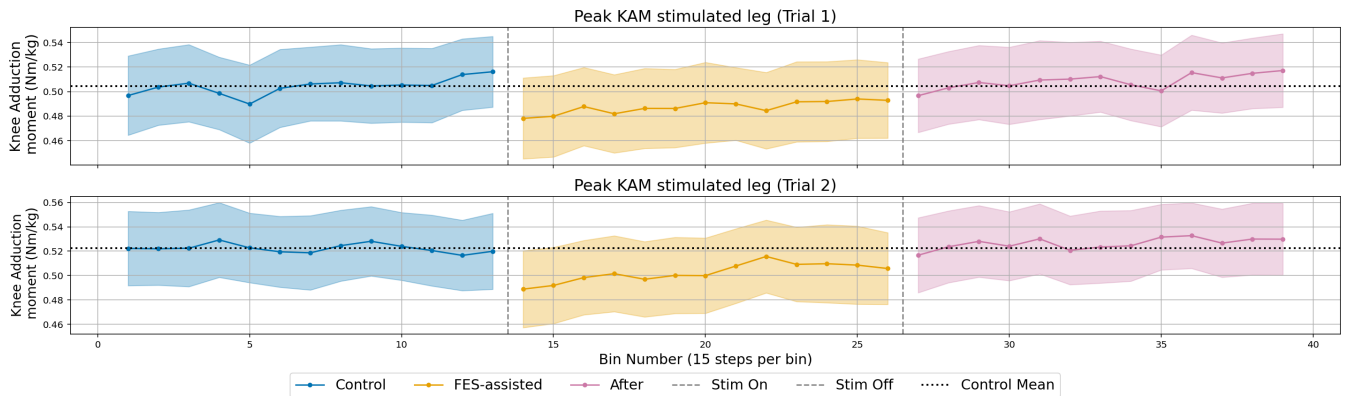


Figure 10: Peak KAM for the stimulated leg during control, FES-assisted, after shown for two trials (top: Trial 1, bottom: Trial 2). Data is binned in 15 steps. The control phase (blue) is followed by the FES-assisted phase (orange) and the after-effects phase (purple). Solid lines show mean Δ KAM; shaded areas represent ± 1 SEM.

IV DISCUSSION

This study investigated whether FES of the BFLH during the stance phase of gait could reduce ACL-relevant knee joint loading, specifically KRM and KAM, without disrupting voluntary motor control. The parametric SPM{t} analysis revealed a statistically significant reduction of 9.47% in KRM in the stimulated leg during 42–48% of the gait cycle (cluster $p = 0.0002$) (see Figure 4 and Figure 5). This finding suggests that FES effectively reduced rotational loading on the knee in a key phase of stance. For KAM, reductions were observed in both the non-stimulated and stimulated legs during early stance; however, these did not reach Bonferroni-corrected significance in either the parametric or confirmatory non-parametric analyses, though the direction and consistency of the effects suggest potential clinical relevance (see Figure 6 and Figure 7). No significant changes were observed for KRA, and only the non-stimulated leg showed a significant reduction in KAA in the non-parametric analysis, indicating a possible compensatory kinematic adaptation (see Figure 8 and Figure 9). Collectively, these results demonstrate that FES can reduce ACL-relevant knee loading, particularly through effects on rotational moments, while having a limited impact on frontal plane loading and joint angles. Building on these biomechanical findings, it was also critical to assess whether such stimulation influenced voluntary motor control. No significant evidence of lasting motor adaptation or disruption of voluntary motor control was found (see Table I). Additionally, sEMG analysis revealed that the timing between GLMAX and BFLH activation was inconsistent, indicating that GLMAX cannot reliably serve as a surrogate measure for BFLH activity during FES. Together, these results support the potential of FES as a short-term assistive intervention during early rehabilitation, without inducing long-term changes in voluntary motor control. However, further research is needed to confirm these preliminary biomechanical effects.

A Effect on kinematics and kinetics

Parametric SPM{t} analysis revealed a statistically significant reduction KRM of 9.47% in the stimulated leg during 42–48% of the gait cycle (cluster $p = 0.0002$). The mean difference was -0.0163 ± 0.0082 Nm/kg (95% CI $[-0.0226, -0.0101]$), with a moderate effect size (Cohen’s $d = -0.418$) (see Figure 4 and Figure 5). This time window corresponds to the typical peak in internal rotation moment during early stance, highlighting the mechanical relevance of the reduction.

This result is directionally consistent with Azmi et al. (2018) [16], who reported a 63% reduction in peak tibial

internal rotation torque, from 0.0012 ± 0.0010 Nm/BW during normal gait to 0.0005 ± 0.0004 Nm/BW with BFLH stimulation ($p = 0.032$). However, methodological differences likely account for the difference in the magnitude of the reduction. Azmi et al. (2018) applied higher stimulation intensities (excluding participants below 40 mA) and delivered stimulation throughout stance with a ramp-up before heel strike to ensure maximum BFLH activation at initial contact. In contrast, our mean stimulation intensity was 37.5 ± 7.62 mA, with some participants receiving less, and stimulation was limited to approximately 300 ms starting at heel strike without ramping, covering only the early stance phase [26]. This shorter, more abrupt stimulation window may have reduced the BFLH’s mechanical contribution during early stance, when internal rotation moments typically peak.

At the individual level, reductions in peak internal rotation moments were consistent across participants (see Figure 5), suggesting a reliable mechanical effect of BFLH stimulation. This outcome likely reflects the anatomical and functional role of the biceps femoris long head (BFLH), which originates from the tuber ischiadicum and inserts on the lateral fibula (see Figure 11). Positioned laterally across the knee, the BFLH generates an external rotation moment during contraction. When stimulated during early stance, a phase when internal rotation moments typically rise, this external moment can counteract ACL-relevant internal rotation loading.

Although the magnitude of reduction in KRM was moderate, the consistency of this effect, its statistical significance, and its alignment with ACL injury mechanisms suggest it may still be clinically meaningful. Similar reductions in internal rotation torque were reported by Azmi et al. (2018), supporting the reliability of this effect across different protocols. The ACL is particularly vulnerable to internal rotation forces, and even modest reductions in KRM could help reduce ligament strain, especially in individuals with compromised joint stability. It is also hypothesized that the unloading effect may be even greater in ACL-injured individuals, who often exhibit increased internal rotation during gait. This increase could lengthen the BFLH moment arm, enhancing its ability to resist internal rotation torque during FES.

These findings emphasize that targeted BFLH stimulation can reduce internal rotation loading during gait. While the observed changes were moderate, their consistency and anatomical basis suggest potential for meaningful clinical application. Future work should investigate longer activation periods, ramped or higher stimulation intensities, and larger, more diverse cohorts, particularly in ACL-deficient populations.

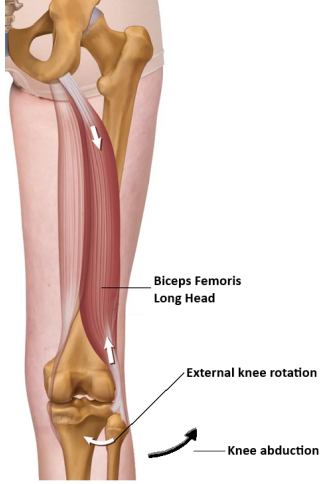


Figure 11: Anatomical position of the BFLH of the right leg (posterior view), arrows indicating the abduction and external rotation moments on the knee by BFLH contraction. (Modified image from Dr. Joe Muscolino. www.learnmuscles.com – art work Giovanni Rimasti)

B Voluntary motor control

The KRM was affected during FES-assisted walking, as indicated by a significant cluster identified in the SPM{t} analysis. Additionally, linear mixed-effects models revealed significant differences across all outcome measures, including peak values and stance excursion, further supporting the immediate impact of the intervention. However, it remains critical to understand how such stimulation influences voluntary motor control over time. To investigate this, we analyzed behavioral responses to assess whether repeated FES leads to lasting motor adaptation or changes in gait dynamics.

No evidence of lasting motor adaptation was found (see Table I), and only KAM showed a significant progression over time during FES-assisted walking compared to control. Specifically, KAM increased gradually across strides at a rate of 0.002 Nm/kg per bin ($p = 0.006$), which might indicate adaptation to the stimulation. A more likely explanation is muscle fatigue caused by repeated BFLH stimulation. Because FES repeatedly activates the same motor units, it accelerates fatigue onset compared to voluntary contractions [27], leading to a decline in muscle force output over time. Therefore, the increasing KAM observed may reflect reduced muscle effectiveness, diminishing the stimulation’s impact on KAM over time. In the retention comparison between FES1 and FES2, two significant differences were observed, most notably an increase of 0.815° ($p = 0.008$) in KRA stance excursion. However, these changes were already present at the start of FES2 and did not progress further across strides, as no progression over time was

found. This lack of within-session progression suggests that the effects observed in FES2 were not due to retained motor adaptation. Instead, they likely reflect residual muscle fatigue from previous trials and habituation to the stimulation sensation. When comparing control and post-stimulation (after) conditions, minimal changes were observed. Although KRM peak progression over time reached statistical significance ($p = 0.031$; $\beta = 0.002$ Nm/kg), the small effect size limits its clinical relevance. Furthermore, no meaningful changes were detected in other biomechanical variables, suggesting that any after-effects of FES were short-lived and functionally negligible.

These findings suggest that FES-assisted gait did not engage classical motor adaptation mechanisms. Classical adaptation is characterized by gradual error reduction across trials, aftereffects post-perturbation, and retention or faster relearning upon reexposure [31]. In contrast, our observed responses were immediate and reversible, without aftereffects or retention. This pattern aligns more with state-dependent motor responses, where motor output is directly influenced by external input (stimulation) without long-term adaptation [32]. In other words, FES applied to the BFLH during early stance produced transient motor behavior changes without lasting improvements in voluntary control. Similar conclusions were drawn by Kim et al. (2024) [33], who observed immediate gait improvements during FES-assisted walking but no aftereffects, gradual adjustment over time, or retention, despite using implicit stimulation. This further supports the interpretation that FES effects primarily reflect state-dependent motor responses rather than classical adaptation. A plausible explanation for this state-dependent response is the nature of the stimulation. The FES was strong and clearly perceived by participants, likely acting as an external cue rather than a subtle perturbation. Because state-dependent responses during walking vary depending on the task and timing within the gait cycle [34], the clearly perceived stimulation likely triggered immediate, context-specific motor adjustments that disappeared once the stimulation stopped.

However, both our study and Kim et al.’s (2024) involved only two brief (~4-minute) FES sessions, which may be insufficient to induce lasting changes in voluntary motor control or motor adaptation effects such as aftereffects. Previous research suggests that sustained motor improvements with FES typically require repeated exposure over many sessions, often up to 40, and are most effective when combined with active voluntary movement practice [19]. This is supported by studies demonstrating that when FES is applied consistently over longer durations, such as 8 weeks, it can lead to measurable improvements in voluntary motor control and functional

outcomes [35], [36].

C BFLH vs GLMAX timing

To further understand how FES influences neuromuscular coordination during gait, we specifically examined the timing between the BFLH and GLMAX muscles using sEMG data. This analysis focused on the non-stimulated leg under both FES-assisted and non-assisted walking conditions. Our findings revealed consistent timing patterns across most conditions, except for FES-assisted trials, where a notable reduction in lag was observed, indicating earlier GLMAX activation relative to BFLH.

The observed timing differences are likely influenced by the effects of FES on the stimulated leg. Our results showed that FES induced significant changes in knee kinematics during walking on the stimulated leg. These biomechanical alterations can lead to compensatory or adaptive changes in gait, affecting muscle activation patterns not only in the stimulated leg but also in the non-stimulated leg. Therefore, the shifts in timing between GLMAX and BFLH activation likely represent adjustments in coordination between limbs responding to changes in knee mechanics caused by FES, rather than changes in the muscle activation control.

Due to these adaptations, the timing relationship between GLMAX and BFLH activation can vary depending on the neuromechanical context induced by FES. As a result, using GLMAX as a proxy for BFLH activation becomes unreliable during FES. Therefore, directly measuring BFLH activation timing remains crucial when assessing muscle coordination during gait, particularly in scenarios involving neuromuscular interventions such as FES.

D Limitations/Future work

This study has several limitations. First, the participant sample was limited to healthy young adults aged 21 to 25. The small sample size of nine further limited the generalizability and statistical power of the findings. A small sample makes it harder to detect subtle but meaningful effects because of increased variability. Including more participants would improve statistical power and reduce the chance of false negatives. It would also increase confidence that any effects found are real. Expanding the sample to include more diverse populations, such as people with ACL deficiencies or older adults, could reveal stronger or more consistent responses to FES. These groups may have different neuromuscular control or joint loading patterns, which might make them more responsive. Such diversity would make the results more relevant and useful. Future research should recruit

larger and more diverse groups. It should also include power analyses to ensure the study can detect meaningful effects.

Second, no ramping was applied during FES, resulting in immediate full contraction of the BFLH muscle upon stimulation. Although this ensured consistent activation, it may have reduced participant comfort and limited the maximum tolerable stimulation intensity. Ramping protocols, which involve gradual increases and decreases in stimulation intensity, are generally better tolerated as they avoid sudden and abrupt sensations [37]. Additionally, stimulation was delivered starting at heel strike and lasted approximately 300 milliseconds, covering only the first half of the stance phase. Other protocols have included earlier activation with a ramp-up phase and longer stimulation durations spanning the entire stance phase. These differences in timing, duration, and intensity may influence the magnitude and detectability of biomechanical effects. Future pilot studies could incorporate ramping, earlier activation timing, and longer stimulation duration to improve participant comfort and maximize the potential mechanical impact of BFLH stimulation.

Third, the FES intervention involved only two brief trials lasting about four minutes each, which may have been too short to capture gradual changes in voluntary motor control. While our findings suggest that short-term use of FES does not affect voluntary control, supporting its potential use as an assistive device in early rehabilitation, this effect may change with repeated exposure, as shown by other studies [35], [36]. Longer-term training protocols with multiple sessions are needed to determine whether BFLH-targeted FES influences voluntary motor control over time and to better understand its potential impact on rehabilitation outcomes [19]. For example, the protocol could be expanded to include increased exposure to FES, such as doubling the number of trials per session from two to four, and repeated over several weeks. This approach would allow for a more comprehensive assessment of changes in muscle activation patterns and joint biomechanics throughout the course of training.

Additionally, the kinematic and kinetic analyses in this study were based on a rigid body model with three degrees of freedom per joint, a common approach that does not account for critical parameters such as ATT. Since ATT is the primary contributor to ACL strain, it plays a crucial role in understanding ACL biomechanics [2]. Future research should incorporate advanced musculoskeletal modeling tools such as OpenSim [38] to enable subject-specific models and provide more anatomically accurate estimates of internal joint mechanics.

For the sEMG timing differences between GLMAX and BFLH activation, it would be valuable to investigate

whether the timing changes observed during FES are consistent across sessions and how varying stimulation intensities influence these timings. This could clarify whether muscle activation adaptations are stable or variable. Additionally, sEMG recordings of the stimulated leg's BFLH before and immediately after stimulation could directly assess whether FES induces changes in voluntary motor control, providing physiological evidence of any after-effects in muscle activation. Incorporating these measures would strengthen the understanding of the intervention's effects.

V CONCLUSION

This study demonstrates that FES of the BFLH during early stance can produce statistically significant biomechanical changes consistent with reduced ACL loading. Both parametric and non-parametric SPM_t analyses, along with LMM models, revealed a meaningful reduction in KRM without disrupting voluntary motor control, supporting the safe short-term use of FES in healthy individuals. The inconsistent timing between GLMAX and BFLH during FES-assisted gait indicates that GLMAX timing is not a reliable proxy for BFLH timing. Future research should focus on optimizing stimulation protocols, leveraging advanced musculoskeletal modeling, and including larger clinical populations to fully assess the therapeutic potential of BFLH-targeted FES as an assistive intervention for ACL injury prevention and rehabilitation.

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VII APPENDIX

A Participant Information

Table II: Participant Characteristics with BMI

Participant	Age (years)	Length (mm)	Weight (kg)	BMI (kg/m ²)	Sex	Dominant Leg	Grip Strength (kg)	Intensity (mA)
1	24	1925	69.0	18.7	M	R	52	35*
2	24	1770	76.6	24.4	M	R	46	40*
3	25	1600	54.1	21.1	F	R	28	25*
4	24	1680	67.5	23.9	F	R	33	35*
5	26	1770	79.9	25.5	M	R	48	29*
6	25	1665	54.4	19.6	F	L	30	37**
7	22	1770	57.8	18.4	F	R	28	48**
8	24	1815	73.1	22.2	M	R	48	50**
9	25	1835	80.7	23.9	M	R	66	40**
10	25	1675	53.0	18.9	F	L	27	36**

* Micromed Energy used

** Odstock ODFS pace used

B IMU

In addition to motion capture using retro-reflective markers, force plates, and surface EMG (sEMG), inertial measurement units (IMUs) (COMETA WaveX MiniX) were also used to track the participants' lower limb movements. This IMU data will be used in a separate project to investigate whether IMUs can replace motion capture with retro-reflective markers for simple kinematic analysis. The IMU data was recorded at a sampling rate of 100 Hz using 5 sensors placed on both the stimulated and non-stimulated limbs.

IMUs were positioned as follows: on the anterior tibia, one-third of the way down the lower leg; midway down the iliotibial tract on the upper leg; and on the pelvis, positioned between the left and right posterior superior iliac spines (PSIS). An overview of the IMU placement locations is shown in [Figure 12](#).

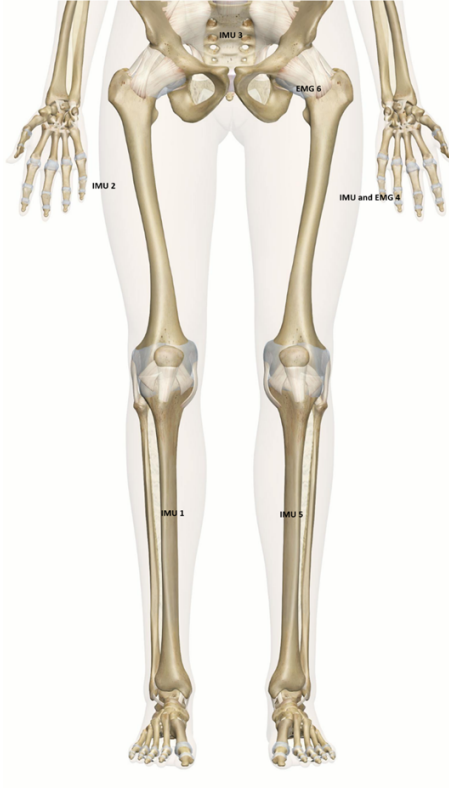


Figure 12: IMU locations 1 till 5 for capturing lowerlimb movements during gait trial

C Design of stimulator add-on device

Electrical stimulation was performed using the Micromed ENERGY stimulator. This stimulator does not natively support the addition of a footswitch to activate stimulation trains. To enable its use in a project requiring stimulation to occur at a specific event, namely the heel strike of the dominant leg, an add-on device was designed. This add-on was inspected and approved by a safety officer at the TU Delft Faculty of Mechanical Engineering (ME).

The Micromed ENERGY can be triggered via a TTL (Transistor-Transistor Logic) pulse signal sent from a computer through an RCA connector located on top of the device. Each stimulation pulse requires a TTL trigger of at least $10\mu\text{s}$ duration, with voltage levels ranging from 0 to 5V. To ensure reliable detection by the stimulator, a pulse duration of $50\mu\text{s}$ is used. The frequency of these pulses directly controls the frequency of stimulation. A visualization of the TTL trigger signal required by the Micromed ENERGY is shown in Figure 13.

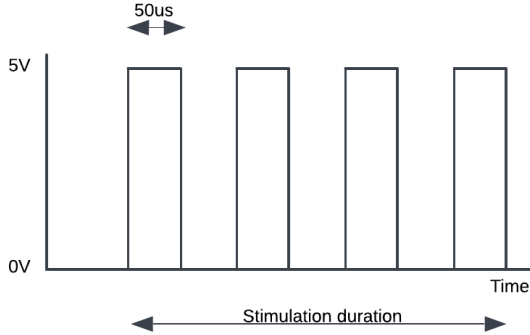


Figure 13: TTL trigger signal for the Micromed ENERGY

To generate the TTL triggers in a portable setup, an Arduino Nano microcontroller was used. A 2.5mm jack connector was used to connect a footswitch to the Arduino, allowing detection of heel strikes. Upon detecting a heel strike, the Arduino sends a preset series of TTL pulses to the Micromed ENERGY via a male RCA connector. The number of pulses is calculated by multiplying the stimulation duration (in seconds) by the stimulation frequency (in Hz). The inter pulse delay, the time between the rising edges of consecutive pulses, is determined by taking the inverse of the frequency and subtracting the pulse duration. For example, at a frequency of 40Hz, this results in an inter pulse delay of 24.5ms. The circuit for the Arduino Nano can be seen in Figure 15.

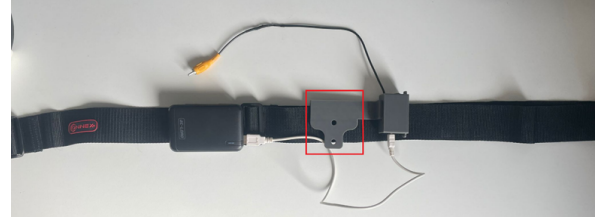


Figure 14: Belt setup with all components required for stimulation activation via footswitch. The red rectangle indicates the mounting location of the stimulator.

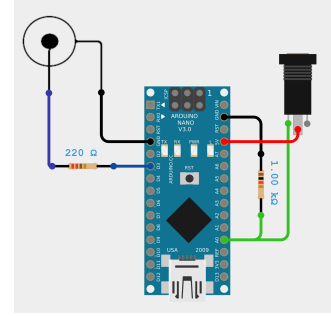


Figure 15: Circuit design of the add-on device (made with CirKit Designer)

The Arduino Nano is housed in a 3D printed PETG enclosure, which also serves as the mounting bracket for the Micromed ENERGY stimulator. The Arduino is powered by an external power bank supplying 5V through a mini USB connection. The complete setup, including the stimulator and power bank, is secured to the participant's hip using a belt, as shown in Figure 14.

D Pilot measurements

The first two measurements of the experiment were conducted using an initial set of stimulation parameters. The stimulation current was set at 15% above the visual activation threshold—defined as the minimal intensity required to elicit a visible contraction in the biceps femoris long head (BFLH) while the participant was standing on the leg designated for stimulation. The stimulation duration was initially set to 100 ms, corresponding to approximately 16.67% of the stance phase during normal gait. For participant 1 the intensity was set to 35mA and for participant 2 it was set to 23mA.

D.1 Knee flexion angles

The knee flexion angle for the stimulated leg (right leg) of Participant 1 increased during the initial footstrike phase (approximately 90–100% of the gait cycle), see [Figure 16](#). This increase was observed in both the initial and second trials, although the second trial showed smaller increases, potentially due to fatigue or a retention effect from prior stimulation. In contrast, the left leg of Participant 1 exhibited a slight decrease in knee flexion during the push-off phase in both trials, which might reflect a compensatory response to the increased activity in the right stimulated leg.

For Participant 2, less pronounced changes in knee flexion angles were observed across both legs and trials, see [Figure 17](#). The left leg in the first trial and the right leg in the second trial showed no significant change. Similar to Participant 1, Participant 2’s left leg demonstrated a slight decrease in knee flexion during the push-off phase of the second trial under FES-assisted gait. Additionally, a decrease in knee flexion was noted during the swing phase (approximately 70–90% of the gait cycle) of the right leg in the first trial.

These results suggest that the higher stimulation intensity used for Participant 1 may have contributed to the more prominent alterations in knee angle kinematics observed during FES-assisted gait.

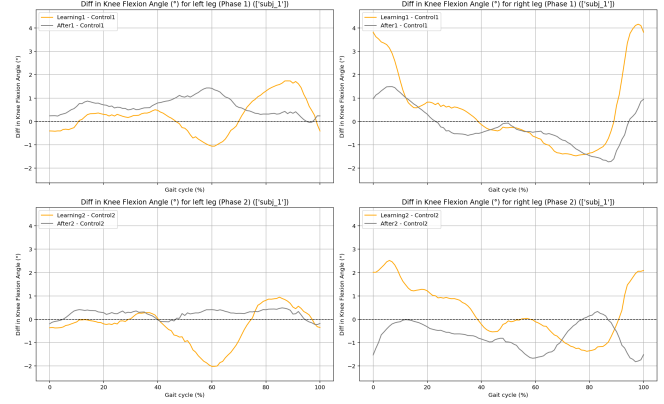


Figure 16: Mean difference between control and conditions for participant 1 of the left(non-stimulated)/right(stimulated) knee flexion angle during the gait cycle within 3 conditions (control1, learning1, After1)

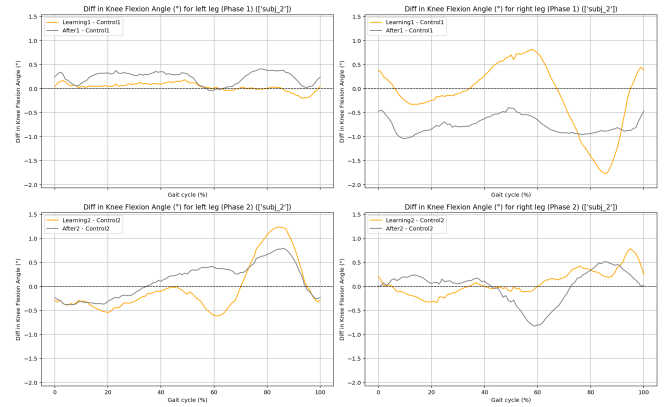


Figure 17: Mean difference between control and conditions for participant 2 of the left(non-stimulated)/right(stimulated) knee flexion angle during the gait cycle within 3 conditions (control1, learning1, After1)

D.2 Hip adduction angles

The hip adduction of the stimulated leg in Participant 1 showed little to no change during the FES-assisted condition in both Trial 1 and Trial 2, with no residual effects observed in the after conditions, see [Figure 18](#). In contrast, the non-stimulated leg displayed a noticeable increase in hip adduction during the swing phase (approximately 60–100% of the gait cycle) under FES-assisted gait, although no significant aftereffects were found. For Participant 2, no immediate effects of FES-assisted gait were observed during the stimulation trials, see [Figure 19](#). However, residual effects were present in both legs during Phase 1: increased hip adduction was seen in the non-stimulated leg after the loading phase

(approximately 30% of the gait cycle), and in the stimulated leg during midstance (approximately 40% of the gait cycle). Overall, no clear or consistent general change in hip adduction was observed across participants and conditions.

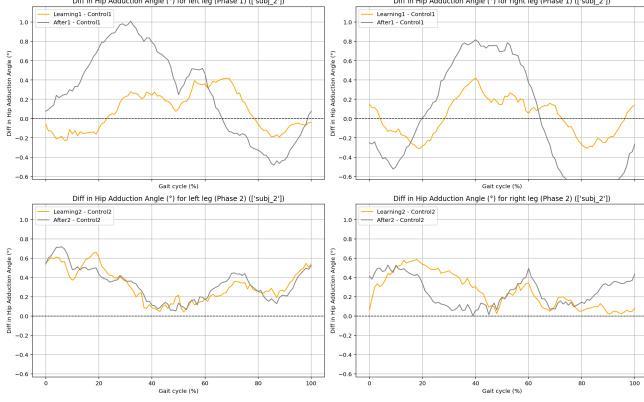


Figure 18: Mean difference between control and conditions for participant 1 of the left(non-stimulated)/right(stimulated) hip adduction angle during the gait cycle within 3 conditions (control1, learning1, After1)

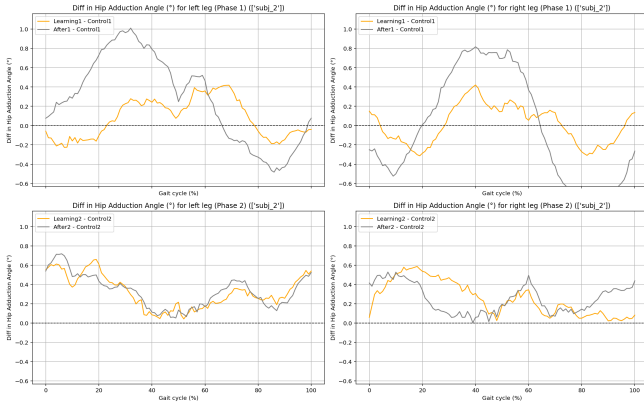


Figure 19: Mean difference between control and conditions for participant 2 of the left(non-stimulated)/right(stimulated) hip adduction angle during the gait cycle within 3 conditions (control1, learning1, After1)

D.3 Verticle ground reaction forces (vGRF)

For Participant 1, changes in vertical ground reaction force (vGRF) during FES-assisted gait were relatively small but consistent, see Figure 20. The vGRF of the stimulated leg decreased during the push-off phase (approximately 60% of the gait cycle) across both trials, while the non-stimulated leg showed an increase in vGRF during the same phase under FES-assisted conditions.

No residual changes were observed in the non-stimulated (left) leg during the after conditions for either trial. However, the stimulated leg exhibited some residual effects: an increased vGRF during the loading phase (approximately 15% of the gait cycle) in the after condition of the first trial, and an increased vGRF during the push-off phase (around 60% gait cycle) in the after condition of the second trial.

For Participant 2, no pronounced changes in vGRF were observed, except for a residual decrease in vGRF found in both the stimulated and non-stimulated legs during the after condition of the first trial, see Figure 21.

The differences in the magnitude of vGRF changes between participants may be explained by the variation in stimulation intensity. Participant 1, who received higher stimulation intensity, showed more pronounced alterations in vGRF patterns, whereas Participant 2's lower intensity likely contributed to the smaller or residual changes observed. This suggests that higher FES intensities may induce more substantial modifications in gait kinetics.

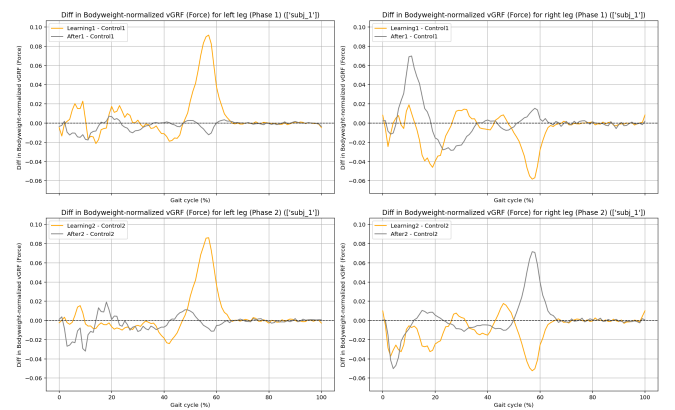


Figure 20: Mean difference between control and conditions for participant 1 of the left(non-stimulated)/right(stimulated) vGRF during the gait cycle within 3 conditions (control1, learning1, After1)

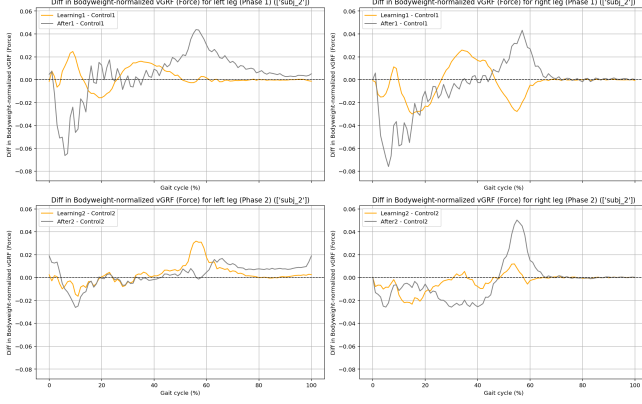


Figure 21: Mean difference between control and conditions for participant 2 of the left(non-stimulated)/right(stimulated) vGRF during the gait cycle within 3 conditions (control1, learning1, After1)

D.4 Duty symmetry

The duty symmetry was not affected by the stimulation, as shown in Figure 22. This indicates that there was no change in the symmetry of stance phase duration between the left and right legs, expressed as a percentage of the gait cycle, across all conditions.

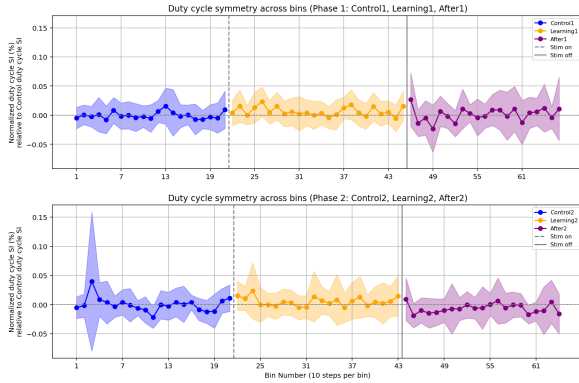


Figure 22: Duty symmetry of the stance phase duration between the left and right leg, shown as the difference from the control mean across phases. Shaded area visualizes the std deviations per bin

D.5 Gait duration

The duration of the gait cycle was slightly reduced for both the left and right legs during FES-assisted gait compared to the control condition, as shown in Figure 23.

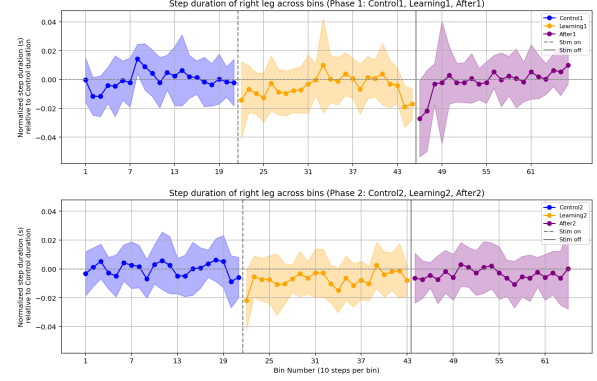


Figure 23: Step duration of the right leg (time for entire gait cycle), shown as the difference from the control mean across phases. Shaded area visualizes the std deviations per bin

D.6 Conclusion

Given that the initial stimulation settings resulted in relatively minor changes in gait kinematics and kinetics, particularly in Participant 2 who exhibited limited variation across conditions, it was decided to adjust the stimulation parameters to elicit more pronounced kinematic responses. In contrast, Participant 1 showed more noticeable changes, likely due to a higher stimulation intensity, including residual effects in both knee kinematics and vertical ground reaction forces (vGRF). These observations motivated the adjustment of stimulation parameters to better assess potential motor learning effects, including aftereffects and retention.

Specifically, the stimulation duration was extended to 300ms to cover approximately 50% of the stance phase, targeting both the peak flexion during the loading response and peak extension during midstance. Additionally, the stimulation intensity was increased from 15% above the visual activation threshold to the participant's maximum tolerable level, aligning with recommendations from comparable studies such as [16].

It is important to note that these observations are based on pilot data from only two participants. Therefore, no definitive statistical conclusions can be drawn at this stage. Nevertheless, these preliminary findings were instrumental in guiding the refinement of stimulation parameters for the main experimental protocol.