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RESEARCH ARTICLE

Adaptive Response of Muscle Activity to Exoskeleton-Assisted Walking

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This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Local Ethical Committee of University Research Ethics Committee of the University of Genoa, under Protocol No. 2023/07, and performed in line with the Declaration of Helsinki.

ABSTRACT The increasing use of robotic devices in clinical settings for rehabilitation and assistance underscores the need to understand their effects on muscle activation patterns. Prior studies have suggested that excessive assistance from robotic devices reduces voluntary control, leading to potential negative consequences on rehabilitation outcomes. However, the observation of muscle activation during exoskeleton-assisted walking in unimpaired individuals suggests the absence of adaptive responses to short-term exposure at high levels of assistance. The objective of this study is to determine whether prolonged exposure to maximum exoskeleton assistance induces adaptive changes in muscle activity and to analyze if distinct muscle activation profiles emerged during assisted versus unassisted walking. To achieve this, we performed the electromyographic analysis of eight bilateral lower limb muscles in ten participants during a one-hour training session. The results revealed a decrease in muscle activity over time. Furthermore, assisted walking exhibited distinct muscle patterns compared to unassisted walking, demonstrating that the level of assistance, along with the exoskeleton's structure, significantly influences muscle activity. These findings hold significance for optimizing assistance regulation in exoskeleton-assisted walking, in terms of levels and timing of assistance changes, to enhance rehabilitative outcomes. Understanding how exoskeletons influence muscle activation can lead to improved rehabilitation strategies, maximizing the benefits of this technology for enhancing walking ability in people with neurological conditions or injuries.

INDEX TERMS Adaptive learning, biomedical engineering, exoskeletons, motion control, muscles.

I. INTRODUCTION

In recent years, the use of robotic devices, particularly of powered exoskeletons, has increased in clinical practice as rehabilitative tools to improve walking in people with neurological diseases or injuries [1], [2], as well as assistive devices that help people with disabilities to stand up and walk [3]. In rehabilitation, these robotic devices assist physical therapists by enabling specific, repeatable exercises and allowing for more intensive training [4]. Robot-assisted gait training can improve locomotor function after stroke, incomplete spinal cord injury [5], [6], [7], [8], [9], [10], [11], [12], [13], or other neurological diseases or injuries [14]. Among these devices, powered exoskeletons are well known for enabling non-ambulatory people to walk at moderate speeds. However, there is limited research on the effectiveness of these devices

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in rehabilitation, and their superiority over conventional gait re-education methods is still debated [7], [8], [15], [16], [17], [18]. Despite their growing use, the mechanisms of motor adaptation during exoskeleton-assisted walking are not yet fully understood and the neuromuscular implications of the assistance provided by exoskeletons are still unclear. Only a few studies have examined the effects of assistive forces [19], [20], [21], [22] or device structure [23], [24], [25] on muscle activation and gait patterns, often with conflicting results when compared to findings from other types of robotic training [26], [27], [28].

Indeed, when facing assistive forces, humans quickly incorporate them into their motor plan, minimizing efforts [29], [30], a phenomenon known as ‘‘slacking’’. Thus, when a robotic device provides too much assistance, users adapt to its guidance by reducing voluntary control [26], [27], [28], [31] with potential negative consequences on rehabilitation and learning outcomes [29], [32], [33].

To prevent this, the recommendation is to use ‘‘assistance-as-needed’’ paradigms [29], [34], [35], [36], [37], [38], [39], [40], [41], i.e. paradigms maintaining the assistance to a minimum level that can evoke a voluntary response so that people do not reduce their voluntary control. While this slacking phenomenon has been consistently observed in upper-limb robots and treadmill-based systems [23], [24], [25], [28], wearable exoskeletons present a unique challenge due to their direct interaction with the lower-limb joints and the motor control coordination required for walking overground as the user progresses forward.

Different studies highlighted an anomaly in exoskeleton-assisted walking: in unimpaired subjects, assistance did not influence muscle activity, as muscle patterns did not change when the assistance level was modified or when it was provided bilaterally or unilaterally, suggesting that subjects did not exploit the possibility of reducing their active participation and effort [20], [24]. However, this was only investigated in the presence of rapid muscle adaptation after short exposure to the same level of assistance. Such short durations may have limited the ability to observe gradual adaptive muscular response, as insufficient time was available for meaningful changes in muscle activity to emerge. Therefore, it is premature to conclude that prolonged exoskeleton assistance does not affect muscle activation patterns. This highlights a clear gap in the literature: the long-term effects of consistently high exoskeleton assistance on muscle activity have not been systematically studied. To address this gap, there is the need to investigate whether prolonged, consistent exoskeleton assistance affects muscle activation patterns, and how this compares to normal walking without assistance. The present study was intended to fill this gap and had two objectives. The first objective was to evaluate whether the muscle activation of unimpaired subjects changed during a one-hour training with a lower limb exoskeleton, UAN.GO [42], at maximum assistance. The second objective was to compare muscle activations during walking with and without the exoskeleton to verify if in line with previous studies [23], [24] the exoskeleton, which has a semi-rigid, not actuated ankle and powered hip and knee joints, alters the timing of muscle activations. By clarifying these mechanisms, this study aims to inform the design of future exoskeleton training protocols and contribute to optimizing the balance between assistance and user-driven control in gait rehabilitation.

II. MATERIALS AND METHODS

A. PARTICIPANTS

Ten unimpaired naïve volunteers (age: 24.4 mean ± 1.4 standard deviation (SD) years, height: 173.8 mean ± 7.6 SD cm, weight: 64.3 mean ± 9.7 SD kg; right-foot dominant, three males and seven females, all university students) participated in this study. Participants were selected to ensure a homogeneous sample with consistent and repeatable gait patterns, minimizing variability that could arise from neurological or orthopedic conditions. An a priori power analysis was conducted to determine the appropriate sample size for detecting meaningful effects: between two time points for the first objective, and between conditions for the second. A paired t-test was used to compare the means at the two time points and between the two conditions, with a target alpha level of 0.05 to control for Type I error. The effect size was estimated based on preliminary data from pilot testing. A power level of 0.9 was targeted, providing a 90% probability of detecting a true effect, if one exists. Based on these parameters, the required sample size was calculated to be 7 participants, ensuring adequate power to detect differences between two time points and between the two conditions for all muscles. This power analysis was performed using G*Power software. Although the power analysis indicated that 7 participants per group would be sufficient, we enrolled 10 participants to account for potential dropouts, ensure adequate statistical power, and mitigate the impact of unanticipated variability, thereby improving the robustness and reliability of the findings. To use the exoskeleton UAN.GO, participants had to meet the following inclusion criteria: (1) functional upper limbs for using the aids, (2) height between 1.55–1.95 m, (3) weight below 100 kg, as these factors ensure proper fit and functionality of the device. Additional inclusion criteria for this study were (4) the absence of any history of neurological or orthopedic disorders, ensuring that motor control and gait patterns were not influenced by underlying conditions, and (5) no prior experience with the exoskeleton, eliminating potential learning effects that could bias the results.

This study conforms to the ethical principles for medical research involving human subjects of the Declaration of Helsinki (revision 2013) and was approved by the local ethical committee (University Research Ethics Committee of the University of Genoa, protocol n. 2023/07). All participants signed an informed consent for the analysis and publication of their data for research purposes. The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to approval by the relevant ethics committee and in compliance with institutional policies.

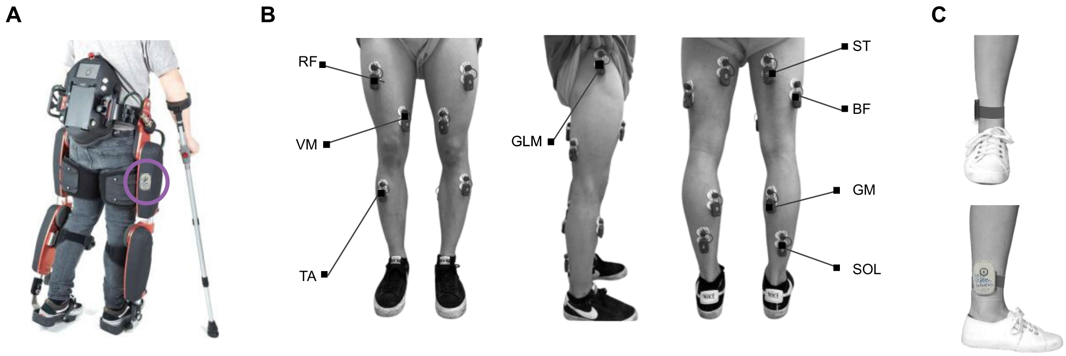

FIGURE 1. Experimental setup. Panel A: UAN.GO exoskeleton and Inertial Measurement Unit (IMU) placement (purple circle) during exoskeleton walking. Panel B: Placement of surface electromyographic (EMG) electrodes, recording bilaterally from tibialis anterior (TA), gastrocnemius medialis (GM), soleus (SOL), rectus femoris (RF), vastus medialis (VM), semitendinosus (ST), biceps femoris (BF), and gluteus medialis (GLM). Panel C: IMU placement during normal walking, frontal (top panel) and lateral view (bottom panel).

B. UAN.GO EXOSKELETON

UAN.GO (U&O S.r.l., Fiorenzuola d'Arda, Piacenza, Italy, Fig. 1 A) is a powered lower limb exoskeleton designed to allow individuals with mobility impairments to walk independently. It features a combination of four motorized joints (located at the hips and knees) and four passive joints (at the ankles and feet). UAN.GO offers multiple movement modes, including overground walking (WALK), standing up and sitting down (UP&DOWN), and ascending and descending stairs (STAIRS). It can be used in two distinct modalities: Assisted mode, in which the operator selects movement patterns from the on-board touchscreen and activates them via start/stop buttons, or Autonomous mode, in which the user autonomously controls the exoskeleton through trunk movements measured by a built-in inertial measurement unit. The level of assistance provided can vary from a maximum value of 100% to a minimum level equivalent to 25% of the maximum [42]. At 100% assistance, the exoskeleton fully supports the user's movement by generating a torque of 60 Nm at each joint, effectively allowing subjects to walk without voluntary effort. In this condition, the exoskeleton controls and executes the entire gait cycle, moving the lower limbs in a predefined pattern. Furthermore, assistance can be applied bilaterally or unilaterally, i.e., the same level of assistance can be provided on both legs or adjusted independently for each limb. The exoskeleton is designed to be lightweight and features active motors that compensate for its inertia during movement. As a result, the user does not perceive the weight of the device, regardless of the level of assistance provided. This design ensures that the exoskeleton's inertia does not influence muscle activity.

C. ELECTROMYOGRAPHIC SYSTEM AND INERTIAL MEASUREMENT UNITS

We used a surface electromyographic system (EMG, FREEEMG, BTS Bioengineering, Milan, Italy), to record the activity of 8 muscles from both legs (Fig. 1 B): tibialis anterior (TA), gastrocnemius medialis (GM), soleus (SOL), rectus femoris (RF), vastus medialis (VM), semitendinosus (ST), biceps femoris (BF) and gluteus medialis (GLM). The selected muscles encompass both proximal and distal regions of the lower limbs and are among the largest and most superficial, making them easily detectable using surface EMG systems. They play a key role in gait and exoskeleton-assisted locomotion and were already considered in previous studies [20], [24]. The EMG electrodes were positioned according to the guidelines of the SENIAM (Surface EMG for Non-Invasive Assessment of Muscles, [43]). An Inertial Measurement Unit (IMU), specifically the G-Sensor (BTS Bioengineering, Milan, Italy), synchronized with the EMG system, was positioned on the femoral segment of the exoskeleton (Fig. 1 A) allowing the offline synchronization with the exoskeleton. The data recorded from the encoders located at the hip and ankle joints of the exoskeleton provided information about walking kinematics and gait cycle events. UAN.GO provides hip and ankle joint angles (°), and the timing of heel strike (HS) and toe-off (TO) events, that allow determining gait spatiotemporal parameters (see data analysis below). For normal walking, we used two IMUs (G-Sensor, BTS Bioengineering, Milan, Italy, and Movendo Technology, Genoa, Italy) positioned on the shank of both legs, right above the ankle joint, with the x-axis aligned with the line connecting the lateral epicondyle and lateral malleolus (Fig. 1 C). The IMUs enabled the detection of HS and TO events by analyzing angular velocity in the mediolateral plane. Specifically, the mid-swing instant was determined by identifying peaks in angular velocity that exceeded 35% of the maximum peak. Then, the HS was defined as the time instant corresponding to the minimum angular velocity value immediately following the mid-swing peak. The TO instant was defined as the time instant of the minimum angular velocity value immediately preceding the mid-swing peak [44].

D. EXPERIMENTAL PROTOCOL

Subjects participated in a single-session experiment. They were instructed to walk back and forth in a ten-meter-long pathway inside the Italian Spinal Cord Italian Laboratory (S.C.I.L.) of the Santa Corona hospital

(Pietra Ligure, SV, Italy). Each participant walked both with and without the exoskeleton. The session lasted approximately 2 hours. It started with 10 minutes of normal walking without the exoskeleton, followed by 1 hour of overground walking with the exoskeleton providing the maximum level of assistance. Participants used crutches for balance support throughout all the training intervals. While crutch use could influence posture and stabilization strategies, it was standardized across all assisted-walking intervals. Moreover, participants were instructed to use the crutches solely for balance and avoid using them to propel themselves.

The detailed protocol consisted of the following phases (Fig. 2):

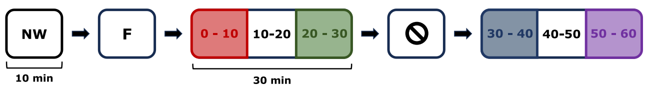

FIGURE 2. Experimental protocol. Subjects performed 10 minutes of normal walking (NW) and then experienced a one-hour training with the exoskeleton providing maximum assistance. The training was composed of two blocks of 30 minutes, the first preceded by a familiarization phase (F) and separated from each other by a pause (prohibition symbol).

-**Normal Walking** (NW). Subjects walked without exoskeleton for ten minutes, wearing flat-soled shoes. They were instructed to walk, as naturally as possible, at the same cadence imposed by the exoskeleton (14 steps/min), following a metronome. Both EMG and IMU data were recorded.

-**Familiarization phase** (F). After learning to use the crutches, subjects had to walk in place while wearing the exoskeleton. This phase lasted 10 minutes and no data was recorded.

-**Exoskeleton Walking with 100% Assistance.** Subjects walked overground wearing the exoskeleton with assistance set to its maximum (100%) in Autonomous mode. This phase lasted 30 minutes. EMG, IMU and data from the exoskeleton were recorded in the first and last 10 minutes.

-**Pause** (Prohibition Symbol). Subjects rested for 10 minutes.

-**Exoskeleton Walking with 100% Assistance.** This phase was repeated for 30 minutes, for 1 hour of walking with the exoskeleton.

E. DATA ANALYSIS

The analysis focused on straight walking strides [24], [45], [46]. The HS and TO events were used to identify the beginning and end of the gait cycle, enabling the calculation of key walking spatiotemporal parameters: stride time (s), duration of the swing and stance phases (% gait cycle), and cadence (steps/min). Regarding the muscle activations, the EMG data, recorded at 1 kHz, were processed using a band-pass FIR filter between 30–450 Hz [47], [48]. The filtered data were rectified, and low-pass filtered using a fourth-order Butterworth filter with 4Hz cut-off frequency to obtain their envelope [49]. The EMG envelopes were segmented in correspondence to each gait cycle (from an HS to the following one). Then, we time-interpolated each gait cycle over a time base with 101 points [20]. The position of the electrodes did not change during the entire acquisition, allowing for direct comparisons of each muscle among all conditions. To directly compare and average the EMG data across subjects and between the two legs within subjects, we normalized each muscle signal for its maximum value [24] computed over all training phases (i.e., 4 training intervals with the exoskeleton at maximum assistance and 1 normal walking interval). We also verified that a different normalization (e.g., for the median value [50], [51]) did not change the main results we obtained [24]. For each gait cycle, we considered the profiles of EMG envelopes and the area under these envelopes computed using trapezoidal integration (iEMG) [52], [53]. In comparison between time intervals, for each muscle, we considered the average of EMG envelopes and the iEMG over each 10-minute time interval.

In Fig. 3 (panels D and H) we reported the iEMG values by dividing each of these training intervals into 5 bins of 2 minutes only for visualization purposes. This division into 5 bins was also used in the within-time interval analysis (see Table 1 in supplementary materials) as explained in the statistical analysis section.

F. STATISTICAL ANALYSIS

We tested two hypotheses:

- Main hypothesis: An adaptive response of the muscle activity to the maximal assistance provided by the device is observable during 1 hour of training.
- Secondary hypothesis: Walking overground with the exoskeleton induces changes in muscle activity compared to non-assisted walking.

In both cases, we used a repeated measures ANOVA (rANOVA) with two within-subject factors: side of the body (dominant and non-dominant side) and time interval. For the main hypothesis, we considered 4 intervals of exoskeleton training with maximal assistance: 0-10 minutes, 20-30 minutes, 30-40 minutes, and 50-60 minutes. For the secondary hypothesis, we considered the muscle activity at the beginning (first 10 min), at the intermediate (30-40 minutes) and at the end (last 10 min) of the training and we compared separately these three-time intervals with the muscle activity during the 10 min of normal walking. As for the main hypothesis, a significant main effect of the time-interval factor associated with lower values at the end compared to the beginning of the session would support the hypothesis that there is an adaptive response of the subject to the maximum level of assistance. Following the significant main effect of this factor, we verified between which training intervals the adaptive response becomes significant. Thus, we performed six comparisons between the four different time intervals of practice at maximum assistance considered in pairs. As for the secondary hypotheses, a significant main effect would support the hypothesis that there is a difference in muscle activation patterns between normal walking and any and/or all of the initial, intermediate, and final exposures to the exoskeleton. To compare the profiles of EMG envelopes during the gait cycle we used the statistical parametric mapping approach (SPM [24], [54]) which allows the statistical analysis of the difference between continuous curves, without extracting specific scalar variables. The statistics of the iEMG values of the different phases described in the protocol were performed with the open statistical software Jamovi [55].

The normality of the data was verified by using the Shapiro-Wilk test. Sphericity was verified with the Mauchly test and corrected for the iEMG data only for the main hypothesis. The statistical significance was set at the family error rate $\alpha = 0.05$. We corrected for multiple comparisons by using the Bonferroni method, adjusting the significance threshold based on the number of comparisons performed.

Before testing the two hypotheses mentioned above, we verified that:

- There were no significant differences in cadence (steps/min) depending on the walking conditions by applying a rANOVA with the within-subject factor 'practice' considering the NW phase and the 4 intervals with maximum assistance (see Fig. 1 and 3 and Table 4 in supplementary materials for details).
- There were no significant differences in the iEMG among the 2 minute-bins of each 10-minute recording interval, using a rANOVA with the within-subject factor 'time sub-interval' (see Table 1 in supplementary materials).

III. RESULTS

The following presents the results for this study's main and secondary hypotheses in separate paragraphs.

A. ADAPTIVE RESPONSE OF MUSCLE ACTIVITY TO EXOSKELETON-ASSISTED WALKING DURING 1 HOUR OF TRAINING

For the first main objective, we compared muscle activity within and between all the training intervals with the exoskeleton at maximum assistance. Specifically, for each gait cycle, we considered the time profile of EMG envelopes and the area under those envelopes, computing the integrated EMG (iEMG) (Fig. 3). Since no significant differences were found between the two legs, we report the results as their average. For all muscles, no differences were observed in the first 10 minutes of training (see Table 1 in supplementary materials). A significant main training effect, corresponding to a decreased activation with practice, was observable for most muscles, starting from the 20-30 minute interval of training. The 10-minute break between training blocks, designed to prevent the onset of fatigue, did not affect the muscle activation patterns. Indeed, although it could potentially have returned muscle activity to baseline levels, the general trend of muscle adaptation remained unchanged, with no significant differences observed between the intervals before and after the break ($t(9) = 1.33$, $p = 0.21$). This suggests that the interruption did not interfere with the adaptive response observed during training.

1) ANTERIOR CHAIN MUSCLES

Prolonged training induced changes in the activity of all the muscles of the anterior chain, particularly in the first 40 minutes of training. No significant changes were evident in the last 20 minutes (i.e., between the 30-40 min, and 50-60 min intervals) both for the envelopes (Fig. 3 A) and the iEMG values (Fig. 3 D). The most notable changes were observed in the upper leg muscles, RF, and VM during the stance (Fig. 3 A, 2nd and 3rd columns).

At the end of the training, their activations were significantly lower than at the beginning (Fig. 3 C). These changes were significant for the iEMG starting from the 20–30 minute training interval (Figure 3 D, 2nd and 3rd columns, for RF $t(9) = 7.74$, $p < 0.001$; for VM $t(9) = 8.77$, $p < 0.001$, see Table 2 in the supplementary materials for more details), while for the envelopes, it was noticeable from the 30–40-minute interval (Fig. 3 B, 2nd and 3rd columns). A further decrease in the iEMG was observed between the first and second half of training (see Table 2 in supplementary materials). Also, the lower limb muscle, TA, had a less marked, but still significant, decrease in activation during training (Figure 3, 1st column). The TA had the most relevant variation at the beginning of the gait cycle, becoming significant for both metrics starting from the 30-40 minute interval of training (Fig. 3 D, 1st column, $t(9) = 2.94$, $p = 0.016$ see Table 2 in supplementary materials for other details).

2) GLUTEUS MEDIUS

The GLM had a low basal activation that slightly decreased with training (Fig. 3 A, 4th column). This decrease was evident when observing the iEMG values (Fig. 3 D, 4th column). The change in the iEMG was significant starting from the 20-30 minute training interval ($t(9) = 2.27$, $p = 0.049$, see Table 2 in supplementary materials), while in the envelope in the second half hour of training.

3) POSTERIOR CHAIN MUSCLES

As for the posterior chain, prolonged training induced changes in the activity of all the muscles. Specifically, the ST muscle activation changed in the terminal swing phase between 80-100%, reaching the threshold for significance in the 50–60 minute training interval (Fig. 3 E, 4th column). The other upper leg muscle, BF, had a small decrease both in the stance and in the swing phases (Fig. 3 E, 3rd column), which became significant for the iEMG in the second half-hour of training ($t(9) = 2,62$, $p = 0.028$, see Table 2 in the supplementary materials). The posterior muscles of the lower limb, SOL, and the GM decreased slightly, but significantly, their activity respectively at 0-20% and 30-40% of the gait cycle (Fig.3 E, 1st and 2nd columns). These changes were observable and significant in muscle envelopes from the 30–40 minute training interval. These changes, lasting a small percentage of the gait cycle, were not detected in the iEMG, accounting for the activation in the entire cycle (see Table 2 in the supplementary materials).

B. WALKING OVERGROUND WITH THE EXOSKELETON INDUCES CHANGES IN THE MUSCLE PATTERNS COMPARED TO NORMAL WALKING

For the secondary objective, we compared muscle activity at the beginning (first 10 min, 0-10 min interval of training), in the middle (30-40 min interval of training), and end (last 10 min, 50-60 min interval of training) of training with muscle activity during 10 minutes of normal walking (Fig. 4). Since no significant differences were observed between the final 10-minute interval (50–60 min) and the intermediate one (30-40 min) (see Table 2 in the Supplementary Materials), and the differences between NW and the 30–40 minute interval were comparable to those between NW and the 50–60 minute interval, we focused our analysis on the initial (0–10 min) and final (50–60 min) intervals, representing early and steady-state exposure, respectively. As for the main hypothesis, no significant differences were found between the two legs, so we report the results as their average.

1) ANTERIOR CHAIN MUSCLES

Walking with and without the exoskeleton affected the activation of the anterior chain muscles. The TA had the same activation timing in both conditions, but when walking with the exoskeleton its amplitude decreased in the terminal phase of the gait cycle (80-100%).

This was observable for both intervals, but more pronounced in the last training interval (50-60 min training interval, Fig. 4, 1st column). As for the upper leg muscles, the activation timing of the RF and VM differed from that of normal walking for both training intervals (Fig. 4, 2nd and 3rd columns).

During the first training interval, peak activation during assisted walking occurred in the stance phase (0-40% of the gait cycle), whereas, in normal walking, the peak activation occurred at 90-100% of the gait cycle. In the last training interval, the activation timing in the stance phase approached that of normal walking, but at the 90-100% gait cycle still differed significantly, as the muscles were not active during this phase (Fig. 4 D, 2nd and 3rd columns). For all anterior chain muscles, no significant differences were captured by the iEMG since these parameters account for the overall amplitude in the gait cycle and did not capture differences in specific time points or phases (see Fig. 2 and Table 3 in supplementary materials).

2) GLUTEUS MEDIUS

Walking with the exoskeleton significantly affected the activation of the gluteus muscle. During normal walking, the gluteus was active throughout the stance phase (0-60% of the gait cycle). However, with the exoskeleton, the gluteus was not active during this phase (Fig. 4 A, last column). This difference from normal walking was even more pronounced in the last training interval. The iEMG values confirmed this observation (initial exposure $F(1,18) = 16.12$, $p = 0.003$, final exposure $F(1,18) = 39.83$, $p < 0.001$, see Figure 2 and Table 3 in supplementary materials).

3) POSTERIOR CHAIN MUSCLES

Walking with the exoskeleton did not affect the activation timing of most posterior chain muscles, except for the ST. Indeed, the ST showed different activation timing compared to normal walking during training. At the beginning of the gait cycle (0-30%), its activation was lower than during normal walking, a difference that was significant in the last interval of training (Fig. 4 H, 4th column). At the end of the gait cycle (90-100%), the ST showed an activation peak during exoskeleton-assisted walking, whereas it remained inactive during normal walking. This difference was consistent even throughout the last interval of training. No significant differences were observed in the iEMG values of the normal and exoskeleton-assisted walking conditions (see Fig. 2 and Table 3 in supplementary materials).

In contrast, the remaining posterior chain muscles showed differences mainly in activation amplitude. The BF muscle exhibited higher activations in the exoskeleton-assisted walking compared to normal walking at the end of the gait cycle (90-100%) (Fig. 4 E, 3rd column). This increase was statistically significant only during the initial exposure, but not in the last time interval. The calf muscles (GM and SOL) exhibited significantly lower activation in both training intervals. This reduction was evident between 40-60% of the gait cycle for the SOL and between 20-60% for GM (Fig. 4 E and F, 1st and 2nd columns). These differences were also observed in the iEMG values comparison between NW and the 0-10 min training interval (for SOL $F(1,18) = 30.1$, $p < 0.001$; for GM $F(1,18) = 21.79$, $p < 0.001$) and between NW and the 50-60 min training interval (for SOL $F(1,18) = 25.12$, $p < 0.001$; for GM $F(1,18) = 43.19$, $p < 0.001$; see Fig. 2 and Table 3 in supplementary materials).

By providing external force and support during movement, the exoskeleton reduces the load on agonist muscles, which are primarily responsible for generating movement. This reduced demand on agonists often leads to a reduction in compensatory co-contraction of antagonist muscles, as less stabilization is required. For example, during initial contact and load response, the controlled shock absorption of the exoskeleton minimizes the need for strong eccentric activation of the tibialis anterior (Fig. 4 A, 1stcolumn), which in turn decreases the compensatory activation of the plantar muscles (Fig. 4 E 1st and 2nd column). Similarly, at the knee level, a reduced engagement of the quadriceps may result in less co-contraction of the hamstrings (Fig. 4 E 3rd and 4th column). In mid-stance, the mechanical support of the exoskeleton stabilizes the joints, limiting the need to activate antagonist muscles to maintain posture. This redistribution of effort allows for more efficient movement patterns, as the muscular effort is no longer directed at maintaining

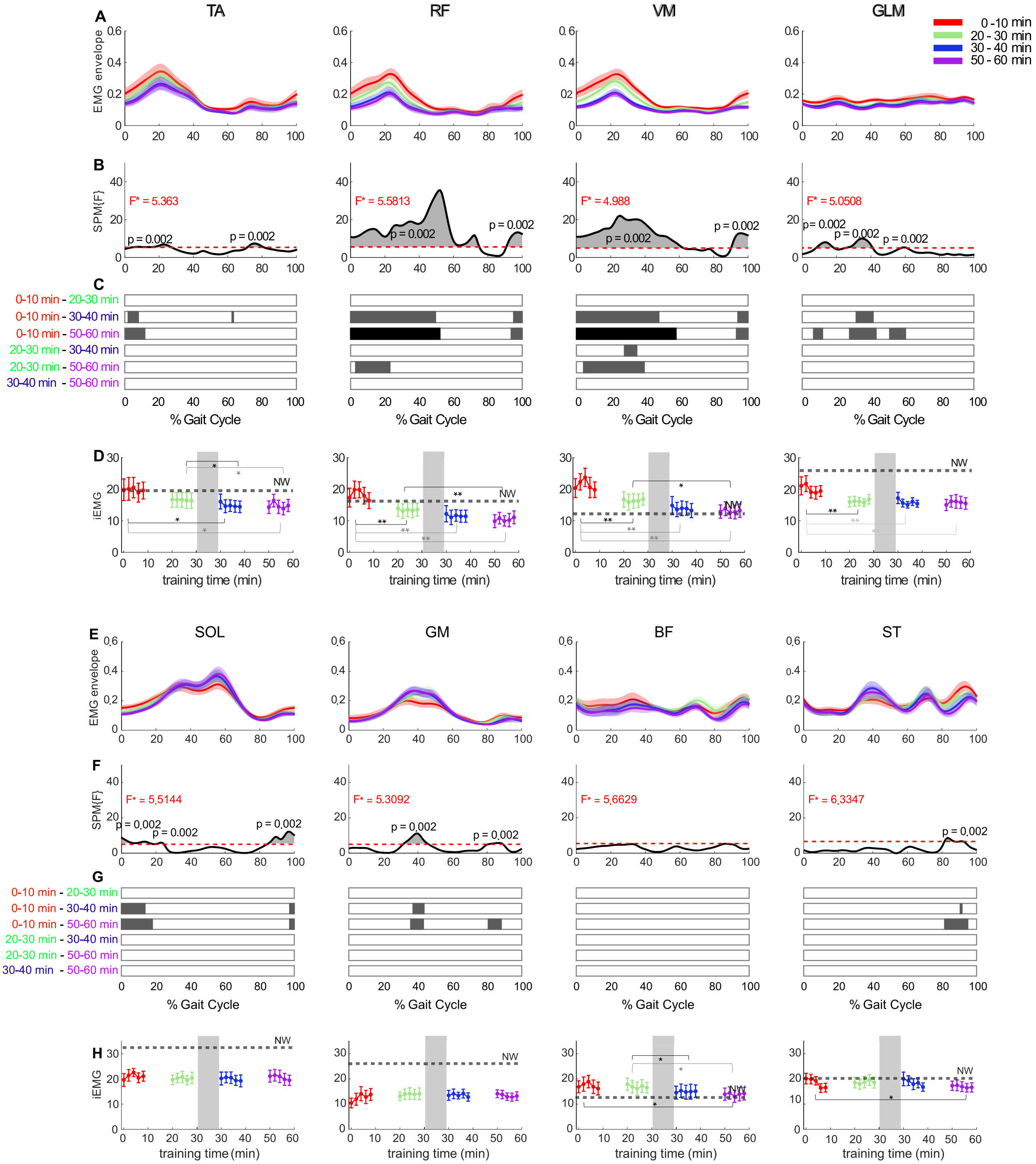

FIGURE 3. **EMG analysis during maximal assistance training. Panels A to D refers to anterior chain muscles and gluteus medius, panels E to H refers to posterior chain muscles. Panels A, E: EMG envelopes (mean ± standard error) during exoskeleton-assisted walking. The red line indicates the 0-10 min interval training; the green, blue, and violet lines indicate respectively the 20-30 min, the 30-40, and the 50-60 min interval of training. Panels B, F: Group-level Statistical Parametric Mapping (SPM{F}) percentage (0-100%) applied to the EMG envelopes in panels A and D. The significance threshold is indicated with the red line. The grey shaded regions indicate portions of the gait cycle with statistically significant effects of the 'time interval' factor ($p < 0.05$). Panels C, G: Paired-level post hoc SPM analysis results on EMG envelopes: black intervals indicate $p < 0.001$, and grey intervals $p < 0.05$. Panels D, H: Paired-level post hoc analysis results on iEMG parameters with the same color code. "**" indicates a value of $p < 0.001$, "*" $p < 0.05$. Black parentheses indicate the first subsequent interval in which a significant difference is found for each interval. Subsequent intervals with significant differences are indicated by grey parentheses. The dashed black horizontal line represents the parameter value for normal walking (NW). The grey vertical rectangle represents the rest phase.**

joint alignment, but at effectively executing the gait cycle. Furthermore, during terminal stance and push-off, the triceps surae (GM and SOL) play a key role in propulsion (Fig. 4 E 1st and 2nd column), while the tibialis anterior remains inactive (Fig. 4 A 1st column). The exoskeleton reduces the demand on these muscles by providing external assistance,

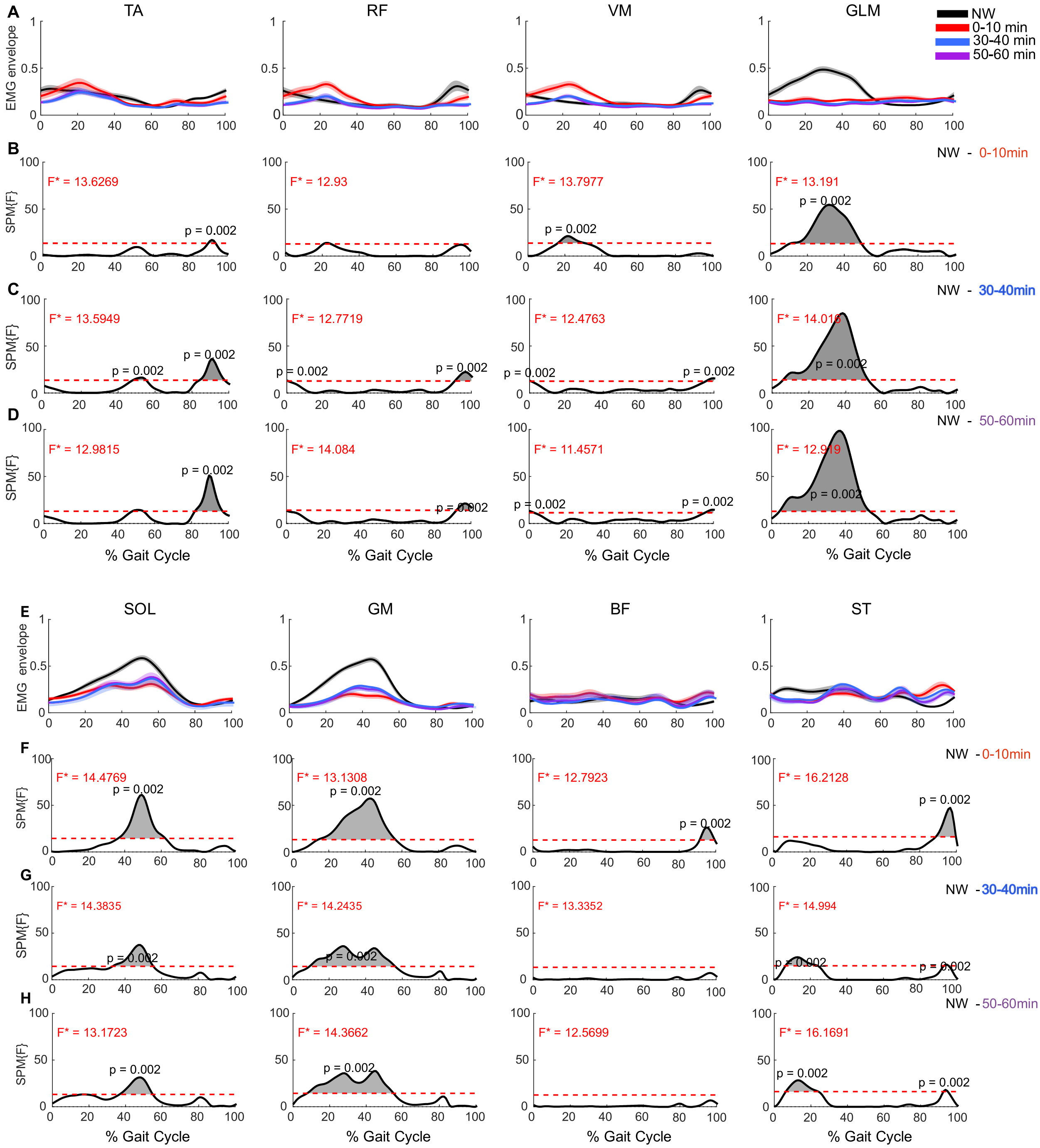

FIGURE 4. EMG analysis during normal walking (NW) phase and first, intermediate and last training interval with the exoskeleton. Panels A to D refers to anterior chain muscles and gluteus medius, panels E to H refer to posterior chain muscles. Panels A, E: EMG envelopes (mean ± standard error). The black line indicates the NW phase; the red, blue and violet lines indicate respectively the first, intermediate (30-40 min) and last (50-60 min) interval of training. Panels B, F, C, G, D, H: Paired-level statistical parametric mapping (SPM{F}) applied to NW and first (B,F), intermediate (C,G) and last (D, H) interval of training profiles. The threshold for significance is indicated in red.

slightly diminishing the need for counteractive stabilization from both the tibialis anterior and the hamstrings. Similarly, in the swing phase, the exoskeleton assists the advancement of the limb, reducing the dependence on the tibialis anterior and minimizing the activation of unnecessary antagonist muscles (Fig. 4 A, 1st column; 4 E 1st and 2nd column). These adaptations indicate that exoskeleton-assisted walking not only modifies the activation of agonist muscles, but also impacts their antagonists, influencing the overall neuromuscular strategy during walking.

IV. DISCUSSION

The results of this study support the hypothesis that prolonged training with an exoskeleton at maximum assistance induces an adaptive response in the muscles, i.e. the muscles' activity decreases in amplitude suggesting that subjects account for the assistive forces in their motor plan. These changes can have different timing depending on the muscles, but there is no rapid adaptation i.e. no significant differences were observable in the first 10 minutes. Finally, this study also supports previous evidence that the structure and/or the assistance provided by the device determine changes in muscle activity compared to normal overground walking.

A. ADAPTIVE RESPONSE OF MUSCLE ACTIVITY TO THE MAXIMAL ASSISTANCE DURING EXOSKELETON-ASSISTED WALKING

Subjects provided with excessive robotic assistance rapidly integrate these assistive forces into their motor plan, reducing their voluntary control, with potential negative effects on recovery outcomes [29], [32]. This stems from the consideration that, in motor control tasks, what drives learning and/or adaptation is the minimization of error and effort [28], [32], [56], [57], [58], [59], [60]. In robotics rehabilitation, if a robot assists a subject in performing a task, and the subject lets the robot work without opposing resistance or contributing to it, the robotic assistance decreases or cancels both error and effort. Although motor learning and recovery are different, they share common characteristics. Thus, if what we observe in motor control studies translates to motor rehabilitation, excessive assistance deprives the subject of error and effort to minimize, resulting in degraded learning performance and decreased recovery outcome. In other words, the hypothesis, suggested by different evidence, is that the effect of excessive assistance is negative in rehabilitation [29], [32]. Because as human beings we are greedy optimizers [28], meaning that we naturally tend to choose solutions that minimize effort and error, when we perceive that there is no need to correct mistakes or contribute effort, we tend to reduce our voluntary control. As a result, the exercise may become passive, involving limited engagement from the central nervous system. Although passive exercise can be beneficial to maintain muscular mass and joint mobility, the intention to actively move and voluntary control is fundamental for brain plasticity processes [61], [62], [63], [64], [65], [66], [67]. Therefore, several protocols aiming to maximize the rehabilitative training effects have been proposed, such as "assistance-as-needed" or "error augmentation" paradigms [68], [69], [70]. Despite this, previous works [20], [24] investigating the assistance of exoskeleton-assisted gait did not find adaptive responses to changes in the level or modality (e.g. bilateral vs unilateral) of robotic assistance. These studies focused on short-term exposure protocols in which a fixed level of assistance was provided for a limited number of steps (i.e., 8–15 strides in [17], 15-meter walking path [21]). These short durations may have limited the ability to observe gradual adaptive muscular response, as they were insufficient for significant changes in muscle activity to emerge. In contrast, our study extended the duration of exposure to one hour, allowing for a more comprehensive assessment of muscle adaptation over time. Importantly, the decision to apply a fixed assistance level (i.e., 100%) was not intended to represent an optimal therapeutic condition. Rather, it was a deliberate and controlled experimental choice, designed to isolate and amplify potential adaptive responses to sustained and maximal support. Within this controlled framework, our results indicate that muscle adaptation can indeed occur under high-assistance conditions, but such responses may remain undetectable in short-term studies, such as those involving only 10-minute sessions [20], [24].

If the training is prolonged up to one hour and the assistance is maintained at the same 'excessive' level, subjects incorporate the forces into their motor plan, leading to an adaptive response that first appears in the anterior chain muscles and gluteus (VM, RF, GLM from 20-30 minutes and VM from 30-40 minutes), then in other muscles (BF, ST from 50-60 minutes), while no response is observed in the GLM and SOL.

B. WALKING OVERGROUND WITH THE EXOSKELETON CHANGES MUSCLE ACTIVITIES WITH RESPECT TO NORMAL WALKING

This study supports previous findings [20], [24] suggesting that together with the assistance, the exoskeleton structure for gait training influences muscle activation. The observed differences in muscle activation between exoskeleton-assisted and normal walking may be due to different factors:

- The exoskeleton structure supports and constrains the movement of the pelvis. The assistance pulls the leg away from the body acting on the hip joint. Thus, this decreases the role of the gluteus muscles in keeping the pelvis stable when the body weight is on one leg. This could cause the low GLM activation [22], [23], [71].

- The ankle joint in the exoskeleton is passive and semi-rigid. Most commercially available exoskeletons do not have active ankle joints; thus, the propulsion of walking is generated by the hip rather than the ankle. In normal not assisted overground walking, the gastrocnemius and soleus are responsible for plantar flexion, i.e. for lifting the heel. They are activated on the rear foot when pushing off the ground to propel the body forward [72]. This ankle movement is not supported by this kind of exoskeleton, determining hip-dependent walking, which is associated with a decrease in the activation of the calf muscle compared to normal walking and a different activation timing in the upper leg muscles (RF, VM and ST). Exoskeletons with active ankle actuation could significantly alter these muscle activation patterns. By actively assisting plantarflexion and dorsiflexion, such devices could restore the push-off contribution of the triceps surae, leading to increased activation of the gastrocnemius and soleus during terminal stance, and can improve the activation of the tibialis anterior during the swing phase. This could, in turn, change the activation of the hip muscles and promote a more physiological gait pattern.

C. IMPLICATIONS FOR REHABILITATION

The control strategy of an exoskeleton should promote both physical and cognitive engagement, thereby enhancing motor learning. To achieve this, control strategies must ensure that the user remains actively involved, providing tailored assistance. By adjusting the level of support according to the user's performance, robotic training systems can stimulate motor learning and promote neuroplasticity [65], [66], [73]. These approaches fall under the concept of 'assistance-as-needed,' which aims to promote voluntary control and reduce passive adaptation by providing only the necessary level of aid to perform the task. This encourages greater muscle recruitment compared to fixed high-assistance strategies and may help sustain or even enhance muscle activity over time rather than reducing it. In this context, biosignals, including EMG, have been used as inputs for the control algorithms [73], [74], [75], [76], [77], [78], [79], [80]. The modulation of assistance based on a biosignal input, as the EMG, may prevent the adaptive muscular response observed in this study. However, muscle activity signals are affected by noise and movement artifacts, which can compromise the detection of muscle activations [78]. Furthermore, the variability in impairments among individuals with neurological disorders or injuries, combined with challenges in sensor placement, further complicates the reliability of this approach [81], [82]. Indeed, muscle signals can also be less reliable in individuals with abnormal activation patterns [83], [84]. These problems become especially relevant in real-time applications.

Another key consideration is the balance between real-time adaptation and the user's ability to adjust to changes in assistance. While real-time adaptation is essential for maintaining voluntary control and ensuring active participation, online adaptation can be highly sensitive to noise. Frequent, rapid adjustments in response to fluctuating signals may make it harder for the user to develop a consistent and reliable motor response. Moreover, frequent adjustments may overwhelm the user. In this context, our study suggests that short training periods (5-10 minutes) do not lead to significant muscle adaptation. If this finding holds in future clinical populations, it implies that continuous real-time adjustments to assistance may not be necessary. Instead, assistance can be provided in brief, consistent intervals, promoting active participation without requiring constant online adaptation. This supports a practical application of the assistance-as-needed paradigm, where assistance is adjusted at fixed intervals—such as every 10–15 minutes—rather than in real time. This timing allows the user to adapt physiologically and cognitively before experiencing a new level of support. Structured training paradigms, where assistance is maintained for short periods before being modified, may be more effective in promoting motor learning while avoiding adaptation. This approach simplifies the challenges of real-time control by adjusting assistance at intervals of 5-10 minutes. These findings are directly applicable to clinical rehabilitation settings. Therapists could design protocols where assistance is progressively adjusted at regular intervals. This would ensure that users stay actively engaged and do not become overly dependent on robotic support, enhancing functional recovery. Moreover, this approach would mitigate the potential negative effects of prolonged high assistance, promoting voluntary control and maximizing recovery.

D. LIMITATIONS OF THE STUDY

This study has specific limitations that need to be acknowledged. First, the study population consisted of a small sample of unimpaired participants, although in line with standards for preliminary research on assistive devices [19], [20], [85], [86], [87], [88]. Indeed, in unimpaired individuals, lower limb muscle activations during walking are highly repeatable, meaning that increasing the number of strides would not significantly alter the outcome [89]. We did not include participants from clinical populations (e.g., individuals with spinal cord injury, stroke, or other neurological conditions) in this study. This decision was driven by the goal of conducting an initial experiment with a homogeneous, unimpaired population to maintain experimental control and isolate the fundamental effects of robotic intervention. Including clinical populations would have introduced additional complexity due to variability in impairment levels and comorbidities. While this approach enhanced internal validity, it limits the generalizability of our findings to clinical populations. In addition, we employed a single, fixed level of excessive assistance (100%) rather than exploring a range of assistance magnitudes. While this allowed us to isolate the effects of prolonged exposure to maximal robotic support, it limits the generalizability of our findings to other assistance levels. It was also not possible to isolate the effect of assistance from the mechanical structure of the exoskeleton, as we only assessed the scenario with maximal assistance. A previous study with a different exoskeleton [24], which allowed a no-assistance modality, demonstrated that variations in activation timing could be related to the structure of the device rather than the level of assistance. This suggests that the timing differences observed in our study would likely occur even without assistance. Participants used crutches for balance during all training intervals, with instructions to avoid using them for propulsion. Although crutch use may influence posture, it was standardized and is considered a minor confounding factor. Finally, NW condition was assessed only once, before the exoskeleton-assisted walking session, and was not repeated at later time points. While this design choice allowed us to preserve the continuity of the one-hour training session and avoid confounding effects such as fatigue or inter-session variability, it also prevented us from directly verifying the temporal stability of NW within the same experimental session. However, given that NW is a familiar and stable motor task, and considering that participants were unimpaired, we relied on existing literature supporting the consistency of gait patterns in similar conditions [90], [91], [92].

E. FUTURE WORK

Future research will include larger and more diverse participant samples, including individuals with neurological conditions (e.g., spinal cord injury, stroke, or multiple sclerosis), to better understand how different populations respond to robotic assistance. Furthermore, future studies will explore different levels of assistance to deepen our understanding of motor adaptation across levels. While previous studies have not shown significant changes in muscle activity in response to variations in assistance level or modality, their findings were based on short-term exposure. Therefore, further research will examine the long-term effects of different assistance levels over extended periods, providing valuable insights for optimizing robotic-assisted rehabilitation and improving outcomes. These studies will contribute to identifying the most effective rehabilitation protocols, ensuring a balance between providing support and encouraging active participation. Additionally, exploring a wider range of exoskeleton designs, including devices with active ankle actuation, will shed light on how the structural characteristics of these devices influence muscle activation patterns, helping to differentiate the effects of mechanical design from assistance. Future studies will explore how alternative control approaches, such as EMG-based assistance strategies, can affect the user's level of active participation and the resulting muscle adaptations. These aspects will lead to more refined and effective rehabilitation strategies. Lastly, future studies will investigate muscle synergies to better understand how different muscles coordinate during exoskeleton-assisted walking, contributing to the development of more effective and targeted rehabilitation techniques.

V. CONCLUSION

To date, the adaptive responses of muscle activity during exoskeleton training, as well as the influence of the device remains a critical gap in research. In response, we focused on prolonged training with maximum assistance, comparing muscle patterns during both assisted and unassisted walking. Our findings suggest that prolonged exposure to the same level of assistance provided by an exoskeleton induces an adaptive muscular response. Specifically, when assistance is maintained for more than 10 minutes, users integrate these assistive forces into their motor plan, reducing voluntary control. This shift leads to more passive exercise and a gradual decline in muscle activity over time. Additionally, our results confirmed that the structure of the exoskeleton and/or the level of assistance influence muscle activity, leading to patterns that differ from those seen during normal overground walking. Using such knowledge, healthcare providers can improve the effectiveness of rehabilitation programs by optimizing the use of exoskeletons to maximize clinical outcomes. In conclusion, our findings underscore the potential drawbacks of prolonged exposure to fixed, high exoskeleton assistance and emphasize the need for customized training protocols. By taking advantage of these insights and periodically adjusting the level of assistance, rehabilitation practices can be refined to ensure active participation and voluntary control, enhancing recovery. This is particularly important for people with neurological disabilities, for whom sustained engagement is critical to maximize the benefits of robotic assistance and support effective motor recovery.

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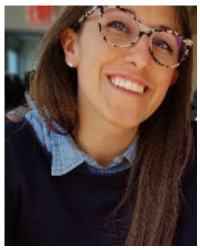

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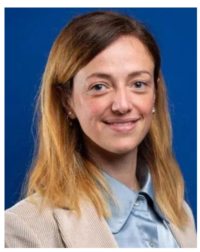

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