Longer Duration of Downslope Treadmill Walking Induces Depression of H-Reflexes Measured during Standing and Walking

Maruf M Hoque, Melissa A Ardizzone, Manning Sabatier, Michael R Borich and Trisha M Kesar*
Department of Rehabilitation Medicine, Division of Physical Therapy, Emory University, Atlanta, Georgia, USA
*Corresponding Author: Trisha Kesar, Department of Rehabilitation Medicine, Division of Physical Therapy, Emory University, Atlanta, Georgia, USA.

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Abstract

Objectives: The Hoffman-reflex (H-reflex) is an electrophysiological technique used to evaluate the excitability of the monosynaptic spinal reflex arc. In individuals with upper motor neuron lesions who show elevated spinal excitability, a depression of spinal excitability may indicate adaptive spinal plasticity. Downslope walking (DSW), an exercise intervention comprising repetitive eccentric muscle activity, has been shown to induce depression of soleus H-reflex amplitudes while seated, however, the dose-response time-course of H-reflex modulation during DSW has not been characterized. The objectives of this study were twofold: (1) to evaluate DSW-induced soleus H-reflex depression in the standing posture and during walking, and (2) to investigate the effect of walking duration (20 minutes and 40 minutes) of DSW (-15% decline) on soleus H-reflexes, with level walking (LW) as a control intervention.

Methods: Soleus H-reflexes were collected Pre, Post-20 minutes, and Post-40 minutes of walking in the standing position; and H-reflexes were also measured at 4 different time points during the terminal stance phase of walking.

Results: Our results showed that soleus H-reflexes evaluated in standing showed a greater % depression after DSW compared to LW, with a statistical trend for greater depression with longer durations (40-minutes). H-reflexes measured during walking showed greater depression after 40 minutes of walking compared to 20- or 30-minutes for both DSW and LW.

Conclusions: Longer duration treadmill walking (40-minutes) may induce a greater acute depressive effect on soleus H-reflex excitability compared to shorter durations (20-minutes) of treadmill walking. Future work will investigate the potential for DSW as a gait training intervention in people with upper motor neuron lesions such as multiple sclerosis and stroke.

Keywords: Hoffman’s Reflex; Spinal Cord Plasticity; Spinal Reflex; Neuroplasticity; Treadmill Training

Introduction

The Hoffman (H)-reflex is a neurophysiologic tool used to evaluate the excitability of the Ia afferent – alpha motor neuron spinal reflex circuitry [1-3]. Individuals with upper motor neuron lesions (such as stroke and multiple sclerosis) demonstrate spasticity and hyperactive reflexes, in conjunction with elevated H-reflex amplitudes [4-6]. Downslope walking (DSW) has been shown to acutely depress soleus H-reflexes after a 20-minute walking session at a greater magnitude compared to level walking [7]. DSW has been characterized by eccentric (muscle lengthening) contractions and greater afferent activation [7,8]. Moreover, 20-minutes of DSW was shown to induce greater soleus H-reflex depression compared to level running [9]. However, the mechanisms underlying this walking-induced soleus H-reflex depression are currently unclear and merit further investigation.

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Previous studies of DSW-induced soleus H-reflex modulation measured soleus H-reflexes while the participant was positioned in a semi-reclined seated posture. The spinal reflex pathway participates in and shows modulation for many motor behaviors, including standing, walking, and running [10,11]. Different motor behaviors are accompanied by task-dependent adaptation in the gain of the reflex pathway, which ensures that input from muscle spindle afferents contributes appropriately to soleus muscle activation during the behavior. Previous research shows task-related modulation of soleus H-reflexes, with H-reflexes being the largest when measured in supine, reduced in standing, and the smallest during walking and running [3,10,11]. Given the known posture-related modulation of soleus H-reflexes, evaluating whether DSW-induced modulation of soleus H-reflexes differs during measurements made during different motor behaviors (standing, walking) may provide detailed insights about the spinal plasticity mechanisms of DSW. For instance, due to the known increase in presynaptic inhibition during standing and walking, evaluation of DSW-induced H-reflex modulation during these tasks may aid with parsing out the role of presynaptic inhibition as a mechanism for the H-reflex depression [11].

Locomotion is the most common functional activity that lower limb muscles engage in, and involves rhythmic modulation of muscle activation and spinal reflex excitability. The human gait cycle is divided into the stance and swing phases, with anti-gravity muscles such as soleus playing an important role in stance phase tasks. Previous studies have shown that both muscle activation measured with electromyography (EMG) and H-reflex amplitudes of the soleus are modulated during different phases of gait cycle [11]. EMG activity of the soleus begins at heel strike, is maintained during most of the stance phase, and decreases just before toe-off [11,12]. Similar to the modulation of EMG activity, soleus H-reflex amplitudes were small at the time of foot contact, but quickly increased to a maximum value during late stance, followed by a decrease after the toes leave the ground [11,12]. Based on the known modulation of soleus H-reflexes during gait, here, we aimed to evaluate DSW-induced modulation of soleus H-reflexes in a task-specific context, i.e. during the terminal stance phase of gait when soleus EMG and soleus H-reflexes are involved.

Previous investigations on changes in H-reflexes following DSW have not systematically evaluated the dose-response relationship between walking duration and H-reflex modulation, and DSW walking durations longer than 20-minutes. It is unknown whether longer durations of DSW walking (e.g. 30- or 40-minutes) induce greater H-reflex depression, or if there is a plateau in DSW-induced H-reflex depression after a 20-minute walking period. In a prior study, we observed that soleus H-reflexes measured in sitting were depressed after DSW at a -15% decline for 20 minutes [8]. Another goal of the present study was to observe the time course of modulation of the soleus H-reflex (measured during walking and standing) during a 40-minute treadmill walking session. The objectives of the current study were to evaluate: 1) if DSW-induced soleus H-reflex depression is observed in the standing position and during walking, and 2) if longer walking durations (> 20-minutes) evoke greater DSW-induced soleus H-reflex depression.

Methods

Participants and Design

This study employed a repeated-measures design, with each participant completing 2 testing sessions, one DSW (-15% slope) session and one LW (0% slope) treadmill walking session. Session order was randomized with a minimum of 24 hours separating the 2 sessions. Nine able-bodied adults participated in this study (8 females; age 22.0 ± 1.7 years; height 163.8 ± 10.7 cm; mass 62.1 ± 11.2 kg; body mass index 23.0 ± 2.28 kg/m²). All participants provided written informed consent before study participation. The study was approved by the Institutional Review Board at Emory University.

To minimize the potential for delayed onset muscle soreness following DSW, at least 24 hours before the first session, the participant was required to walk DSW on the treadmill for 10 minutes [8,13]. In a previous study, participants underwent a battery of DSW walking tasks of varying durations (10 or 20-minutes) and varying slope grades (-15% or -25%), and participants who completed 10-minutes of -15% DSW session first did not report muscle soreness 24 and 48 hours post-exercise [8]. Except for the difference in the treadmill walking slope (DSW versus LW), identical procedures were followed during both testing sessions (Figure 1). Each walking session was subdivided into two 20-minute blocks of treadmill walking, with a total of 40-minutes of treadmill walking completed during the session (Figure 1). Soleus H-reflex recruitment curves were collected in a standing posture at 3 different time points: prior to the first 20-minute walking block (Pre), immediately after the first 20-minute walking block (Post20), and immediately after the 2nd 20-minute walking block (Post40). Soleus H-reflexes during the terminal stance phase of gait (50% gait cycle) were collected at 4-time points during the 40-minute walking session at 10 minutes (Walk10min), 20 minutes (Walk20min), 30 minutes (Walk30min), and 40 minutes (Walk40min). Participants walked on a Sole Fitness F85 Folding Treadmill at 2.5 mph. Each of the two 20-minute walking blocks were divided into five-minute epochs. Detailed procedures for H-reflex measurement are described below (Figure 1).

Figure 1: Overview of experimental protocol. A schematic shows the time points during the experimental sessions.
**H-reflex measurements**

Each participant’s dominant leg was tested during both sessions. At the beginning of each session, two surface electrodes (EL503, Biopac Systems Inc., Goleta, CA) were placed over the skin overlying the soleus muscle 2-cm apart to record electromyographic (EMG) activity. A ground electrode was placed on the lateral malleolus. EMG data were collected at 2 kHz, band-pass filtered (5Hz-1000Hz), and amplified by 2000 (BN-EMG2, Biopac Systems Inc., Goleta, CA). A cathodal pen electrode (Model G. MPPE, Digitimer, Hertfordshire, AL7 3BE, England) was placed at the popliteal fossa to deliver electrical stimulation to the tibial nerve and to search for the appropriate site to deliver peripheral nerve stimulation. With the participant in a prone position with the knee flexed, optimal nerve stimulation electrode placement was identified as the location that yielded an H-reflex without an M-response, and a plantar flexion movement at the ankle without eversion or inversion.

**Collection of soleus H-reflex recruitment curves in the standing posture**

During each session, H-reflexes were recorded in the standing posture before walking (Pre), after 20 minutes of walking (Post20), and after 40-minutes of walking (Post40) (Figure 1). During the standing H-reflex measurements, the participants were instructed to maintain a consistent standing posture and maintain a low-level (20% of maximum voluntary contraction) background EMG activity in the soleus. Participants were provided visual feedback regarding ongoing EMG activity to maintain background EMG activation. Soleus H-reflexes were evoked by stimulating the tibial nerve in the popliteal fossa through a monopolar adhesive electrode (round, 2.5 cm diameter) with the anode (square, 5-cm) placed above the patella (Medical Products Online, Danbury, CT). Using a constant-current electrical stimulator (STIMSOLA; BIOPAC Systems Inc., Goleta, CA), approximately fifty, 1-ms rectangular pulses were delivered at random intervals (5 - 8 seconds in duration) with progressively increasing stimulation intensities [8]. H-reflex and M-wave amplitudes were measured as the peak-to-peak amplitudes of the EMG signal. The standing H-reflex amplitude was expressed as the ratio of Hmax (average of the three largest H-reflex responses) to Mmax (average of the three largest M-wave responses).

**Collection of H-reflexes during walking**

During each walking session, H-reflexes during terminal stance phase were recorded at 4-time points during the 40-minute walking duration, at 10 minutes (Walk_{10min}), 20 minutes (Walk_{20min}), 30 minutes (Walk_{30min}), and 40 minutes (Walk_{40min}). The H-reflexes collected at Walk_{10min} served as the “baseline” or “Pre” H-reflexes during walking. All responses collected during the later time points would be compared to Walk_{10min}. H-reflexes during walking were evoked the terminal stance phase of the gait cycle (half way point between consecutive heel-strikes at approximately 50% of the gait cycle) (Figure 2). Accelerometers attached to the ankle were used to identify initial contact gait event (BIOPAC Systems Inc., Goleta, CA). The electrical stimulation intensity was set to evoke an H-reflex response whose amplitude was equal to 5%Mmax [14]. Each stimulus was delivered approximately every 3 - 5 gait cycles. As stated above, during each visit, the walking sessions were divided into two 20-minute blocks, and soleus responses were collected every 10 minutes of walking. For each 20-minute walking period, Mmax was determined for the terminal stance phase of the gait cycle to control for the effect of muscle force and length on Mmax. Prior to walking, the participant’s gait cycle duration, and timing for terminal stance phase was computed using data collected during one minute of treadmill walking. After gait cycle timing was determined, soleus H-reflexes were evoked for 30 gait cycles every 10-minutes (Figure 2). Each walking H-reflex trial acquisition was initiated at heel-strike. Electrical stimulation was delivered every 5 seconds at the pre-determined duration after occurrence of heel-strike (to match 50% gait cycle duration) (Figure 2). Soleus H-reflexes that did not correspond to 5% Mmax were discarded from analysis. An average of 12 H-reflex responses was used for analysis for each time point.

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Figure 2: Raw data from a representative subject demonstrating the timing of delivery of peripheral nerve stimulation to elicit H-reflexes with respect to the gait cycle. Graphs show raw data from (A) the accelerometer, (B) Soleus EMG, and (C) timing of delivery of the peripheral nerve stimulation. The accelerometer signal was used to identify heel strike, demarcating the beginning and end of a gait cycle (marked with dashed vertical lines). Stimulation was delivered at 50% of the gait cycle, which corresponds to the terminal stance phase of gait. The H-reflex elicited from the soleus muscle during gait is shown enlarged in the inset.

Statistical Analysis

The Hmax/Mmax ratio was the primary measure of H-reflex amplitude collected in standing at 3 time points during each session: Pre-walking (Pre-Hmax/Mmax), Post-20 minutes of walking (Post20-Hmax/Mmax), and Post-40 minutes of walking (Post40-Hmax/Mmax). The H-reflexes recorded during walking were normalized to the Mmax derived during walking as well as the Hmax/Mmax obtained in the standing posture (Hresponse/Walking Mmax)/(Hmax/Mmax).

To evaluate the magnitude of H-reflex depression observed in standing, for each session, % depression in the Hmax/Mmax with respect to baseline (Pre-Hmax/Mmax) was calculated following 20-minutes and 40-minutes of walking. Similarly, to evaluate the magnitude of H-response depression observed during walking, for each session, the % depression of H-response during walking with respect to the Walk10min was calculated at 20, 30, and 40 minutes of walking. Separate statistical analyses were performed for H-reflex data collected in standing and during walking. For H-reflexes evaluated in standing, a two-way repeated measures ANOVA was performed to evaluate the effect of walking condition (DSW, LW) and walking duration (20 minutes, 40 minutes) on the % depression in Hmax/Mmax. For H-responses collected during walking, a two-way ANOVA was performed to determine the effect of walking condition (DSW, LW) and walking duration (20, 30, 40 minutes) on the % depression in H-response with respect to H-response collected during the first 10-minutes of walking. IBM SPSS Statistics 23 was used for all statistical analysis and the significance level was set at p ≤ 0.05.

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Results

Standing Soleus Hmax/Mmax shows greater depression after DSW compared to LW

An H-reflex recruitment curve for a representative subject at Pre, Post-20-minute, and Post-40-minute time points for both treadmill walking sessions (DSW and LW) is displayed in figure 3. Figure 4A shows group Hmax/Mmax values for both walking conditions (DSW or LW) and all 3 time points (Pre, Post20, Post40) measured in the standing posture. Results of the 2-way ANOVA showed a main effect of walking condition (F = 6.78, p = 0.03) on % change in Hmax/Mmax. There was no significant main effect observed for time (F = 1.98, p = 0.196) nor was there a significant interaction effect (F = 0.428, p = 0.53) (Figure 4B). Post hoc paired comparisons displayed that there was no statistical difference in % depression at 20 minutes (p = 0.237) but there was a statistical trend for greater depression at 40 minutes following DSW compared to LW (p = 0.069). Additionally, the planned, paired t-test comparing the “Pre” Hmax/Mmax responses collected during standing showed no significant difference between DSW and LW (p = 0.82).

Walking soleus H-reflexes display depression after 40-minutes of walking for both DSW and LW

Of the 4 time points when H-reflexes during walking were measured, a large soleus H-reflex depression was observed after 40 minutes of walking (-87.1 ± 2.3% for DSW and -82.3 ± 4.1% for LW). The 2-way ANOVA evaluating the effect of walking condition (DSW, LW) and time (20 minutes, 30 minutes, and 40 minutes) on % depression of H-reflexes measured during gait (normalized to the 10-minute time point) showed a significant main effect of time (F = 29.32, p < 0.001), but no effect of walking condition (F = 0.05, p = 0.83) or interaction effect (F = 0.302, p = 0.75) (Figure 4D). Post-hoc pairwise comparisons (pooled for DSW and LW conditions) showed significantly greater % depression of soleus H-responses measured during walking at 40-minutes compared to 20-minutes (p < 0.001) and 30-minutes (p < 0.001). There was no significant difference in % depression between the 20-minute and 30-minute timepoints (p = 0.13). Additionally,
the planned, paired t-test comparing the "Pre" H responses collected during walking between the 2 slope conditions showed significantly smaller H-responses during DSW versus LW (p = 0.016).

Discussion

Our first objective was to investigate whether DSW-induced H-reflex depression, previously observed in the seated posture, is also observed for H-reflex measurements made during standing and walking. Similar to our previous findings in the seated posture, soleus H-reflexes measured in standing showed a greater H-reflex depression following DSW versus LW. However, DSW did not induce greater soleus H-reflex depression for reflexes measured during walking. Our second study objective was to evaluate the effect of walking duration on soleus H-reflexes during treadmill exercise sessions comprising DSW and LW. For H-reflexes measured during standing, we observed that walking longer durations (40-minutes compared to 20-minutes) showed a statistical trend for inducing a greater magnitude

![Figure 4](image)

*Figure 4: H-reflex data measured during standing and walking. (A) Group average (N = 9) and standard deviation for Hmax/Mmax values for the 2 walking conditions (DSW, LW) at three different time points (Pre, Post 20-min, and Post-40min of walking). (B) Average % depression of soleus Hmax/Mmax post 20-minutes and post 40-minutes (compared to the Hmax/Mmax value at Pre) for each of the 2 walking conditions (DSW, LW) measured in the standing position. (*p > 0.05). (C) Group average (N = 9) and standard deviation for H-reflex amplitudes values for the 2 walking conditions (DSW, LW) collected during the terminal stance phase of gait at 4 different time epochs (Pre, Post 10-min, Post 20-min, and Post-40min of walking). The H-reflex amplitudes measured during walking were normalized to the Mmax obtained during the same phase of the walking cycle, and then normalized to Hmax/Mmax obtained in the "Pre" standing position. Thus, these are H-responses as a percentage of the standing baseline Hmax/Mmax. (D) Average % depression of the H-responses measured during walking compared to the H-reflexes collected during the first 10-minute walking block.*

of soleus H-reflex depression for DSW than LW. Similarly, we observed that the % depression of soleus H-reflexes elicited during the late stance phase of gait was significantly greater after 40-minutes of walking compared to the 20-minute or 30-minute of both DSW and LW.

When soleus H-responses are collected while the participant is in the standing or seated positions, the ankle and knee joints are maintained in a relatively stable, static position, and consistent background EMG activation can be feasibly maintained throughout the experiment [1-3]. In our present study, we provided visual biofeedback to participants during the standing trials to ensure that a consistent magnitude of background soleus EMG was maintained during the H-reflex measurements made in standing. Thus, static postures (standing or sitting) create a more ideal scenario for H-reflex data collection by enabling better standardization of factors such as muscle length, ankle angle, and background EMG activation. Analysis of H-reflexes collected in the standing posture showed a main effect of walking condition, with DSW resulting in greater H-reflex depression than the control LW condition. Thus, similar to our previous work demonstrating that 20-minutes of DSW induced depression of soleus H-reflexes measured in the seated posture [8], in the current study, 40-minutes of DSW induced greater depression of H-reflexes collected in the standing posture compared to LW. Also, post-hoc comparisons showed that after 20-minutes of treadmill walking, there were no significant differences in H-reflex % depression between DSW and LW, but following 40-minutes of walking, there was a statistical trend for DSW inducing larger % depression of H-reflex amplitude compared to LW. Thus, our results suggest that longer durations of DSW may induce a greater depression of soleus H-reflexes measured in standing.

A disadvantage of H-reflex measurements during static postures (standing, seated) is that they evaluate spinal excitability for the muscle of interest during a state dissimilar to the functional role of the muscle during movement. H-reflex measurements during a functional task such as gait, albeit challenging to collect, can provide a valuable, task-specific index of spinal reflex excitability [10,11]. During gait, soleus muscle activity is dynamically modulated; increasing from heel-contact to push-off, at which time the EMG level peaks, reducing almost no activity during the swing phase [10,11,14]. In neurologically-unimpaired individuals, the soleus stretch reflex (and H-reflex) pathways are modulated during the stance phase to assist force production, and are suppressed during the swing phase to prevent foot drop [10]. A key finding from the H-response data recorded during walking in our study was that prolonged durations of walking (40 minutes), regardless of slope, induced substantial depression of H-reflexes measured during the late stance phase of gait. After prolonged durations of walking (40-minutes), both DSW and LW induced a large decrease in soleus H-responses (-70.1 ± 2.3% for DSW and -82.3 ± 4.1% for LW). However, our current results failed to show a differential effect of walking condition on H-responses measured during walking. This finding is in contrast to findings of larger magnitude of H-reflex depression observed post-DSW (compared to post-LW) when collected in both seated (prior study) [7] and standing positions (current study). One factor responsible for the lack of greater H-depression during gait following DSW versus LW could be that at baseline (during first 10-minutes of walking), H-responses measured during the DSW walking condition (34.23 ± 28.63%) were significantly smaller compared to LW (75.13 ± 63.24%). During DSW, starting out with a much smaller H-response may limit or ‘saturate’ the capacity for further down-regulation of H-responses. Smaller H-responses (at baseline) during DSW versus LW could be influenced by differences in ankle joint position and excursion during terminal stance for DSW [15]. Also, because presynaptic inhibition has been shown to be reduced during walking, the modulation of H-responses recorded during gait following 40-minutes of walking cannot likely be attributed to increased presynaptic inhibition [11,16]. The mechanisms underlying DSW- and LW-mediated modulation of soleus spinal reflex excitability require additional investigation in future studies.

Our results suggest that longer durations of walking exercise (40-minutes) may be more successful at inducing plasticity in spinal circuits compared to shorter walking durations studied previously (20-minutes). Another previous study evaluating the dose-response effects of DSW compared H-reflex depression induced after DSW durations of 20-minutes and 10-minutes, as well as 2 different treadmill slopes (-15% and -25% DSW) [8]. However, treadmill walking durations longer than 20-minutes were not investigated previously, and a dose-dependent effect of DSW on soleus H-reflexes was only found previously when comparing the largest DSW dose (20 minutes at -25% slope) to the smallest DSW dose (10 minutes at -15% slope) [8]. Overall, with regards to walking-induced H-reflex down-regulation, our

results demonstrate an advantage of longer (40 minutes) duration of DSW and LW exercise.

DSW has been purported as an exercise that modulates patterns of sensory, motor, and spinal inter-neuronal activity occurring during walking [7]. By inducing depression of soleus spinal reflex excitability, DSW may have promise as an exercise intervention for reducing hyperactive reflex responses in individuals with spasticity following upper motor neuron lesions. Our current findings demonstrate that the effects of walking slope and walking duration on DSW-induced modulation of soleus H-reflexes may vary with the static posture or dynamic task condition in which H-reflex measurements are made. Our results suggest that if the goal is to induce depression of H-reflex amplitudes during walking, 40-minutes of walking (either DSW or LW) may provide an advantage compared to shorter duration walking bouts. If the goal is to induce depression of H-reflex amplitudes in a static posture (sitting, standing), DSW provides an advantage compared to LW, and longer durations of DSW may be more effective.

Our study has a few limitations. We did not collect H-reflexes in the seated posture at any time point, preventing a direct comparison of our current results with changes observed in the seated posture. We did not include additional measures to probe the mechanisms of DSW-induced H-reflex modulation in-depth. For example, measurement of presynaptic inhibition and homosynaptic depression could help parse out the source of H-reflex depression [16]. Another interesting question that remains unanswered from our current dataset is why do the H-responses measured during walking show a large drop at the 40-minute time epoch, an effect that was not observed at 20- or 30-minutes. Also, given the longer durations of walking tested here, fatigue may be a potential factor influencing H-reflex depression. Future studies will evaluate the extended walking duration (40 minutes) in patients with spasticity and elevated H-reflexes, such as stroke, spinal cord injury, and multiple sclerosis. Lastly, the last H-reflex measurement was made immediately after walking, thus the persistence of H-reflex depression beyond the walking period was not evaluated in the current study.

Conclusions

Our study results demonstrate that soleus H-reflexes, measured in the standing posture, are depressed to a greater extent by DSW compared to LW. Soleus H-reflexes measured during the terminal stance phase of walking showed a large magnitude of depression after 40-minutes of walking for both DSW and LW. Our results also suggest that when the goal of walking exercise is to induce soleus H-reflex depression, longer durations (40-minutes) of walking may provide a greater advantage. The effects of walking exercise interventions on soleus H-reflexes may vary according to the posture (seated, standing) and task (standing, gait) during which the H-reflex measurements were obtained. Interventions such as DSW hold promise as rehabilitative strategies for individuals with hyperactive spinal reflexes due to upper motor neuron insults. However, for better clinical decision-making related to the prescription of DSW as an intervention (e.g. for balance versus gait retraining), the task- and posture-dependence of the reflex-modulatory effects of DSW need further study. This study provides the groundwork for future evaluations of the dose-response relationships of the DSW-induced H-reflex depression, effects of multiple sessions of DSW treadmill training on the H-reflex pathway, and the exploration of DSW as an exercise intervention to aid with spasticity management in individuals with upper motor neuron lesions such as multiple sclerosis or stroke.

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Statement of Conflicts of Interest

The authors have no conflicts of interest to disclose related to this study.

Bibliography


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