RESEARCH ARTICLE

# Lower leg muscle function: a contributory risk factor of changes in gait and balance after six minutes walk among people with multiple sclerosis

**Authors**
Adeola A. Sanni[1*], Robert C. Lynall[1], Deborah Backus[2], Kevin K. McCully[1]
**Affiliations**
[1]Department of Kinesiology, University of Georgia. USA.
[2]Shepherd Rehabilitation Center, Atlanta Georgia. USA.
***Corresponding author**
Adeola A. Sanni
Email: aas56767@uga.edu

**Abstract**
**Background/Purpose:** Multiple sclerosis (MS) is a progressive neurological disease that results in increased fatigue, decreased muscle function, and impaired gait and balance. The purpose of this study was to evaluate the relationship between lower leg muscle function and changes in gait and balance immediately and 20 minutes after 6mins walk-induced fatigue.
**Methods:** Six persons with MS (Patient-Determined Disease Steps 3-5) participated in the study. Perceived fatigue and demographic information were taken at baseline. Muscle fatigability and mitochondria capacity was measured in the lower leg muscles before walking. Reported fatigue (VAFS, 0-10), gait variability, gait asymmetry, and static balance were measured before and immediately after a self-paced 6 minutes treadmill walk with slight elevation, and after 20 minutes of rest.
**Results:** Participant's baseline MFIS score ranged from 14 - 71. There was a 173% increase in reported fatigue scores after walking. Gait was impaired with a ~26% increase in gait variability and ~40% increase in gait asymmetry immediately after walking (Cohen's D = 0.4, 0.3 respectively). There was also ~69% increase in balance postural sway with eyes opened and a ~20% increase with eyes closed (Cohen's D = 0.5, 0.2 respectively). Gait and balance remained impaired after 20 minutes of rest. The decline in gait parameters after walking had a negative correlation with muscle endurance ($r=-0.80$, $p=0.03$), and mitochondrial capacity ($r=-0.92$, $p<0.01$). The changes in gait and balance were more evident in participants with a higher disability.
**Conclusions**: Six minutes of self-paced walking with slight elevation worsened gait and balance among people with MS (PwMS), which did not completely recover after 20 minutes of rest. The changes in gait after walking were associated with lower leg muscle function. Lower leg muscle function might be an important intervention target to improve gait and balance fall risk among PwMS.

**Keywords**: Fall risk, Muscle fatigue, Muscle Mitochondria capacity, Multiple Sclerosis, Physical activity, gait, and balance

## 1.0. Introduction

Multiple Sclerosis (MS) is one of the world's most common progressive autoimmune diseases, affecting approximately 2.3 million people worldwide as of 2016 [1, 2]. People with MS (PwMS) have increased fatigue, gait abnormalities[3, 4], poor balance, and loss of mobility [5-8]. Mobility/disability in people with MS is evaluated using the timed 25-foot walk test (T25FWT) [9] and the Patients Determined Disease Step (PDDS) [10]. The six-minute walk test is used to measure endurance and fitness in people with MS [11].

Studies have shown declines in muscle function in people with MS. This includes the decrease in muscle strength, increase in muscle fatigability [12, 13], and impaired muscle mitochondria capacity[14, 15]. Muscle fatigability is a measure of the decline in muscle contractile force which mostly occurs during/after physical activity[16]. Muscle fatigability interferes with the central perception system leading to a lack of motor control and falls [17, 18]. Muscle fatigability is objectively measured using voluntary muscle contraction [12], and involuntary contractions (electrical neuromuscular stimulation) [13, 19, 20]. Perceptions of fatigue among PwMS have been subjectively measured using perceived fatigue scales such as the Modified Fatigue Impact Scale (MFIS) and visual Analog Fatigue Scale (VAS-F) [21].

Studies have shown that gait variability [22, 23], gait asymmetry [24], and poor postural balance are leading fall risk factors among PwMS. Increased disease severity is associated with greater declines in gait and balance [22, 24]. About 50%-80% of PwMS report poor balance [25], this affects their quality of life and functionality [26, 27]. The gastrocnemius (GA) and the Tibialis Anterior (TA) muscles play important roles in maintaining an upright position and good balance [28], thus fatiguability in these muscles could be a contributory risk factor of gait and balance impairment.

While multiple studies have measured gait and balance among PwMS at rest [6, 8, 29, 30], there is little information on these measures after practical daily physical activity such as walking among PwMS. Therefore, the purpose of this study was to evaluate associations between changes in gait and balance immediately and 20 minutes after a 6 minutes self-paced treadmill walk in a sample of PwMS. We hypothesized that a 6 minutes walk would produce an increase in gait variability, gait asymmetry, and postural sway that will not be recovered to baseline values after 20 minutes of rest. We also hypothesized that muscle function of the lower leg will correlate with the changes in gait and balance after the 6 minutes walk.

Acquiring information on changes in gait and balance after physical activity among PwMS, and the role of muscle function of the lower leg on these changes might provide direction to developing improved interventions to prevent falls among this population.

## 2.0. Methodology

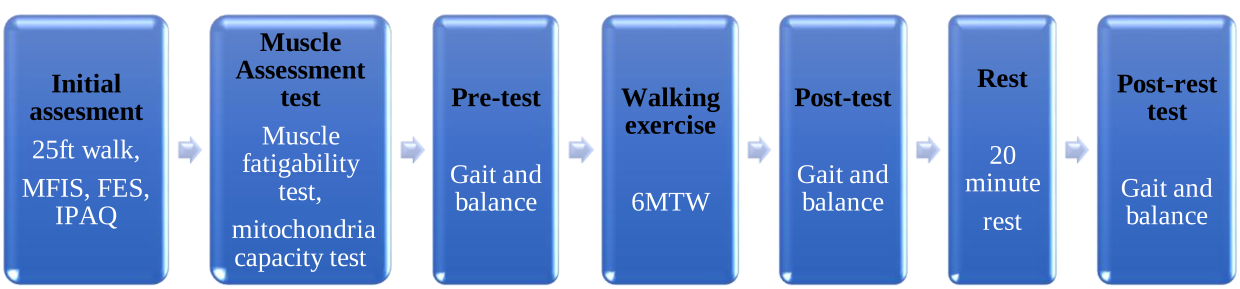
The study was conducted with the approval of the Institutional Review Boards at the University of Georgia and Shepherd Center in Atlanta, Georgia. All participants signed the informed consent form before been tested. All testing took place in a laboratory setting.

**2.1. Participants:** Participants were included if they were 18 years or older, had been diagnosed with MS, had a Patient-Determined Disease Steps (PDDS) score of 3-5, and could walk 25ft with or without a single walking cane. PDDS of 3 is defined as gait disability where the MS interferes with the participant's activities but can walk without support except for when in an attack. PDDS of 4 is defined as an early cane, where the participant walks with a can for long distances but can walk 25 feet in 20 seconds without a cane. PDDS of 5 is defined as a late cane, where the participant needs a cane to walk 25 feet and needs a scooter or crutch to walk long distances. Participants were excluded if they had any other neurological disease, unmanaged cardiovascular disease, or any other musculoskeletal condition which might affect their walking patterns. Participants who reported a fall or a relapse in the past 30 days were also excluded. Exclusion criteria also included any sensitivity on the skin of the lower leg which might make the electrical stimulation for the muscle fatigability test uncomfortable. Participants were recruited in collaboration with the Shepherd Center in Atlanta. Nine PwMS were recruited, one was excluded because he reported he had a relapse in the last 30 days and two were excluded because their PDDS was below 3. Six persons with MS with minimal walking aid within the PDDS score of 3-5 completed the study. This study was shortened due to COVID-19 related restrictions resulting in a pilot study due to the small sample size.

**2.2. Study design:** Pretest-posttest experimental study design was adopted for this study. The participants were screened for eligibility using a pre-designed questionnaire. Figure 1 shows an illustrative outline of the study protocol.

Each participant had an initial assessment that involved completing questionnaires, performing a Timed 25-foot walk test (T25FWT). The muscle assessment test included a muscle fatigability test, and muscle mitochondria capacity test. The muscle fatigability test was performed on the Gastrocnemius (GA) muscle and Tibialis Anterior (TA) muscles of both legs, while the muscle mitochondria capacity test was done on the GA muscle of the dominant leg reported by the participant. Outcome measures of gait and static balance were taken before and after a 6 minutes treadmill walk. The same outcome measures were also taken after 20 minutes of rest.

**Figure 1** The illustration of the study protocol.




## 2.3. Experimental procedure

Initial assessments*:* All assessments were performed by a trained research doctoral candidate with the help of some trained research assistants

Questionnaires/forms:

Modified Fatigue Impact Scale (MFIS): This is a standardized 21-item questionnaire that measures how perceived fatigue influences daily living in terms of physical, cognitive, and psychosocial functioning with an Interclass correlation (ICC) of 0.85 among PwMS[31].

Modified Fall Efficacy Scale: The participants answered questions on the Modified Fall Efficacy Scale (FES). This is an expanded version of the fall efficacy scale. It is a 14-item questionnaire used to identify fear of fall when performing some daily activities mostly at home, it has an ICC of 0.93 [32].

International Physical Activity Questionnaire (IPAQ): The 7-day IPAQ is a generally accepted questionnaire used to identify the participants' previous activity level. This has been validated both in healthy and clinical populations [33].

Demographic Information: Participants provided demographic information including years since diagnosis, age, type of MS, and current list of medications using a self-developed questionnaire.

Mobility assessment (T25FWT): The T25FWT is a validated and reliable clinically approved test for mobility which was developed in 1960 by the National Multiple Sclerosis Society [34]. Participants were asked to walk from one end of a marked 25ft course to the other end as quickly and safely as possible. The time was initiated as soon as the participant was asked to start at the beginning of the measured walkway, and the time was stopped as soon as the participant crossed the end mark. Two trials were performed and the average of the two trials was recorded.

Muscle endurance test to measure muscle fatigability: The muscle fatigability test was done using the involuntary contraction method which bypasses the CNS thereby requiring less physical effort from the participants. It was calculated as a percent of endurance index using a previously established protocol [20, 35]. The test was done on the participants' GA and TA muscles of both legs by measuring the acceleration of involuntary muscle contractions. Muscle contractions were elicited with twitch electrical stimulation using an electrical stimulation device (RICH-MAR, theratouch 4.7, Version 15). Two surface neuromuscular stimulation electrodes (superstim, richmar) sized (4cm x 5cm) were placed proximal and distal to the muscle belly [35]. Muscle twitch acceleration was measured with a tri-axial accelerometer (Axivity-AX3, Newcastle Upon Tyne, UK) placed on the belly of the muscle tested (between the two electrodes) and secured with double-sided tape while the participants laid in a supine position. The accelerometer was set at ± 2 g, with a 13-bit resolution, and 400 Hz sampling rate. The muscle was stimulated at 5Hz; Biphasic square wave pulses (200 µs with a 50 µs inter-pulse delay) with submaximal current levels enough to cause visible muscle twitch but tolerable by the participant. The endurance test uses a 5-minute stimulation period with 30 seconds baseline before and after the test. The decline in muscle twitch contraction was used to calculate the muscle endurance index/muscle fatigability (EI) [20].

Mitochondria capacity test: This was non-invasively measured on the GA muscle of the reported dominant leg, using the Near-Infrared spectroscopy (NIRS) following an established protocol [13, 36]. The NIRS device (Portamon; Artninis medical system, Eistenweing, the Netherlands) measures recovery kinetics after a bout of exercise/electrical muscle stimulation. The device was placed on the belly of the medial GA and two surface neuromuscular stimulation electrodes (superstim, richmar) were placed proximal and distal to the NIRS device. A blood pressure cuff (10 cm width) was placed on the thigh just above the knee joint. The cuff was rapidly inflated to 230-

280 mmHg to temporarily occlude blood flow to the muscle. The test protocol involved an initial 30-second occlusion to measure resting muscle metabolism. Next, muscle activation was tested using 30-second stimulation and another 30-second occlusion. Mitochondrial capacity was then measured using 30 seconds of electrical stimulation to activate the muscle, followed by six temporary occlusions. There were four trials of the mitochondrial capacity test, after the fourth stimulation, 5 minutes of recovery was allowed and a final 30-second occlusion (final resting metabolism) was performed to calibrate the mitochondrial capacity trials test [36].

Six minutes' Treadmill walk (6MTW): This was adapted from the clinical 6 minutes walk test which was used to measure walking endurance among PwMS. The walking exercise was done on a treadmill for six minutes with the participant's preferred inclination between 1-5% to mimic unleveled ground and to activate the GA muscle. Preferred inclination and speed were selected by the participants. Walking was done with minimal or no touch on the sidebar.
Walking exertion was assessed by asking the participants for their Rating of Perceived Exertion (RPE) using a 20-point BORG scale (6-no exertion at all and 20- Maximal exertion) [37]. Their heart rate was taken with a finger pulsometer, and a visual analog scale for fatigue (VAS-F) 0-10 was used to monitor fatigue level at every minute.

*The gait and balance measures were taken before the walk, after the walk, and after 20 minutes of rest*

Gait: Spatiotemporal gait parameters were measured while participants walked comfortably across a 20-foot gait mat (Protokinetics Zeno walkway, Havertown, PA, USA). There was a ten feet walkway before and after the gait mat to allow the participants to accelerate and maintain a consistent speed across the gait mat. Gait variability is a measure of fluctuation in walking patterns, while gait asymmetry is the difference between the right and the left limb walking patterns. Selected spatiotemporal gait variability and asymmetry parameters that have been previously associated with fall risk were measured [38, 39], and included double support time, stride length, single support time, and step length. Gait variability was defined by the percent coefficient of variation (%CV) of double support time and the stride length separately. Double support time is the amount of time when both feet are in contact with the ground while stride length is the distance between the heel of one foot to the heel of the same foot. Gait asymmetry was defined as the difference between the right and the left foot for the mean step length (cm) and mean single support time (s), this was calculated using a previously established equation $(\frac{right-left}{\frac{1}{2}(right+left)} * 100)$ [40]. Step length is the distance between the heel of one foot to the heel of the next foot, while single support time is the amount of time each foot is in contact with the ground. All gait parameters have been shown to have an intraclass correlation coefficient (ICC) above 0.84 except step length variability which has an ICC above 0.95 [41].

Balance: The participant's static balance was measured on a force plate (Bertec, Columbus, Ohio USA) with a data collection rate of 1000Hz. Among the various methods used to measure balance, force platform technology provides a quantifiable measure of postural sway among clinical populations [42]. Two trials of 30s data collection were made each, with participant's eyes opened and eyes closed with double leg stance (2 trials each). Postural sway was assessed by recording the area of an ellipse that encapsulated 95% confidence of the participants' sway (center of pressure) throughout the trials [43]. Postural control measures on a force platform have an ICC greater than 0.70 with an average of at least two trials [44].

The gait and balance tests were repeated immediately (average of 10s) after the walk. Then participants were asked to sit and rest for 20 minutes before the gait and balance measurements were taken again. For each measurement, the gait data was collected before the balance for all the participants.

### 2.4. Data analysis

Muscle Fatigability/Endurance test: Data from the accelerometer was transferred to Microsoft Excel and a resultant vector was calculated from the three axes $Ar = \sqrt{(X^2 + Y^2 + Z^2)}$. Further analysis was done in MATLAB R2017b (Mathworks inc., Natick, Massachusetts, USA) using a customized written program. The peak-to-peak analysis was used to determine the magnitude of acceleration for each contraction frequency. Muscle fatigability was reported as an endurance index which was calculated as the average acceleration over 20 seconds at the end of the stimulation in ratio to the peak values at the first 20 seconds. $((EI = a_e/a_p) \times 100))$. [20, 35, 45].

Mitochondria capacity test: This analysis was done with a pre-written MatLab program [36] using oxygen recovery kinetic after the electrical stimulation by fitting the oxygen metabolic rates to the exponential equation, $y(t) = End - \Delta \times e - kt$. Four trials of the mitochondria protocol were done and an average value of rate constant ($min^{-1}$) was recorded.

Gait: Selected gait parameter measures were exported from the PKMAS software into Microsoft Excel. %CV of double support time and stride length was selected to calculate gait variability. The mean difference between the right leg and left leg step length (cm) and single support time(s) was calculated as the gait asymmetry. An average of the two trials was calculated for each value. Equation $\frac{(pre-post)}{pre} * 100$ was used to calculate the %difference in gait before and after the 6MTW, while $\frac{pre-rest}{pre} * 100$ was used to calculate the %difference in gait after taking 20 minutes rest compared to before the 6MTW [46].

Balance: Data collected from the force platform were exported and a custom-written Matlab code was used to calculate the 95% ellipse area [43]. An average of the two trials was calculated for each value. Percent difference in balance before and after the 6MTW was calculated $\frac{(pre-post)}{pre} * 100$, while $\frac{pre-rest}{pre} * 100$ was used to calculate the %difference between the balance before the 6MTW and after taking 20 minutes of rest.

### 2.4. Statistical analysis

Descriptive analysis of participants was done by calculating the mean and standard deviation. All statistical analysis was done with SPSS IBM version 24.0. Significance was accepted at 0.1 alpha level. The use of a significance level of 0.1 was done because of the pilot nature of the study due to the small sample size as a result of the restrictions related to COVID-19. The effect size was calculated and reported as Cohen's. Paired t.test was used to find the difference in HR, RPE, and VASF pre and post the 6 minutes walk. Pearson r was also used to find the correlation between MFIS and 25-foot walk score. Pearson correlation analysis was used to find the relationship between the muscle fatigability of the GA and the TA on both legs, and mitochondria capacity. Independent t.test was used to find the difference in muscle fatigability of the GA muscles between the right and the left leg. Paired sample t-test was used to find the difference in gait and balance before and after the walk, and also the difference before the walk and after 20 minutes of rest. Pearson correlations were calculated to access the relationship between %difference in gait and balance post-walk and muscle function.

## 3.0. Results

*Descriptive analysis:* Six persons with MS completed the study. Participants MFIS ranged between 14 and 71. There was a moderate but not significant correlation between participants' MFIS and T25FWT walk time (r = 0.48, p = 0.17). Table 1 shows the demographic characteristics of the participants and their treadmill walk performance.

**Table 1: Participant's Demographic Characteristics/ Treadmill Walking**

| Gender | Age (yrs) | Height (cm) | Mass (kg) | BMI ($kg/m^2$) | Diag (yrs) | Type of MS | PDDS | T25F walk (s) | MFIS | Walk speed |
|---|---|---|---|---|---|---|---|---|---|---|
| Female | 65 | 172.0 | 96.8 | 32.7 | 7 | PPMS | 4 | 8.14 | 54 | 1.5 |
| Male | 60 | 181.0 | 77.6 | 23.7 | 25 | RRMS | 5 | 10.50 | 27 | 1.0 |
| Female | 31 | 170.3 | 60.6 | 20.9 | 6 | RRMS | 3 | 4.41 | 14 | 4.0 |
| Female | 52 | 172.6 | 98.9 | 33.2 | 20 | RRMS | 3 | 6.26 | 35 | 2.0 |
| Male | 37 | 190.0 | 75.6 | 20.9 | 9 | PPMS | 5 | 7.95 | 70 | 1.0 |
| Male | 39 | 171.4 | 77.1 | 26.2 | 2 | RRMS | 4 | 9.35 | 71 | 2.0 |

**Note;** BMI (Body Mass Index), Diag (duration since Diagnosis), PPMS (Primary Progressive MS), RRMS (Relapsing-Remitting MS), PDDS (Patient Determine Disease Step), MFIS (Modified Fatigue Index Scale).

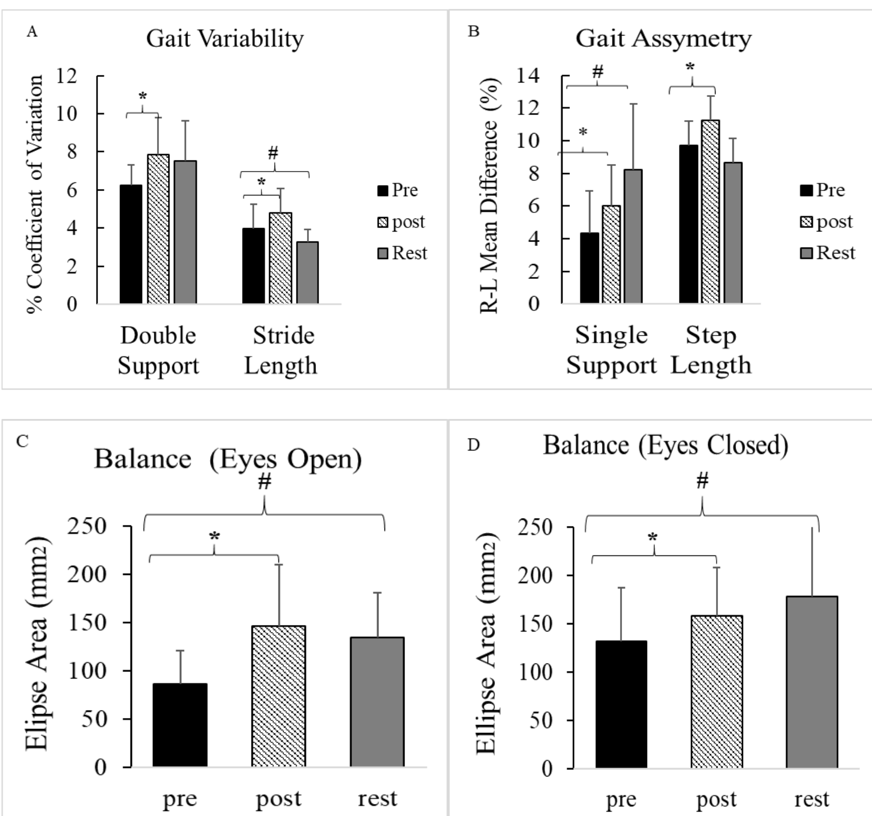
*Six minutes walking:* The average walking speed was 1.8 ± 0.9 mph and the average inclination was 2.3 ± 1.4 degrees. Heart rate increased from 95 bpm in the first minute to 112 bpm at 6 minutes (p<0.01), cohen's D = 2.07. RPE increased from 8.6 at one minute to 13.8 at 6 minutes (p<0.01), Cohen's D = 2.91. VAS-F also increased from 1.7 at one minute to 4.7 at 6 minutes (p<0.01), Cohen's D = 2.30.

*Muscle function:* All participants reported the right leg as their dominant leg. There was a significant positive relationship between the muscle fatigability of the right GA and the right TA (r = 0.94, p<0.001), and between the left GA and the left TA (r = 0.96, p<0.001). There was no significant difference between the fatigability of the right and left GA (p = 0.79), so the fatigability (endurance index) of the right GA muscle was used for further analysis. Mitochondrial capacity and endurance index of the GA muscle were significantly correlated (r = 0.77, p=0.037).

*Changes in gait and balance post-walk:* There was a 26% increase in double support time variability, 25% increase in stride length variability, a 16% increase in step length asymmetry, and a 40% increase in single support time asymmetry after the 6MTW with a Cohen's D of 0.4, 0.3, 0.3 and 0.1 respectively. There was also a 69% increase in the COP ellipse sway area, Cohen's D of 0.5 with eyes opened and a 20% increase in the COP ellipse sway area, Cohen's D of 0.2 with eyes closed after the 6MTW.

*Changes in gait and balance post rest:* After 20 minutes of rest, there was still a 21% increase in double support time variability, an 18% decrease in stride length variability, an 11% decrease in step length asymmetry, and a 90% increase in single support time asymmetry compared to the baseline values with a Cohen's D of 0.5, -0.3, -0.1 and 0.7 respectively. There was a 55% increase in the COP ellipse area, Cohen's D of 0.5 with eyes opened, and a 35% increase in the COP ellipse area, Cohen's D of 0.3 with eyes closed. Figure 2 shows the mean value and standard deviation for each gait parameter before the 6 minutes walk, after the 6 minutes walk, and after the 20 minutes rest.

**Figure 2**




**Figures 2 A)** gait variability, **B)** gait asymmetry before the 6-min walk, after the 6-min walk, and after 20 minutes rest. Balance ellipse area before (pre) and after the 6MTW (post) and after 20 minutes rest (rest) with participants' eyes opened (C) and eyes closed (D). * shows a significant increase after 6MTW, # indicates a significant increase after 20 minutes of rest. Values are mean and standard deviation.

*Individual change in gait and balance:* Figure 3 shows the change in gait and balance after the 6 minutes walk and after the 20 minutes rest for each participant. The participants with higher disability (PDDS 4-5) walked slower with an average speed of 1±0.3mph and inclination of 1 ± 0.3 degrees, they had a 45% increase in gait variability and a 78% increase in COP ellipse area after the 6mins walk with a Cohen's D of 0.8 and 0.8 respectively, and a 36% increase in gait variability and 67% increase in COP ellipse area after 20 minutes of rest with a Cohen's D of 1.0 and 1.2 respectively. The participants with a lower disability (PDDS 3-4) walked faster with an average speed of 3 ± 1.2mph and inclination of 3 ± 1.0 degrees had a 3.4% decrease in gait variability and a 0.9% increase in COP ellipse area with a Cohen's D of 0.3 and 0.01 respectively, and a 2.8% decrease in gait variability and 20% increase in postural sway with a cohen's D of 0.06 and 0.1 respectively.

**Figure 3**

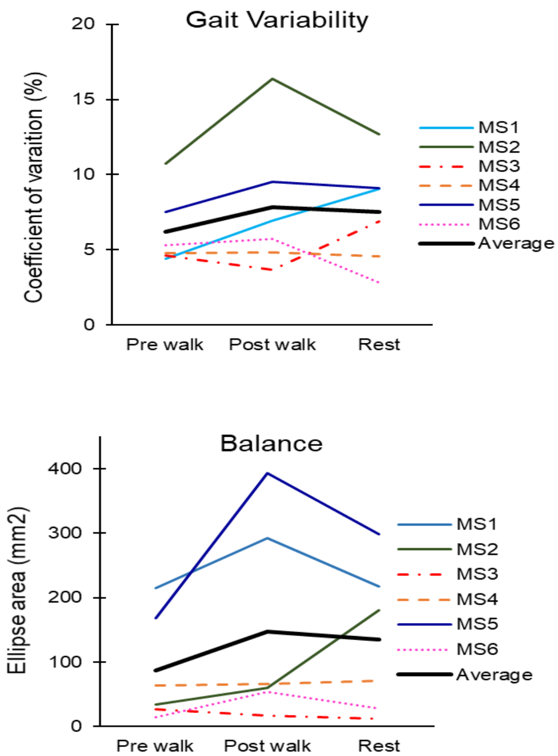



**Figure 3** shows an illustration of each participant as a case study. The figure shows the participant's **A)** gait variability and **B)** balance ellipse area before the walk, after the walk, and after 20 minutes of rest. The participants with the lower disease severity are with broken lines, while participants with higher disease severity are with the solid lines

*Relationship between muscle function, Gait, and Balance:* There was a significant negative relationship between %change in the gait variability measure of double support time post-walk and muscle fatigability (r = -0.80, p = 0.028), and muscle mitochondria capacity (r = -0.92, p = 0.004). There was also a significant negative relationship between the %change in the gait asymmetry measure of single support time post-walk and muscle fatigability (r = -0.99, p < 0.001), and muscle mitochondrial capacity (r = -0.78, p=0.035). There was no significant relationship between % change neither in balance post-walk and muscle fatigability (p = 0.38), nor balance post-walk and muscle mitochondria capacity (p = 0.45) (Figure 4). There was a positive correlation between muscle fatigability and T25FWT time (r= -0.83, p = 0.02).

**Figure 4**

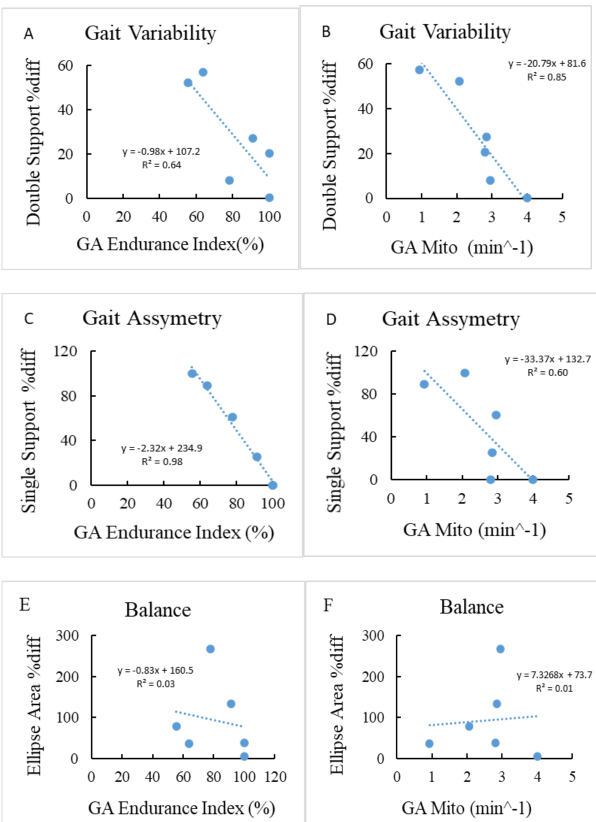



**Figure 4 A)** The correlation between % change in gait variability measure of double support time and gastrocnemius (GA) muscle fatigability/endurance index. **B)** The correlation between gait variability of double support time percent difference (pre and post) and GA muscle mitochondria capacity. **C)** The correlation between gait asymmetry measure of single support time and the GA muscle fatigability/endurance Index. **D)** The correlation between gait variability and GA muscle mitochondria capacity. **E)** The correlation between the balance variable of ellipse percent difference (pre and post) and GA muscle fatigability/endurance. **F)** The correlation between the balance variable of ellipse Area % difference and GA muscle mitochondria capacity.

## 4.0. Discussion

The major finding in this study was that there was a decline in gait and balance after the 6 minutes treadmill walk among participants with mild to moderate MS. Previous studies have measured the gait and balance of PwMS at rest but few studies have measured the changes in gait and balance after they have been physically active/fatigued. A recent study showed that there was an increase in gait asymmetry every minute during a fast-paced 6 minutes walk test among people with mild to severe MS [24], and the increase in gait asymmetry was highest among the severe group. This was consistent with the findings of our study. The walking exercise was self-paced by the participant and was chosen to reflect everyday walking activities among PwMS who are ambulatory. This suggests that the changes in gait and balance reported in our study could be expected in people with MS in the course of their daily activities.

Changes in gait variability and balance have been associated with increased fall risk. The increases in gait variability and reduced balance measures in our study suggest that people with MS could be at greater fall risk after they have walked. In addition, the evidence that gait variability and balance variables didn't completely return to baseline after 20 minutes of rest suggest the potential increase in fall risk remains for at least 20 minutes after walking.

The increase in gait variability and gait asymmetry after walking suggests that PwMS might be at the risk of falling after their day-to-day physical activity such as walking their dogs, shopping, trail walking, etc. The 95% COP area measure used in this study has been reported to be more reliable, accurate, and sensitive than the clinical Berg Balance Scale in predicting an accidental fall over a 3-months study [47]. Participants' balance got worse after the 6 Minutes treadmill Walk. Our study showed a 69% difference in balance with eyes opened and a 20% difference with eyes opened compared to the baseline balance measure. An exercise intervention on the balance of PwMS only reported a 15% - 17% improvement in force plate COP measures after a 12-weeks home-based balance training compared to their baseline measures [48]. This shows that the 69% decline reported here may be clinically significant despite the small sample size. The risk of accidental falls was reported to be a difference in 8% in COP value [47], how much more a 69% difference which was reported in our study. This shows that PwMS need proper safeguard after a short bout of physical activity which might reduce their risk of falls. However, the relationship between falling, gait, and balance parameters can be influenced by other fall risk factors, such that it is difficult to assign absolute fall risk to the changes seen in this study. Therefore, future research could evaluate the magnitude of changes in gait and balance, and its association with fall risk.

In this study, the impairment in gait and balance persisted after 20 minutes of rest. We did not go longer, but the idea that gait and balance did not recover adds to the importance of looking out for PwMS after an ideal daily walking activity. To the best of our knowledge, previous studies have not precisely looked at the recovery of gait and balance after fatigue-related activities among PwMS. We think more emphasis needs to be placed on allowing PwMS to recover after they have been active, and 20 minutes might not be sufficient. More studies need to emphasize the recovery rate of fatigue, gait, and balance after physical activity among PwMS to ensure the safety of this population.

An important aspect of our study was the choice of physical activity. This study found that 6 minutes of walking at a self-selected speed and a slight inclination produced changes in gait and balance. The 6 minutes walk is often used as a clinical test of endurance among PwMS [24], it was chosen in this study because it is practical and within the intensity of normal daily activities among this population. When used for functional evaluation, the 6 minutes walk test is performed without elevation. The use of elevation in this study should have increased activation of the GA muscles. This could help explain the relationships between the changes in gait and balance and the muscle function tests of the lower leg muscles. The 6 minutes walk is not considered a maximal exercise test, although it did result in significant changes in heart rate, perceived exertion, and fatigue. Karpatkin and Reztelny also reported an increase in fatigue among PwMS after a continuous 6 minutes walk, although they did not use increased elevation in their study [49].

Studies have shown that gait variability [22, 23], gait asymmetry [24], and poor postural balance are leading fall risk factors among PwMS. The increase in gait variability, gait asymmetry, and postural sway could suggest an increased risk of falls among these participants. Falls lead to an increased rate of injury and death among PwMS [50]. Mazumder et.al showed that out of 58 PwMS, 70% reported a fall at least once and 48% reported a fall at least twice in six months; the participants concluded that most of their falls were a result of fatigue [51]. A previous study also shows that most falls occur when performing relevant daily activities such as walking [52]. Alterations in gait (walking pattern) and balance are the most common mobility challenge faced by PwMS, and these are the leading independent risk factors and predictors of falls among this population [25, 53].

When evaluating the responses of individual subjects, three of the participants showed greater evidence of impaired gait and balance after the 6 minutes walk and after the 20 minutes rest. The other three subjects had little or no change in their gait and balance measures after the six-minute walk. In the three subjects who changed after walking, the magnitude of change was as large as doubling the balance ellipse area or greater than a 10% increase in gait asymmetry and variability. These changes could be large enough to influence the risk of falling. The three subjects who showed evidence of impaired gait and balance after walking also walked the slowest and used the lowest walking incline, they had the highest PDDS values and lowest muscle fatigability and muscle mitochondrial capacity values. This suggests that the potential impact of walking on gait and balance might be predicted based on higher disability scores or lower muscle function values.

The change in gait variability and asymmetry was related to lower leg muscle function, this was true for fatigability and mitochondrial capacity of two important muscles responsible for walking. The increase in gait variability and asymmetry are common measures and predictors of fall risk among PwMS and older adults[22, 24]. Studies have reported that most falls among PwMS occur during locomotion [52]. This suggests that decreased function of the lower leg muscles might play a role in fall incidence among PwMS especially after a short bout of physical activity. It might be interesting to see if increasing muscle function would improve the gait and balance of PwMS thereby reducing fall risk. A previous study shows muscle dysfunction is positively associated with walking impairments (25 foot and 6 minutes walk) among PwMS, and that walking training can improve muscle function [13]. There was an association between muscle mitochondria capacity and muscle fatigability of the GA, this is in line with the previous study by Willingham et.al [13], where the authors reported that there was a significant relationship between GA muscle fatigability and mitochondria capacity in 20 persons with mild to moderate MS. The mitochondria play an important role in energy (ATP) production in the skeletal muscle [54]. Studies have shown that there is a 40% deficit in mitochondria capacity among PwMS compared to their control group, and those with lower mitochondria walked slower during the 25ft walk test [14]. There was a strong positive correlation between walking distance, muscle fatigability, and muscle mitochondria capacity [13]. Hansens et.al concluded that muscle mitochondria capacity is primarily associated with walking distance among PwMS [55]. It is recommended that PwMS should engage in frequent physical activities such as walking to slow down the progression of their disease and improve quality of life, but they engage in less physical activity mostly due to an increase in fatigue, mobility impairment, and fear of fall as a result of a previous fall [56, 57]. Engaging in safeguarded exercises to increase the skeletal muscle fatigability and mitochondria capacity might play a role in improving the mobility and balance among this population which could result in an increased level of physical activity.

The fatigability measurements in this study used an accelerometer to measure the acceleration of the muscle during submaximal electrical stimulation; this bypasses the central nervous system. This method has been used to study muscle fatigue both in healthy and clinical populations[20, 35]. Previous studies measured muscle fatigue using voluntary contactions[12] or reported fatigue [21, 58]. Moris et.al concluded that objective measures should be used to measure muscle performance in PwMS rather than perceived reports [58]. Most voluntary contraction test results to general fatigue rather than localized muscle fatigue.

There are several limitations to this study. One limitation was the relatively small sample size, due to restrictions as a result of the COVID-19 pandemic. Despite the small sample size, there were moderate to large effect sizes, which suggests clinical relevance despite the small sample sizes. Also, the wide range in MFIS suggest a wide difference in impairment among the participants in this study, future studies can look into participants with a closer range of disability. One other limitation of this study was that there was no control group to compare the measures to, however, this was a pre-post study design. Other studies can look at the changes compared to matched healthy control. The information in this study might provide direction towards a mechanism in preventing falls among PwMS. Future studies can perform an experimental study on the effect of lower leg muscle endurance training on gait and balance after physical activity as a means to reduce falls among PwMS.

## 5.0. Conclusions

Our study found that 6 minutes of treadmill walking at self-selected speeds with an average inclination of two degrees resulted in a large increase in perceived fatigue. Following the 6 minutes walk, there was an impairment in gait and balance, which remain after 20 minutes of rest. This could suggest an increase in fall risk immediately after a walking exercise which might need a longer rest time to recover fully. The gait and balance impairment was more evident among three of the participants who had higher disability using the PDDS scale. There was a significant correlation between the change in gait and muscle function (fatigability and mitochondria capacity). Performing safeguarded exercises to improves the muscle fatigability and mitochondria capacity of the lower limb could be one of the mechanisms to improve gait and balance among PwMS. The information on the alteration in gait and balance after practical daily locomotion and the influence of the lower leg muscle function might guide therapeutic in the management of falls among PwMS. Future studies are needed to confirm and extend these findings. These studies should focus on people with higher PDDS scores and they should include measurements both immediately and after a recovery period.

**Acknowledgments:** The authors will like to thank the participants for their time, and Megan Ware for helping through the recruitment process. The research assistants who helped with data collection are also well appreciated.

## References

1. Global, regional, and national burden of multiple sclerosis 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*. Mar 2019;18(3):269-285. doi:10.1016/s1474-4422(18)30443-5
2. Stenager E. A global perspective on the burden of multiple sclerosis. *The Lancet Neurology*. Mar 2019;18(3):227-228. doi:10.1016/s1474-4422(18)30498-8
3. Kalron A, Dvir Z, Frid L, Achiron A. Quantifying gait impairment using an instrumented treadmill in people with multiple sclerosis. *ISRN Neurol*. 2013;2013:867575-867575. doi:10.1155/2013/867575
4. Socie MJ, Motl RW, Pula JH, Sandroff BM, Sosnoff JJ. Gait variability and disability in multiple sclerosis. *Gait & posture*. 2013/05/01/ 2013;38(1):51-55. doi:https://doi.org/10.1016/j.gaitpost.2012.10.012
5. Comber L, Sosnoff JJ, Galvin R, Coote S. Postural control deficits in people with Multiple Sclerosis: A systematic review and meta-analysis. *Gait & posture*. Mar 2018;61:445-452. doi:10.1016/j.gaitpost.2018.02.018
6. Socie MJ, Sosnoff JJ. Gait variability and multiple sclerosis. *Multiple sclerosis international*. 2013;2013:645197-645197. doi:10.1155/2013/645197
7. Comber L, Galvin R, Coote S. Gait deficits in people with multiple sclerosis: A systematic review and meta-analysis. *Gait & posture*. Jan 2017;51:25-35. doi:10.1016/j.gaitpost.2016.09.026
8. Cameron MH, Wagner JMJCN, Reports N. Gait Abnormalities in Multiple Sclerosis: Pathogenesis, Evaluation, and Advances in Treatment. journal article. July 22 2011;11(5):507. doi:10.1007/s11910-011-0214-y
9. Society M. National MS Society Updated 10/8/2019. https://www.nationalmssociety.org/For-Professionals/Researchers/Resources-for-Researchers/Clinical-Study-Measures/Timed-25-Foot-Walk-(T25-FW)
10. Learmonth YC, Motl RW, Sandroff BM, Pula JH, Cadavid D. Validation of patient determined disease steps (PDDS) scale scores in persons with multiple sclerosis. *BMC Neurol*. 2013;13:37-37. doi:10.1186/1471-2377-13-37
11. Goldman MD, Marrie RA, Cohen JA. Evaluation of the six-minute walk in multiple sclerosis subjects and healthy controls. *Multiple sclerosis (Houndmills, Basingstoke, England)*. Apr 2008;14(3):383-90. doi:10.1177/1352458507082607
12. Braley TJ, Chervin RD. Fatigue in multiple sclerosis: mechanisms, evaluation, and treatment. *Sleep*. 2010;33(8):1061-1067. doi:10.1093/sleep/33.8.1061
13. Willingham TB, Backus D, McCully KK. Muscle Dysfunction and Walking Impairment in Women with Multiple Sclerosis. *International Journal of MS Care*. 2019;21(6):249-256. doi:10.7224/1537-2073.2018-020 %J International Journal of MS Care
14. Harp MA, McCully KK, Moldavskiy M, Backus D. Skeletal muscle mitochondrial capacity in people with multiple sclerosis. 2016;2:2055217316678020. doi:10.1177/2055217316678020
15. Barcelos IPd, Troxell RM, Graves JS. Mitochondrial Dysfunction and Multiple Sclerosis. *Biology (Basel)*. 2019;8(2):37. doi:10.3390/biology8020037
16. Enoka RM, Duchateau J. Muscle fatigue: what, why and how it influences muscle function. 2008;586(1):11-23. doi:10.1113/jphysiol.2007.139477
17. Enoka R, Baudry S, Rudroff T, Farina D, Klass M, Duchateau J. Unraveling the

neurophysiology of muscle fatigue. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*. 11/01 2010;21:208-19. doi:10.1016/j.jelekin.2010.10.006

18. Tripp BL, Yochem EM, Uhl TL. Functional fatigue and upper extremity sensorimotor system acuity in baseball athletes. *J Athl Train*. Jan-Mar 2007;42(1):90-98.

19. Willingham TB, Backus D, McCully KK. Muscle Dysfunction and Walking Impairment in African American and Caucasian American Women with Multiple Sclerosis. 0(0):null. doi:10.7224/1537-2073.2018-020

20. B Willingham T, McCully K. *ASSESSMENT OF SKELETAL MUSCLE ENDURANCE USING TWITCH ELECTRICAL STIMULATION AND ACCELEROMETER-BASED MECHANOMYOGRAPHY*. vol 1. 2017.

21. Hebert JR, Corboy JR. The association between multiple sclerosis-related fatigue and balance as a function of central sensory integration. *Gait & posture*. 2013/05/01/ 2013;38(1):37-42. doi:[https://doi.org/10.1016/j.gaitpost.2012.10.015](https://doi.org/10.1016/j.gaitpost.2012.10.015)

22. Kalron A. Association between gait variability, falls and mobility in people with multiple sclerosis: A specific observation on the EDSS 4.0-4.5 level. *Neurorehabilitation*. 02/13 2017;40doi:10.3233/NRE-171445

23. Kalron A, Frid L, Gurevich M. Concern about falling is associated with step length in persons with multiple sclerosis. *European journal of physical and rehabilitation medicine*. Apr 2015;51(2):197-205.

24. Plotnik M, Wagner JM, Adusumilli G, Gottlieb A, Naismith RT. Gait asymmetry, and bilateral coordination of gait during a six-minute walk test in persons with multiple sclerosis. *Scientific Reports*. 2020/07/24 2020;10(1):12382. doi:10.1038/s41598-020-68263-0

25. Cameron MH, Nilsagard Y. Balance, gait, and falls in multiple sclerosis. *Handbook of clinical neurology*. 2018;159:237-250. doi:10.1016/b978-0-444-63916-5.00015-x

26. Karst GM, Venema DM, Roehrs TG, Tyler AE. Center of Pressure Measures during Standing Tasks in Minimally Impaired Persons with Multiple Sclerosis. 2005;29(4):170-180. doi:10.1097/01.Npt.0000282314.40230.40

27. Soyuer F, Mirza M, Erkorkmaz Ü. Balance performance in three forms of multiple sclerosis. *Neurological Research*. 2006/07/01 2006;28(5):555-562. doi:10.1179/016164105X49373

28. Di Giulio I, Maganaris CN, Baltzopoulos V, Loram ID. The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture. *J Physiol*. 2009;587(Pt 10):2399-2416. doi:10.1113/jphysiol.2009.168690

29. Azizah Mbourou G, Lajoie Y, Teasdale N. Step Length Variability at Gait Initiation in Elderly Fallers and Non-Fallers, and Young Adults. *Gerontology*. 2003;49(1):21-26. doi:10.1159/000066506

30. Crenshaw SJ, Royer TD, Richards JG, Hudson DJ. Gait variability in people with multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)*. Oct 2006;12(5):613-9. doi:10.1177/1352458505070609

31. Rietberg MB, Van Wegen EE, Kwakkel G. Measuring fatigue in patients with multiple sclerosis: reproducibility, responsiveness and concurrent validity of three Dutch self-report questionnaires. *Disability and rehabilitation*. 2010;32(22):1870-6. doi:10.3109/09638281003734458

32. Hill KD, Schwarz JA, Kalogeropoulos AJ, Gibson SJ. Fear of

falling revisited. *Arch Phys Med Rehabil*. Oct 1996;77(10):1025-9. doi:10.1016/s0003-9993(96)90063-5
33. Hagströmer M, Oja P, Sjöström M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutrition*. 2006;9(6):755-762. doi:10.1079/PHN2005898
34. Society NMS. Timed 25Foot Walk (T25-FW). 2020;
35. Liss CM, Sanni AA, McCully KK. Endurance of the Dorsal and Ventral Muscles in the Neck. 2020;5(3):47.
36. Sumner MD, Beard S, Pryor EK, Das I, McCully KK. Near Infrared Spectroscopy Measurements of Mitochondrial Capacity Using Partial Recovery Curves. *Frontiers in physiology*. 2020;11:111. doi:10.3389/fphys.2020.00111
37. Borg G, Dahlstrom H. A pilot study of perceived exertion and physical working capacity. *Acta Soc Med Ups*. 1962;67:21-7.
38. Hausdorff J, A. Rios D, K. Edelberg H. *Gait Variability and Fall Risk in Community-Living Older Adults: A 1-Year Prospective Study*. vol 82. 2001:1050-6.
39. Callisaya ML, Blizzard L, Schmidt MD, et al. Gait, gait variability and the risk of multiple incident falls in older people: a population-based study. *Age and ageing*. Jul 2011;40(4):481-7. doi:10.1093/ageing/afr055
40. Wong DW-C, Lam W-K, Lee WC-C. Gait asymmetry and variability in older adults during long-distance walking: Implications for gait instability. *Clinical Biomechanics*. 2020/02/01/ 2020;72:37-43. doi:https://doi.org/10.1016/j.clinbiomech.2019.11.023
41. Egerton T, Thingstad P, Helbostad JL. Comparison of programs for determining temporal-spatial gait variables from instrumented walkway data: PKmas versus GAITRite. *BMC Res Notes*. 2014;7:542-542. doi:10.1186/1756-0500-7-542
42. Barclay-Goddard R, Stevenson T, Poluha W, Moffatt ME, Taback SP. Force platform feedback for standing balance training after stroke. *The Cochrane database of systematic reviews*. Oct 18 2004;2004(4):Cd004129. doi:10.1002/14651858.CD004129.pub2
43. Prieto TE, Myklebust JB, Hoffmann RG, Lovett EG, Myklebust BM. Measures of postural steadiness: differences between healthy young and elderly adults. *IEEE Transactions on Biomedical Engineering*. 1996;43(9):956-966. doi:10.1109/10.532130
44. Golriz S, Hebert JJ, Foreman KB, Walker BF. The reliability of a portable clinical force plate used for the assessment of static postural control: repeated measures reliability study. *Chiropr Man Therap*. 2012;20(1):14-14. doi:10.1186/2045-709X-20-14
45. McCully KK, Prins P, Mistry K, Willingham TB. Muscle-specific endurance of the trapezius muscles using electrical twitch mechanomyography. *Shoulder Elbow*. Apr 2018;10(2):136-143. doi:10.1177/1758573217726269
46. Escudero-Uribe S, Hochsprung A, Izquierdo-Ayuso G. Gait pattern changes after six-minute walk test in persons with multiple sclerosis. 2019;24(1):e1741. doi:10.1002/pri.1741
47. Prosperini L, Fortuna D, Giannì C, Leonardi L, Pozzilli C. The Diagnostic Accuracy of Static Posturography in Predicting Accidental Falls in People With Multiple Sclerosis. *Neurorehabilitation and neural repair*. 2013/01/01 2012;27(1):45-52. doi:10.1177/1545968312445638
48. Prosperini L, Fortuna D, Giannì C, Leonardi L, Marchetti MR, Pozzilli C. Home-based balance training using the Wii balance board: a randomized, crossover pilot study in multiple sclerosis. *Neurorehabilitation and neural repair*. Jul-Aug 2013;27(6):516-25. doi:10.1177/1545968313478484

49. Karpatkin H, Rzetelny A. Effect of a Single Bout of Intermittent versus Continuous Walking on Perceptions of Fatigue in People with Multiple Sclerosis. *International journal of MS care*. Fall 2012;14(3):124-131. doi:10.7224/1537-2073-14.3.124

50. Coote S, Sosnoff JJ, Gunn H. Fall Incidence as the Primary Outcome in Multiple Sclerosis Falls-Prevention Trials: Recommendation from the International MS Falls Prevention Research Network. *International journal of MS care*. Winter 2014;16(4):178-184. doi:10.7224/1537-2073.2014-059

51. Mazumder R, Murchison C, Bourdette D, Cameron M. Falls in people with multiple sclerosis compared with falls in healthy controls. *PLoS One*. 2014;9(9):e107620-e107620. doi:10.1371/journal.pone.0107620

52. Gunn H, Creanor S, Haas B, Marsden J, Freeman J. Frequency, Characteristics, and Consequences of Falls in Multiple Sclerosis: Findings From a Cohort Study. *Archives of Physical Medicine and Rehabilitation*. 2014;95(3):538-545. doi:10.1016/j.apmr.2013.08.244

53. Society NMS. Walking (Gait) difficulties 2020;

54. Health NIo. Muscle Mitochondria May form Energy Power Grid. July 14, 2020. Accessed July 14, 2020. https://www.nih.gov/news-events/nih-research-matters/muscle-mitochondria-may-form-energy-power-grid#:~:text=At%20a%20Glance.%20Skeletal%20muscles%20are%20made%20of,meet%20this%20energy%20demand%2C%20muscle%20cells%20contain%20mitochondria.

55. Hansen D, Feys P, Wens I, Eijnde BO. Is walking capacity in subjects with multiple sclerosis primarily related to muscle oxidative capacity or maximal muscle strength? A pilot study. *Multiple sclerosis international*. 2014;2014:759030-759030. doi:10.1155/2014/759030

56. Kinnett-Hopkins D, Adamson B, Rougeau K, Motl RW. People with MS are less physically active than healthy controls but as active as those with other chronic diseases: An updated meta-analysis. *Multiple sclerosis and related disorders*. Apr 2017;13:38-43. doi:10.1016/j.msard.2017.01.016

57. Kalb R, Brown TR, Coote S, et al. Exercise and lifestyle physical activity recommendations for people with multiple sclerosis throughout the disease course. *Multiple Sclerosis Journal*. 2020:1352458520915629. doi:10.1177/1352458520915629

58. Morris ME, Cantwell C, Vowels L, Dodd K. Changes in gait and fatigue from morning to afternoon in people with multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2002;72(3):361-365. doi:10.1136/jnnp.72.3.361