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Faculteit Revalidatiewetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

Spatiotemporal Gait and Fatigability in Multiple Sclerosis: Overground vs Treadmill Walking

Yannick Surgeon

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij neurologische aandoeningen

PROMOTOR :

Prof. dr. Peter FEYS

BEGELEIDER :

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2024
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Abstract

Background: Multiple sclerosis (MS) is a neurodegenerative condition that leads to gait impairments, often worsened by fatigability. While spatiotemporal gait characteristics in MS have been studied extensively, little is known about how prolonged walking under fixed-speed conditions affects gait. Fixed-speed walking may exacerbate deviations, while overground walking allows self-regulation.

Objective: To compare spatiotemporal gait characteristics in people with MS (pwMS) and healthy controls (HC) during prolonged overground and fixed-speed treadmill walking.

Method: Nine pwMS and 18 HC (aged 29-64 years) performed a 6-minute walk test (6MWT) under three different conditions: overground and treadmill walking at 50% and 80% of maximal speed from the Timed 25-foot Walk Test (T25FW). Wearable inertial sensors (overground) or the GRAIL system (treadmill) recorded cadence, double support, single limb support (SLS), step duration (SD), stride length (SL), and swing time. A linear mixed model assessed effects of group, condition and their interaction; percentage changes from minute one to six were calculated for each parameter.

Results: SLS and SD changed significantly more during overground walking than both treadmill conditions, whereas SL changed less overground compared to the slow treadmill. Mean T25FW speeds were 2.13m/s (pwMS) and 2.10m/s (HC); mean 6MWT speeds were 1.63m/s (pwMS) and 1.72m/s, with no group differences in either test.

Conclusion: Walking condition significantly influenced gait adaptation: overground walking elicited greater changes in SLS and SD but smaller changes in SL, and yielded greater walking distance than treadmill conditions.

Keywords: Spatiotemporal; gait characteristics; walking fatigability; multiple sclerosis; overground; treadmill

Research Context

This master's thesis is in the domain of gait and balance, more specifically, the spatiotemporal parameters of gait. The relevance of this master's thesis is to see whether the spatiotemporal

gait characteristics of people with multiple sclerosis (pwMS) deviate when compared to a healthy control group, when walking for a long time. It is known that pwMS decreases walking performance and gait quality over prolonged periods. However, it is not known how one that slows down in a self-paced overground condition would behave when changes in walking speed are not possible. Additionally, this study tried to capture gait deviations that self-paced conditions would not be able to find and to understand how pwMS compensate when they are not able to modulate their walking speed.

This master's thesis is part of an ongoing doctoral study. The doctoral study is about Gait and cortical activity profiles of people with multiple sclerosis presenting with walking fatigability (project code: 11O5823N and 11O5825N, funding: Fonds Wetenschappelijk Onderzoek- FWO) and is conducted by Felipe BALISTIERI SANTINELLI, a PhD student of the Department of Physiotherapy and Biomechanics.

The testing was divided into two experimental days: on the first day, both pwMS and healthy controls (HC) performed a maximum self-selected overground walking speed (i.e., 6-minute walking test); on the second day, both groups performed two fixed-speed treadmill 6-minute walking test corresponding to either 50% and 80% of the walking speed on the timed 25-foot walking test. The conditions were randomized. The overground and treadmill part was performed at REVAL Research Centre at UHasselt. The 6-minute walking test was conducted in a 25m walkway. First, wearable inertial measurement units (APDM sensors) were placed on specific body parts, and then the actual testing began. For the treadmill part, reflective markers were placed onto specific body landmarks for movement tracking (HBM model) during treadmill walking.

Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative and inflammatory disease, and the leading cause of disability in young adults (Palace & Robertson, 2014). Specifically, the presence of gait impairments stands out as a primary cause of disability (Dalgas, 2011; Heesen et al., 2008). Among people with MS (pwMS), impairments in walking and elevated fatigue levels are the most common symptoms (LaRocca, 2011; Oliva Ramirez et al., 2021). When combined, fatigability can amplify existing gait pattern alterations in pwMS (Sehle et al., 2011). Moreover, fatigue in MS negatively impacts quality of life (Oliva Ramirez et al., 2021). More

specifically, there are two forms of fatigue, namely trait and state fatigue. These two forms are distinct and may occur independently of one another (Kluger et al., 2013). Trait fatigue reflects a general, long-term perception of fatigue and is typically assessed using questionnaires like the Modified Fatigue Impact Scale (MFIS) (Flachenecker et al., 2002). In contrast, state fatigue (fatigability) refers to a decline in performance during specific tasks, such as walking, and can be measured objectively and subjectively. In particular, walking fatigability is clinically defined as a 10% decline in distance walked during the 6-minute walking test (6MWT) (Van Geel, Veldkamp, et al., 2020). Walking fatigability is calculated using the distance walking index (DWI), resulting from the difference between distances walked at minute six and one, divided by the distance at minute one, and multiplied by 100. (Leone et al., 2016). In addition, the prevalence of fatigability increases with the severity of MS, particularly among individuals with an Expanded disability Status Scale (EDSS) score between 4.5–5.5 (Santinelli, Abasiyanik, Dalgas, et al., 2024). Furthermore, pwMS presents walking impairments when compared to age- and sex-matched healthy controls (HC). These are shown in reductions in gait speed, stride length, swing phase (Comber et al., 2017), and an increased double support time compared to HC (Comber et al., 2017; Richmond et al., 2020). In this study, the DWI formula is used, but the gait speed is changed by the different spatiotemporal gait characteristics to see the change over time during the 6MWT between overground and treadmill walking.

Prolonged walking protocols, such as fixed-speed treadmill walking (Sehle et al., 2011) and the 6MWT (Van Geel et al., 2020), have shown that pwMS exhibit a worsening of their gait patterns during extended walking, as impairments may remain unnoticed during shorter walking tests. Sehle et al. (2011) observed that step length increased when pwMS became fatigued during prolonged treadmill walking at a comfortable speed. Similarly, Santinelli et al. (2024) reported that during 6MWT during overground walking, pwMS showed a decreased cadence and increased step duration. This study distinguishes itself from previous research by directly comparing three prolonged walking conditions (slow treadmill, fast treadmill, and overground walking), each performed for six minutes in both pwMS and HC. While earlier studies examined a single walking condition, this design allows for a more comprehensive analysis of group and condition differences. As such, it provides novel insight into how gait patterns are influenced

when speed modulations are restricted, and how pwMS adapt their gait across different walking contexts.

Protocols that allow people to pace themselves, such as the 6MWT, might be hiding some worsening in gait impairments, as some people might slow down in order to preserve gait quality. Although changes are seen even when pwMS are slowing down (and surpassing the cutoff for distance walking fatigability), there are cases where pwMS slow down without changes in any other gait characteristics (Santinelli, Abasiyanik, Ramari, et al., 2024). In contrast, Theunissen et al. (2023) reported only an increase in walking speed during the 6MWT performed on a self-paced treadmill, and no changes in gait characteristics were found after adjusting for gait speed. A potential reason prior studies failed to detect differences between pwMS and HC is the use of protocols that were either too short (Remelius et al., 2012; Sloot et al., 2014) or allowed participants to self-pace (Theunissen et al., 2023), all of which were conducted on a treadmill. These conditions may enable pwMS to compensate by slowing down when fatigued, whereas shorter protocols may not be sufficiently challenging. This highlights the need for more challenging walking protocols, such as prolonged, fixed-speed treadmill walking in less impaired pwMS.

We hypothesise that spatiotemporal gait characteristics and gait variability have a greater change over time during overground walking compared to fixed-speed treadmill walking, and that these changes are more pronounced in pwMS compared to HC. This study aims to investigate whether spatiotemporal gait characteristics change over time during the 6MWT under different walking conditions, fixed-speed treadmill walking (slow and fast) and self-paced overground walking, and whether these characteristics differ between the three conditions.

Method

Participants

This study included 9 pwMS and 18 HC. Patients were recruited from the Rehabilitation- and MS Centre in Pelt, National MS Centre Melsbroek or the REVAL Research Centre at UHasselt. The inclusion criteria were (1) age between 18 and 65 years old; (2) a diagnosis of MS (2017 revisions of the McDonald criteria) with an EDSS score between 1 and 5.5; (3) the ability to

walk for 6 minutes without rest; (4) report at least a score of 2 in the following questions of the MSWS-12: “7. Increased the effort needed for you to walk?”, “10. Slowed down your walking?”, “11. Affected how smoothly you walk?”, and “12. Made you concentrate on your walking?”. PwMS who had a relapse less than one month before the start of this study were not included. Other exclusion criteria were (1) cognitive impairments hindering understanding of study instructions; (2) pregnancy; (3) musculoskeletal disorders in the lower limbs not related to MS; and (4) an assistive device needed to walk.

Clinical Outcomes

Questionnaires and clinical tests were applied to characterize our study population, including Risk of falling: Falls Efficacy Scale International (FES-I)-16 questions (van Vliet et al., 2013), Perceived walking fatigability: MSWS-12 plus gait items-12 questionnaire (Strzok et al., 2018), Perceived fatigability: Pittsburgh Fatigability Scale (PFS) 10 questions (Carlozzi et al., 2021) and Modified Fatigue Impacts Scale (MFIS)-21 questions (Mills et al., 2010), Self-control: Brief Self-Control Scale (BSCS)-13 questions (Manapat et al., 2021), the Symbol Digit Modality Test (SDMT) (Sandry et al., 2021) for information processing speed.

After assessing the cognitive function, the motricity index (MI) (Rasova et al., 2012) was performed to measure the muscle strength of the lower limbs, and lastly, the timed 25-foot walking test (T25FWT) (Motl et al., 2017) to evaluate the maximum walking speed. The maximum walking speed was used to determine the treadmill speed, at 50% and 80% of the participants' maximum walking speed.

6-Minute Walking Test

For the overground walking, the participants performed the 6MWT to measure the walking characteristics during overground walking. When performing the 6MWT, the participants were instructed to walk as fast and safely as possible. The goal is to cover a maximal individual distance for 6 minutes. The participants walked on a 25-meter walkway with turns at both ends. No verbal encouragement was provided during the test. The participants were asked to complete the 6MWT without taking any rest (Goldman et al., 2008).

For the 6MWT with a fixed speed, a treadmill was used. The 6MWT was performed on a two-belt treadmill, with two force platforms embedded, in the Gait Real-Time Interactive Laboratory (GRAIL). During this part, the participant walked in two conditions: (1) 80% of the

walking speed obtained in the T25FW and (2) 50% of the walking speed obtained in the T25FW. The performance of the two fixed-speed conditions was randomised.

Gait Measurement

For the overground walking, eight wearable sensors (OPAL V.2, APDM, USA), with an acquisition frequency of 128Hz, were positioned to measure gait characteristics. Two sensors were strapped on the feet, two on each tibia, two on each thigh, two on each wrist, one at the lumbar (L4) vertebra, and one on the sternum. The gait characteristics were extracted minute by minute during the 6MWT. The software Moveo Explorer (APDM, USA) acquired and processed the data. After the acquisition of the data, the data was segmented in a minute-based manner using a custom code build-up in Matlab®. The following variables were extracted: cadence, step length, double limb support, single support time, step duration, swing phase, and gait speed. Lastly, to investigate the changes in gait quality in minute 6 of the 6MWT, the same formula, DWI, was used but replacing the distance walked with the value of the spatiotemporal gait characteristics, was used.

The GRAIL (Motekforce Link, Amsterdam, the Netherlands) was used to assess the gait characteristics changes over the fixed speed 6MWT. For movement track, a 3D motion capture system (ten infrared cameras (Vicon Motion System®)), with a data acquisition frequency of 100Hz, was used to record twenty-two passive reflective markers (HBM lower body model, Motek). The spatiotemporal gait characteristics were acquired and processed using the D-Flow software (Motek).

The primary measures of this study were spatiotemporal gait characteristics measured during overground, treadmill slow, and treadmill fast walking. Specifically, cadence, double support, single limb support, step duration, step length, and swing time were used to quantify gait performance across the three different walking conditions. Minute-by-minute data were collected over the 6MWT, but for the analysis, only minute 1 and minute 6 were used to calculate the change of the specific spatiotemporal gait characteristics. These changes were compared between conditions and groups.

The secondary measures included gait variability measures, calculated by the coefficient of variation of step duration and stride length. These outcomes provided insight into the consistency and stability of gait. Furthermore, walking distance across the three conditions

(overground, treadmill slow, and treadmill fast) was also considered as part of the secondary outcomes. In addition, changes in Borg Rating of Perceived Exertion (BORG RPE) scores across the three walking conditions were evaluated.

Statistical Analysis

Statistical analyses were conducted using JMP Pro 17. A p-value < .05 was considered statistically significant. The Shapiro-Wilk test was used to assess normality, and the Brown-Forsythe test was applied to evaluate variance between groups.

To assess group differences in demographic data, a two-sample t-test was used for normally distributed variables with equal variances, and the Mann-Whitney U test (rank-sum) was used for non-normally distributed variables.

For the primary outcome measures, a linear mixed model was used to analyse the effects of walking conditions, group and their interaction on spatiotemporal gait characteristics. Conditions (overground, treadmill slow, and treadmill fast), group (pwMS and HC), and their interaction (group x condition) were included as fixed effects. Participants were included as a random effect to account for repeated measures within individuals. For the secondary outcome measure, gait variability was assessed using the coefficient of variation of step duration and stride length, calculated as the standard deviation divided by the mean, multiplied by 100. The same linear mixed model structured as used for the primary outcome was applied, with condition, group, and their interaction as fixed effects, and participants as a random effect. Finally, differences in walking distance and BORG RPE score between the three walking conditions were examined using the previously described statistical approach.

A post-hoc pairwise comparison was performed with Tukey correction where appropriate. Effect sizes were reported using Cohen's d for pairwise comparisons.

For an overview of the statistical test selection used for the demographic data, as well as the primary and secondary outcome measures, see Appendices A and B.

Results

In Table 1, an overview of all the demographic data, questionnaires and clinical tests is provided. There are more females in both pwMS and HC. There are no significant differences between the groups in all demographic data. There is only a significant difference in the MFIS-

total and cognitive score, FES-I and MI ankle and knee (Table 1). PwMS had a significantly higher score on the MFIS-total score ($p = .0205$) and the MFIS-cognitive score ($p = .0301$), indicating a higher level of fatigue. Also, a significant higher score in FES-I ($p = .0120$), suggesting a higher fear of falling and a significantly lower score in MI ankle left ($p = .0414$) and right ($p = .0414$), knee left ($p = .0415$), right ($p = .0414$), implying a lower muscle force in the knee and ankle joint.

Table 1*Demographic and Clinical Data from the Total Sample*

	PwMS (N = 9)	HC (N = 18)	P-value (parametric)	P-value (non- parametric)
Sex (M/F)	1/8	4/14	-	.6361
Age (years)	42 ± 9 (32 – 57)	47 ± 11 (29 – 64)	.2576	.3538
Weight (kg)	73 ± 16 (55 – 105)	68 ± 16 (49 -118)	-	.5333
Height (cm)	170 ± 5 (162 – 180)	168 ± 10 (151 – 190)	.7061	.4548
MS type (RR/SP/PP/NP)	6/2/0/0	-	-	-
EDSS (0-10)	2 ± 1 (1 – 4)	-	-	-
MS onset (years)	9.33 ± 6.20 (2 – 22)	-	-	-
T25FW (s)	4 ± 0.5 (3.1 – 4.6)	3.6 ± 0.4 (2.9 – 4.3)	.0578	.0506
MSWS-12 (%)	38 ± 21 (20 – 78)	-	-	-
SDMT (N)	65 ± 12 (47 – 79)	57 ± 9 (44 – 72)	.1260	.1818
MFIS-Total (0-84)	32 ± 19 (3 – 59)	15 ± 13 (0 – 42)	.0154	.0326
MFIS-Physical (0-36)	12 ± 7 (1 – 21)	6 ± 6 (0 – 21)	.0593	.0707
MFIS-Cognitive (0-40)	17 ± 11 (0 – 38)	7 ± 6 (0 – 23)	-	.0321
MFIS-Psychosocial (0-8)	2 ± 2 (0 – 5)	1 ± 1 (0 – 7)	-	.1597
FES-I	22 ± 6 (17 – 34)	17 ± 2 (16 – 23)	-	.0129
PFS-P	19 ± 10 (6 – 40)	13 ± 7 (0 – 26)	.0629	.1160
PFS-M	13 ± 11 (4 – 38)	7 ± 6 (0 – 30)	-	.1216
BSCS	45 ± 6 (37 – 56)	48 ± 7 (32 – 59)	.4879	.3583
MI left ankle	27 ± 10 (9 – 33)	33 ± 0 (33)	-	.0471
MI right ankle	27 ± 10 (9 – 33)	33 ± 0 (33)	-	.0474
MI left knee	29 ± 7 (14 – 33)	33 ± 0 (33)	-	.0475
MI right knee	29 ± 6 (19 – 33)	33 ± 0 (33)	-	.0474
MI left hip	29 ± 7 (14 – 33)	33 ± 0 (33)	-	.0544
MI right hip	29 ± 7 (14 – 33)	33 ± 0 (33)	-	.0544
6MWT (m)	579 ± 60 (426 – 639)	617 ± 59 (485 – 711)	.1357	.2169

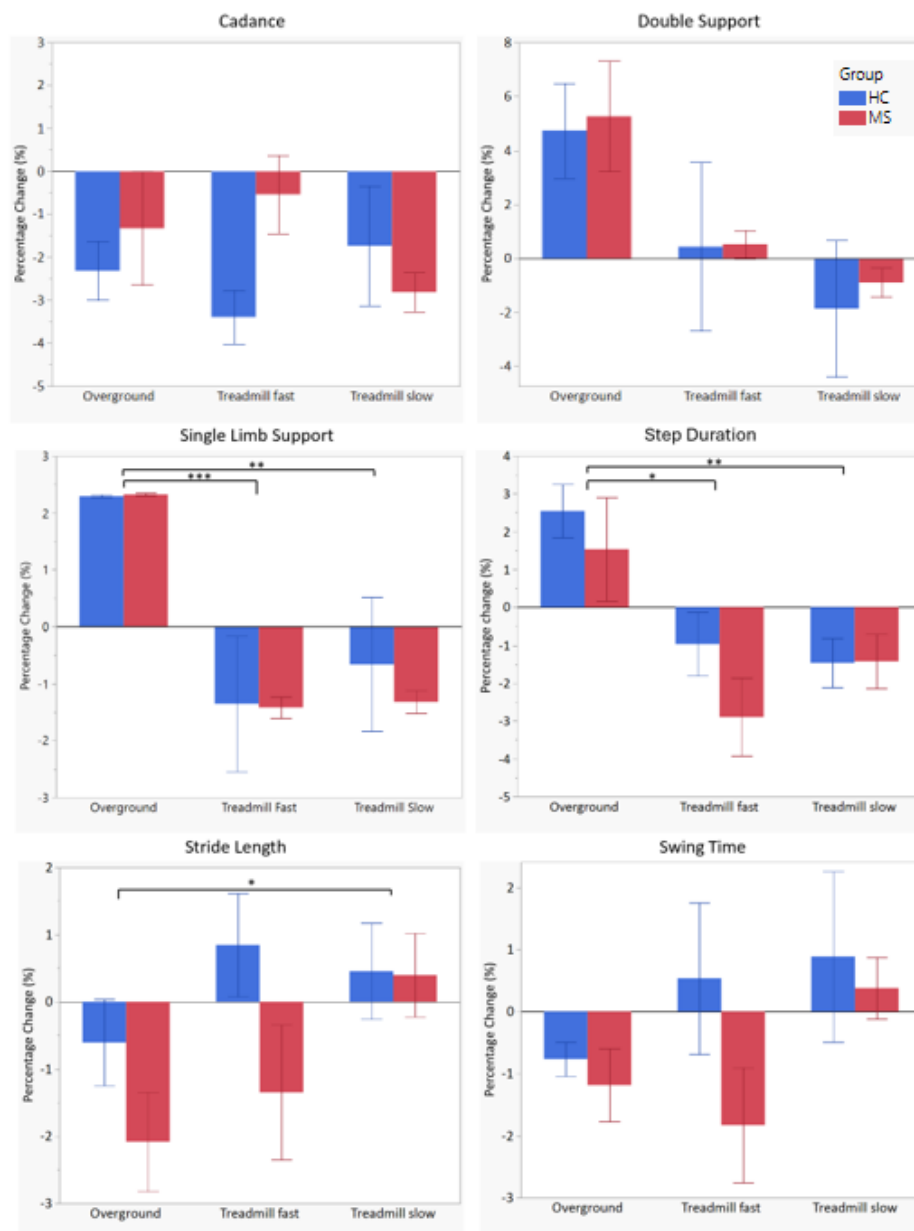
Note. In **bold**, significant differences between groups. A±B (C-D), A: mean value, B: standard deviation, and C-D: range. pwMS = people with MS, HC = healthy control, RR = relapsing-remitting, SP = secondary progressive, PP = primary progressive, NP = non provided, EDSS = Expanded Disability Status Scale , T25FW = Timed 25-foot Walking, MSWS-12 = Multiple Sclerosis Walking scale, SDMT = Symbol Digit Modality Test, MFIS = Modified Fatigue Scale, FES-I = Falls Efficacy Scale International , PFS-P = Pittsburgh Fatigability Scale - physical PFS-M = Pittsburgh Fatigability Scale – mental , BSCS = Brief Self-Control Scale, MI = motricity index , 6MWT = 6 Minute Walking Test. The parametric test is the two-sample t-test, and the non-parametric test Wilcoxon Rank-Sum Test

Primary Outcome Measures

Linear mixed model analysis revealed a significant main effect of walking condition for single limb support ($p = .0002$), step duration ($p = .0001$), and stride length ($p = .0411$). No significant difference was found for cadence, double support, and swing time. No significant group $\times$ condition interaction effects were observed. Post hoc comparison showed that change in step duration was significantly greater during overground walking compared to both treadmill fast (M difference = 3.9, 95% CI [1.67, 6.27], $p = .0004$, $d^z = 0.99$) and treadmill slow (M difference = 3.48, 95% CI [1.31, 5.65], $p = .0010$, $d^z = 1.04$). Similarly, the change in single limb support was significantly greater during overground walking than during treadmill fast (M difference = 3.68, 95% CI [1.42, 5.95], $p = .0008$, $d^z = 1.03$) and treadmill slow (M difference = 3.30, 95% CI [1.12, 5.48], $p = .0018$, $d^z = 2.72$). However, the change in stride length was significantly smaller during overground walking compared to treadmill slow walking (M difference = -1.80, 95% CI [-3.49, -0.11], $p = .0349$, $d^z = -0.15$). For more details, see Figure 1. Descriptive mean values of spatiotemporal gait characteristics during the first and last minute of overground and treadmill walking are presented in Tables 2-4. For a more detailed minute-by-minute overview of changes in all spatiotemporal gait characteristics across the three different walking conditions, see Appendix C.

Figure 1

Change in Spatiotemporal Gait Characteristics Across Walking Conditions



Note: Mean values are presented, with healthy control (HC) shown in blue and people with multiple sclerosis (MS) in red. Error bars indicate standard error. The x-axis represents the different walking conditions (self-paced overground walking, treadmill slow (50% of Timed 25-Foot Walking Test (T25FW)) and treadmill fast (80% of T25FW)). The y-axis shows the percentage changes in minute six compared to minute one of the different spatiotemporal gait characteristics.

Table 2

Descriptive Mean Data of Spatiotemporal Gait Parameters During the First and Last Minute of Overground Walking During the 6MWT

Parameter	pwMS		HC	
	Min 1	Min 6	Min 1	Min 6
Cadance	131.39 ± 7.49	129.68 ± 9.29	139.34 ± 11.78	136.01 ± 10.79
Double support	14.69 ± 2.12	15.94 ± 2.66	14.10 ± 2.51	14.71 ± 2.40
Single limb support	42.54 ± 1.10	42.02 ± 1.33	43.28 ± 1.72	42.91 ± 1.62
Step duration	0.45 ± 0.02	0.46 ± 0.03	0.43 ± 0.03	0.44 ± 0.03
Stride length	1.48 ± 0.12	1.45 ± 0.13	1.47 ± 0.11	1.46 ± 0.11
Swing Time	42.55 ± 1.12	42.05 ± 1.31	43.27 ± 1.68	42.94 ± 1.63

Note: Mean and standard deviation of all spatiotemporal gait characteristics during the 6-minute walking test (6MWT). Values are presented for minute 1 and minute 6, separately for people with multiple sclerosis (pwMS) and healthy controls (HC). Parameters are expressed as follows: Cadence (steps/min), double support, single limb, and swing time (% gait cycle), step duration (s), and stride length (m).

Table 3

Descriptive Mean Data of Spatiotemporal Gait Parameters During the First and Last Minute of Treadmill Slow Walking During the 6MWT

Parameter	pwMS		HC	
	Min 1	Min 6	Min 1	Min 6
Cadance	101.02 ± 6.16	98.14 ± 5.43	106.48 ± 7.51	1104.80 ± 8.22
Double support	34.78 ± 2.48	34.46 ± 2.35	32.66 ± 2.43	32.13 ± 3.62
Single limb support	32.61 ± 1.24	32.18 ± 1.21	33.62 ± 1.19	33.37 ± 1.84
Step duration	0.60 ± 0.04	0.59 ± 0.04	0.57 ± 0.04	0.56 ± 0.04
Stride length	0.57 ± 0.05	0.57 ± 0.06	0.59 ± 0.06	0.59 ± 0.06
Swing time	0.39 ± 0.02	0.39 ± 0.02	0.38 ± 0.03	0.38 ± 0.03

Note: Mean and standard deviation of all spatiotemporal gait characteristics during the 6-minute walking test (6MWT). Values are presented for minute 1 and minute 6, separately for people with multiple sclerosis (pwMS) and healthy controls (HC). Parameters are expressed as follows: Cadence (steps/min), double support, single limb, and swing time (% gait cycle), step duration (s), and stride length (m)

Table 4

Descriptive Mean Data of Spatiotemporal Gait Parameters During the First and Last Minute of Treadmill Fast Walking During the 6MWT

Parameter	pwMS		HC	
	Min 1	Min 6	Min 1	Min 6
Cadance	121.23 ± 7.63	120.51 ± 6.45	131.99 ± 10.59	118.41 ± 32.33
Double support	29.53 ± 2.09	29.69 ± 2.22	26.95 ± 2.11	25.39 ± 7.91
Single limb support	35.23 ± 1.04	34.73 ± 1.10	36.45 ± 1.02	33.44 ± 9.22
Step duration	0.50 ± 0.03	0.48 ± 0.03	0.46 ± 0.03	0.36 ± 0.04
Stride length	0.72 ± 0.03	0.71 ± 0.04	0.76 ± 0.07	0.71 ± 0.20
Swing time	0.35 ± 0.02	0.34 ± 0.01	0.34 ± 0.02	0.32 ± 0.08

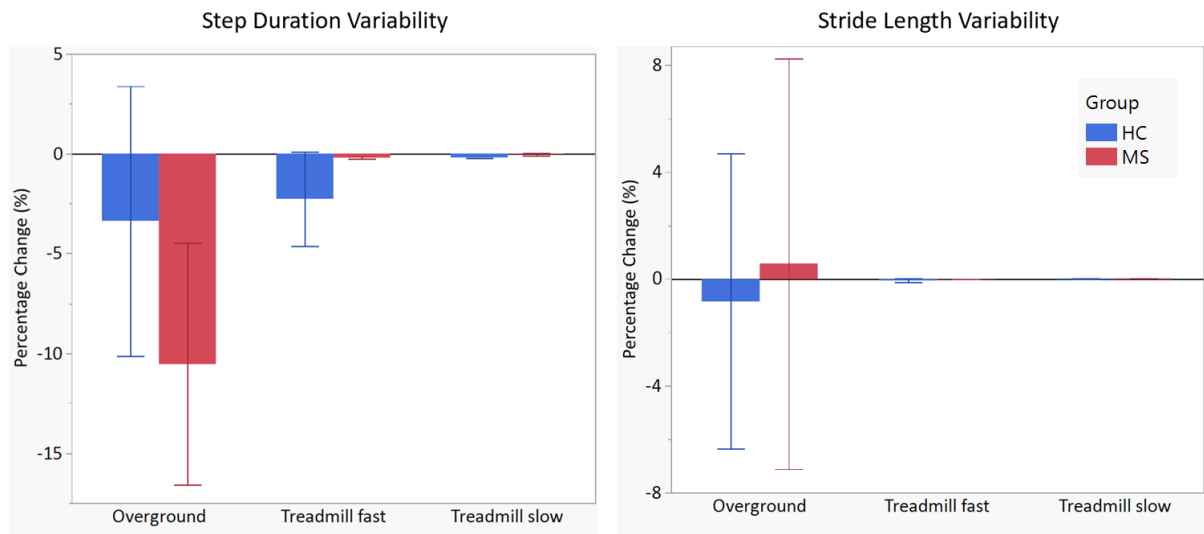
Note: Mean and standard deviation of all spatiotemporal gait characteristics during the 6-minute walking test (6MWT). Values are presented for minute 1 and minute 6, separately for people with multiple sclerosis (pwMS) and healthy controls (HC). Parameters are expressed as follows: Cadence (steps/min), double support, single limb, and swing time (% gait cycle), step duration (s), and stride length (m)

Secondary Outcome Measure

Linear mixed model analysis revealed no significant differences in step duration variability for the main effects of condition ($p = .3726$), group ($p = .7366$), and their interaction (group x condition: $p = .6414$). Similarly, no significant differences were found in stride length variability for the main effects of condition ($p = .9020$), group ($p = .9944$), and their interaction (group x condition: $p = .9844$). See Figure 2 for a graphical representation.

Figure 2

Change in Step Duration Variability and Stride Length Variability

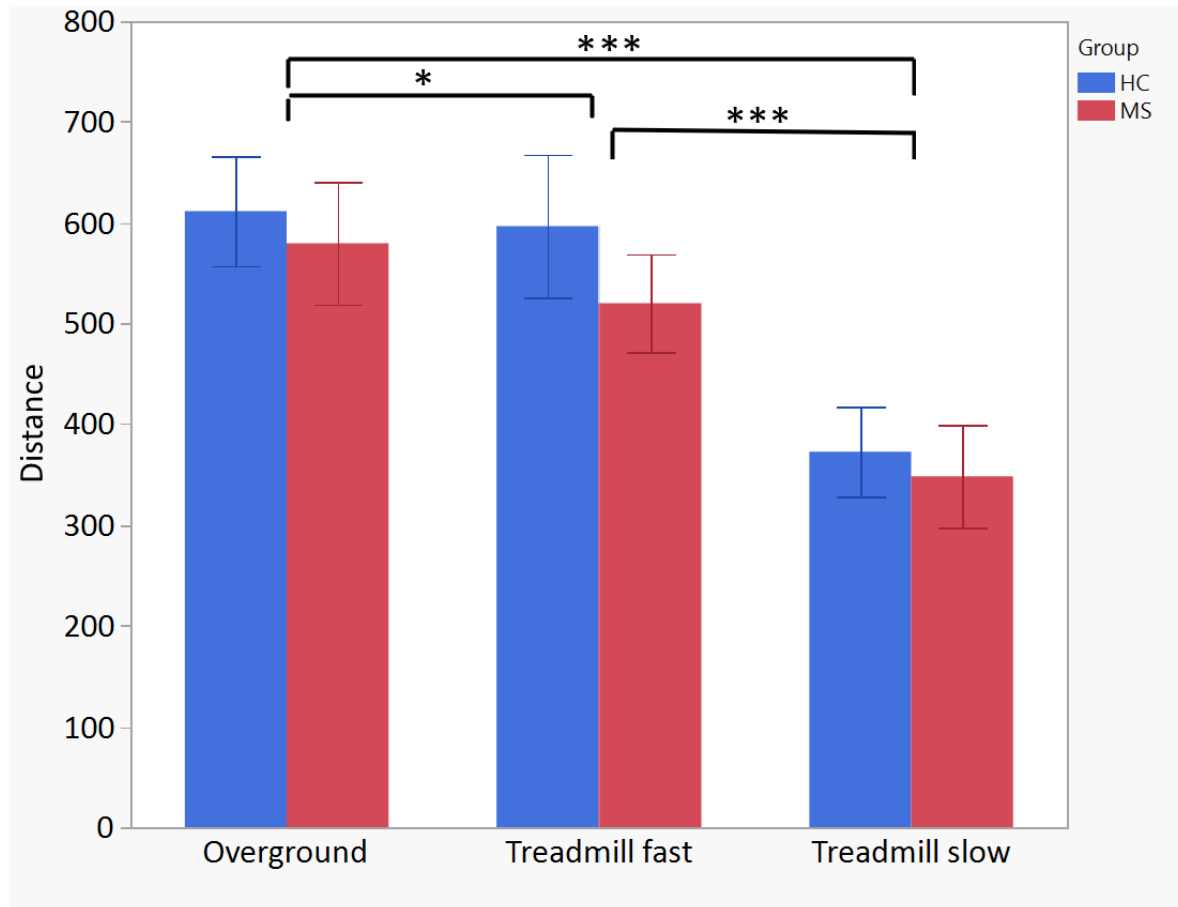


Note: Mean values are presented, with healthy controls (HC) shown in blue and people with multiple sclerosis (MS) in red. The x-axis represents the different walking conditions (self-paced overground walking, treadmill slow (50% of Timed 25-Foot Walking Test (T25FW)) and treadmill fast (80% of T25FW)). The y-axis shows the percentage changes in minute six compared to minute one in step duration variability and stride length variability. Error bars represent the standard error of the mean

Furthermore, a significant difference was found in the distance walked, specifically for the main effect of condition ($p = <.0001$). However, there was nearly a significant difference for the effect group ($p = .0570$) and no significant difference in the interaction group x condition ($p = .2821$). During overground walking, participants walked significantly farther compared to treadmill fast (M difference = 31.63; 95% CI [1.24, 62.02]; $p = .0398$; $d^2 = 0.48$) and treadmill slow (M difference = 235.47; 95% CI [207.13, 263.80]; $p = <.0001$; $d^2 = 3.19$). Additionally, participants walked significantly farther in the treadmill fast condition compared to the treadmill slow (M difference = 203.84; 95% CI [173.23, 234.44]; $p = <.0001$; $d^2 = 2.53$). A visual summary is provided in Figure 3.

Figure 3

Distance Walked During the 6-Minute Walking Test in Three Different Walking Conditions



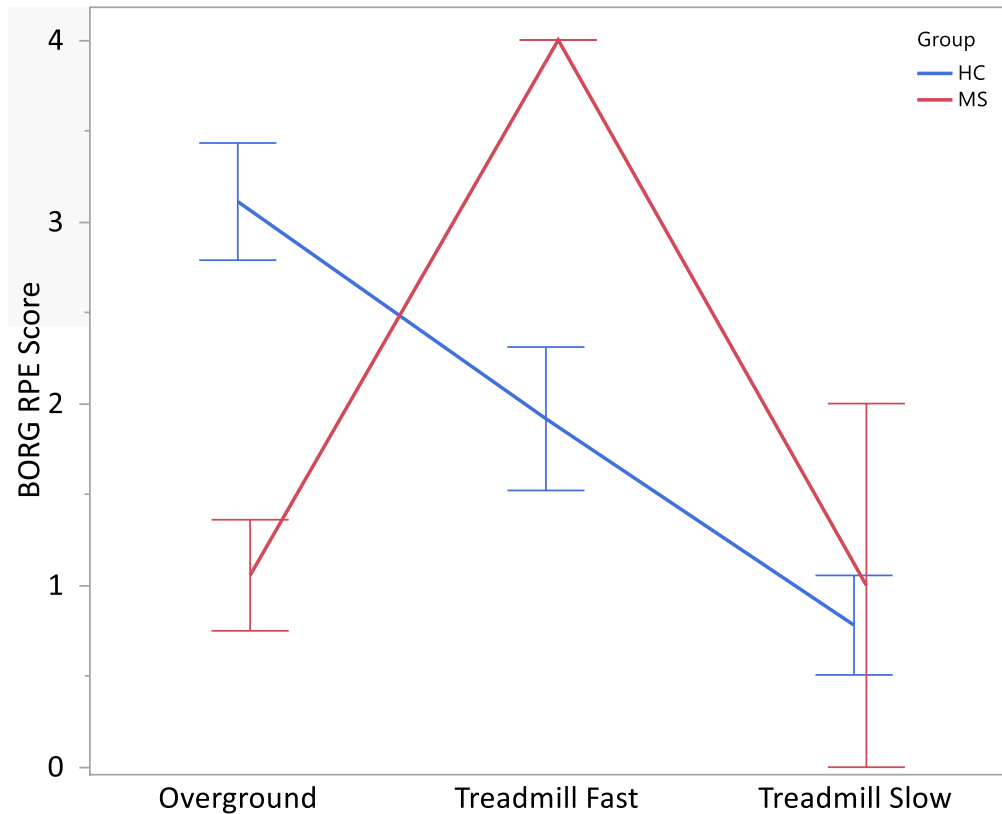
Note: The x-axis represents the different walking conditions (self-paced overground walking, treadmill slow (50% of Timed 25-Foot Walking Test (T25FW)) and treadmill fast (80% of T25FW)). The y-axis represents the distance walked during the 6-minute walking test. Mean values are presented, with healthy control (HC) shown in blue and people with multiple sclerosis (MS) shown in red. Error bars represent the standard error of the mean.

A significant difference was found in the BORG RPE score in walking condition ($p = .0024$) and condition $\times$ group interaction ($p = .0007$). The post-hoc analysis shows that overground is more fatiguing than treadmill slow walking (M difference = 1.24; 95% CI [0.01, 2.47]; $p = 0.0456$; $d^2 = 1.20$) and that treadmill fast is more fatiguing than treadmill slow (M difference = 2.08; 95% CI [0.72, 3.45]; $p = 0.0017$; $d^2 = 0.92$). Additionally, a significant interaction effect ($p = .0007$) was found between group and walking conditions for perceived exertion, as measured by the

BORG RPE score (Figure 4). Specifically, pwMS reported higher exertion during the fast treadmill condition compared to overground and treadmill slow walking

Figure 4

Mean BORG RPE Score after the 6-Minute Walking Test in the Different Walking Conditions.



Note: Mean BORG RPE Score reported after each walking condition (Overground, Treadmill Fast, and Treadmill Slow) for people with multiple sclerosis (pwMS) and healthy controls (HC). A significant interaction effect was found between group and condition, indicating that perceived across the three different walking conditions differed between groups.

Discussion

The aim of this study was to investigate how prolonged walking under different conditions affects spatiotemporal gait characteristics in pwMS compared to HC. It was hypothesised that overground walking had greater changes compared to fixed-speed treadmill walking, and that these changes are more pronounced in pwMS. The results partly support this hypothesis, some spatiotemporal parameters had greater changes during overground walking compared

to fixed-speed treadmill walking. No significant differences were found between pwMS and HC. More specifically, step duration and single limb support had a greater change during overground walking. These greater changes could be that during overground walking, the participants walked farther and with a higher perception of fatigability. A potential explanation is that, unlike fixed-speed treadmill walking, overground walking allows participants to self-regulate their pace. This may have led them to cover more distance and adjust their gait more dynamically in response to fatigue. These adaptations could explain the greater changes observed in these two spatiotemporal gait parameters. In contrast, the magnitude of change in stride length was greater during the slow treadmill condition than in overground walking. This suggests that when pace is externally imposed, participants adapt spatial aspects of their gait more strongly. Perhaps because a fixed speed limits their ability to adjust timing, forcing them to modify step size instead. Furthermore, no significant changes were found over time in cadence, double support, and swing time. This might indicate that these parameters are less affected by walking speed or fatigability.

These results suggest that the walking condition has a greater influence on the change in spatiotemporal gait characteristics than the presence of MS. This could be the case because, during overground walking, the participants could pace what is not possible during the treadmill conditions. Furthermore, no significant differences were found for the main effects or their interaction in step duration variability and stride length variability. Additionally, participants walked significantly farther during overground walking compared to both treadmill fast and treadmill slow conditions. Also, the distance covered during treadmill fast was significantly greater than during treadmill slow walking. A possible explanation for the longer distance observed during overground walking may lie in the relatively low EDSS score of the participants, which may have allowed for greater self-selected pacing and endurance.

Moreover, despite overground walking being rated as more fatiguing based on the BORG-scale, participants still walked farther during this condition. This may be explained by the self-selected pacing allowed during overground walking, enabling participants to regulate their effort and maintain a higher walking speed even with increased perceived exertion. Additionally, the nature of overground walking may encourage greater effort, even when fatigue is perceived as higher. In contrast, fixed-speed treadmill walking limits pace

adjustments, potentially resulting in lower perceived fatigue but also shorter walking distances.

Two studies have examined gait characteristics in pwMS using short treadmill walking. Remelius et al. (2012) reported no significant differences in stride length and swing time between pwMS and HC when walking at a self-selected speed. However, they observed that pwMS demonstrated significantly greater double support time and reduced swing time compared to HC. When pwMS walked at a fixed speed, they demonstrated a longer double support, stride length and a shorter swing phase. Sloot et al. (2014) found slightly reduced values in most kinematic and kinetic parameters during self-paced treadmill walking in pwMS. It is important to note that both studies assessed short treadmill walking only, under either self-selected or fixed speed conditions. Two previous studies examined prolonged overground or self-paced treadmill walking. Santinelli et al. (2024) reported a decrease in cadence from minute two onwards and a steady increase in step duration over the 6MWT. In contrast, when adjusted for walking speed, Theunissen et al. (2023) found no significant changes in cadence, step length, and step width during self-paced treadmill walking. In our study, by directly comparing prolonged overground walking with two fixed-speed treadmill conditions, we observed significant main effects of walking conditions on single limb support, step duration, and stride length. These results are partially in line with those of Santinelli et al. (2024) and align with Theunissen et al. (2023) in showing that not all spatiotemporal parameters change under certain treadmill conditions. Furthermore, the significant differences observed between walking conditions, but not between groups, suggest that walking conditions had a greater impact on spatiotemporal gait characteristics than the presence of MS itself. This may be partially explained by the relatively low EDSS scores of the pwMS participants. At such low levels of disability, gait patterns often remain comparable to those of HC, and compensatory mechanisms may help maintain functional performance during walking tasks. As such, group differences in spatiotemporal gait characteristics may not yet be detectable. Previous systematic reviews have suggested that gait abnormalities in pwMS may not be apparent during early stages of the disease but tend to worsen with disease progression (Chee et al., 2021; Coca-Tapia et al., 2021). This could account for the lack of significant differences between pwMS and HC in the present study.

Furthermore, the study of Socie and Sosnoff (2013) investigated gait variability in pwMS and its influence on the energetic cost of walking (CW). They found that a greater variability in spatiotemporal gait characteristics is associated with a higher CW, and found that EDSS is positively correlated with CW. They suggest that disease progression, along with increased variability in stance phase and step length, contributes to a higher energetic penalty during overground walking (Socie & Sosnoff, 2013). The present study examined only the change in variability in step duration and stride length and found no significant difference in all main effects and their interaction. A possible explanation for the present study found no significant difference in the main effects could be due to the low EDSS score of the pwMS. Additionally, the presence of high standard error reduces the precision of the main estimates and may have contributed to the lack of statistically significant effects. Future research with larger sample sizes is needed to further investigate spatiotemporal gait characteristics and their change during prolonged walking in overground and treadmill walking conditions, as well as the influence of walking fatigability. To improve comparability, future studies could consider equalising walking distance across conditions, which may help better isolate the effect on spatiotemporal gait characteristics. Furthermore, these findings suggest that future research could benefit from exploring more challenging and varied walking protocols to better understand how different conditions influence gait and fatigue in pwMS. Finally, the results of this study suggest that overground walking may induce greater changes in certain spatiotemporal gait characteristics compared to fixed-speed treadmill walking. However, further research is needed to determine whether these changes are clinically relevant and how they may inform rehabilitation or gait assessment strategies.

Limitations

Although significant differences and large effect sizes were observed, the relatively small sample size and unequal group sizes warrant caution in interpreting these results. These factors may inflate the effect size and limit the generalizability of the findings.

Given that spatiotemporal gait characteristics are strongly affected by walking speed, future research should consider complementing these measures with more quality-based analyses.

Assessing joint angles of the hip, knee, and ankle during gait may offer a more comprehensive understanding of motor impairment in pwMS.

Another limitation is that participants walked a longer distance during the overground condition compared to the treadmill conditions. This increased physical effort may have led to greater fatigue, potentially influencing the observed gait changes. Future studies should consider matching walking intensity of distance across conditions to control for this effect.

Conclusion

There was a significant main effect of walking condition, indicating that, in general, walking condition influenced single limb support, step duration, and step length. Specifically, the change from minute 1 to minute 6 in single limb support and step duration was significantly greater during overground walking compared to both treadmill slow and treadmill fast walking. Additionally, stride length showed a significantly smaller change during overground walking compared to treadmill slow walking. Furthermore, no significant differences were found in step duration and stride length variability. Finally, the participants in this study walked farther during overground walking than during treadmill fast walking and during treadmill slow walking.

Acknowledgement

I would like to express my sincere appreciation to my promoter, Prof. Dr. P. Feys, for the opportunity to conduct this thesis under his supervision. I also want to thank Felipe Balistieri Santinelli, for his continuous feedback and assistance with the statistical analyses. I am grateful to all participants who took part in the study. Furthermore, I would like to thank my family and my girlfriend for their encouragement and understanding throughout this process.

Finally, I acknowledge the use of AI-based language support (ChatGPT, OpenAI) to assist with English grammar, phrasing, and improving the readability of the text. The content and ideas are entirely my own and the tool was used only to refine language based on my original drafts.

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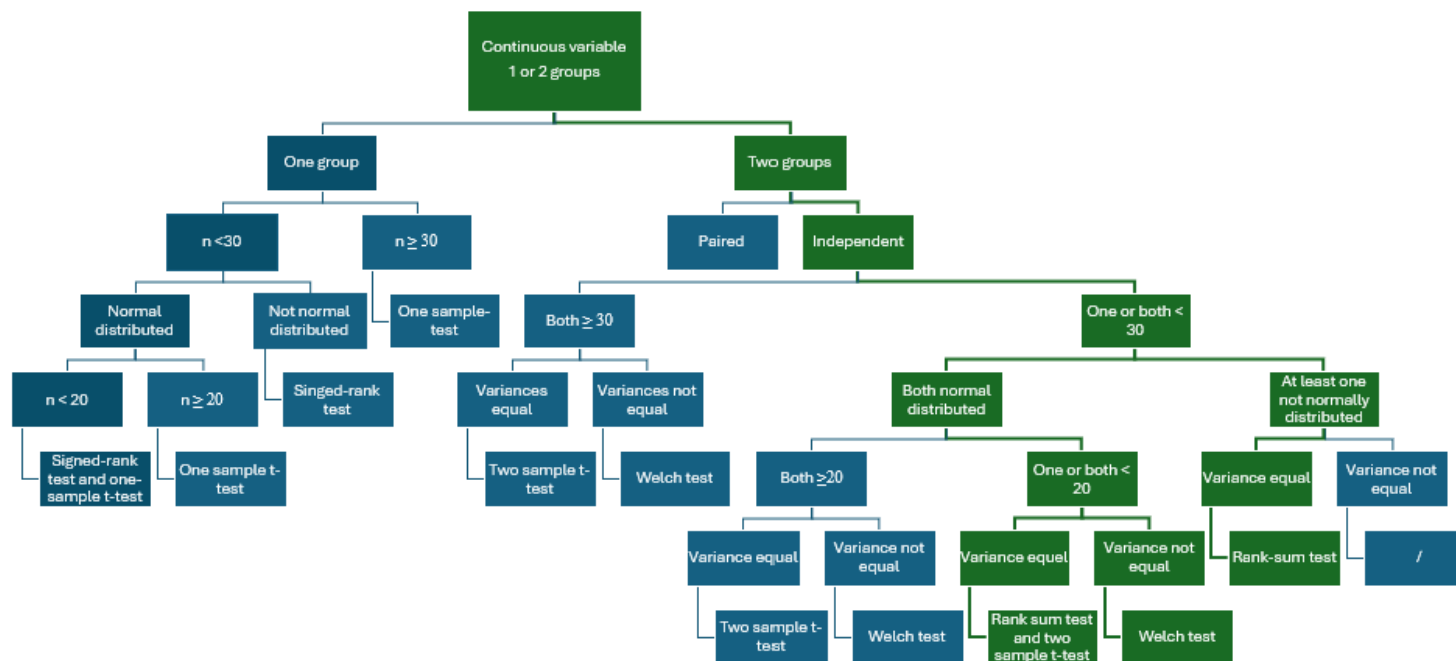
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Appendix A

Figure 5

Decision Tree for Selecting the Statistical Test for the Demographic Data

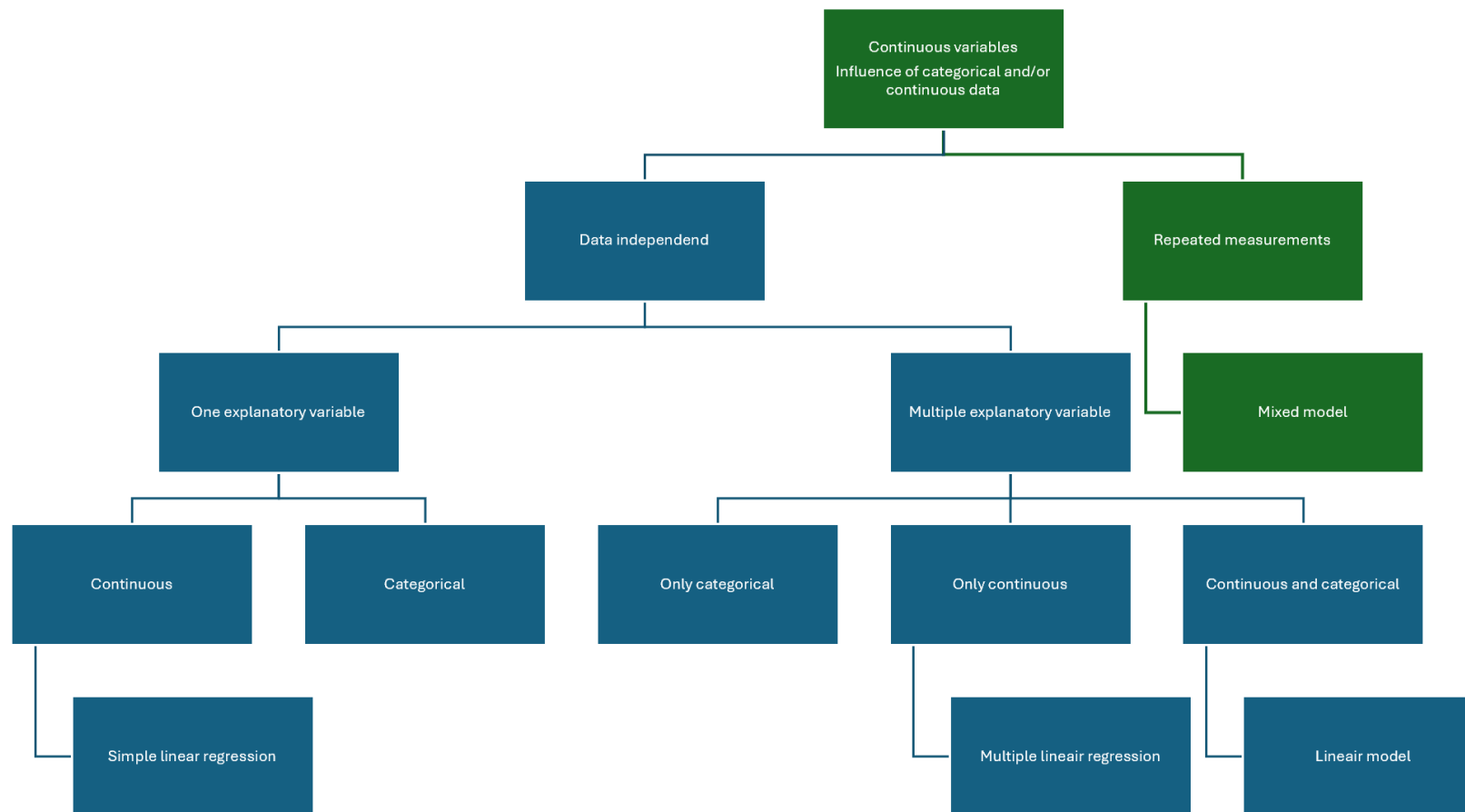


Note: Decision tree for selecting the statistical test to determine the differences in the demographic data of all the participants. The path in green was followed. For the analysis of demographic data, a two-sample t-test (for normally distributed variables) or a rank-rank sum (for non-normally distributed variables) was used to assess group differences.

Appendix B

Figure 6

Decision Tree for Selecting the Statistical Test for the Primary and Secondary Outcome Measures

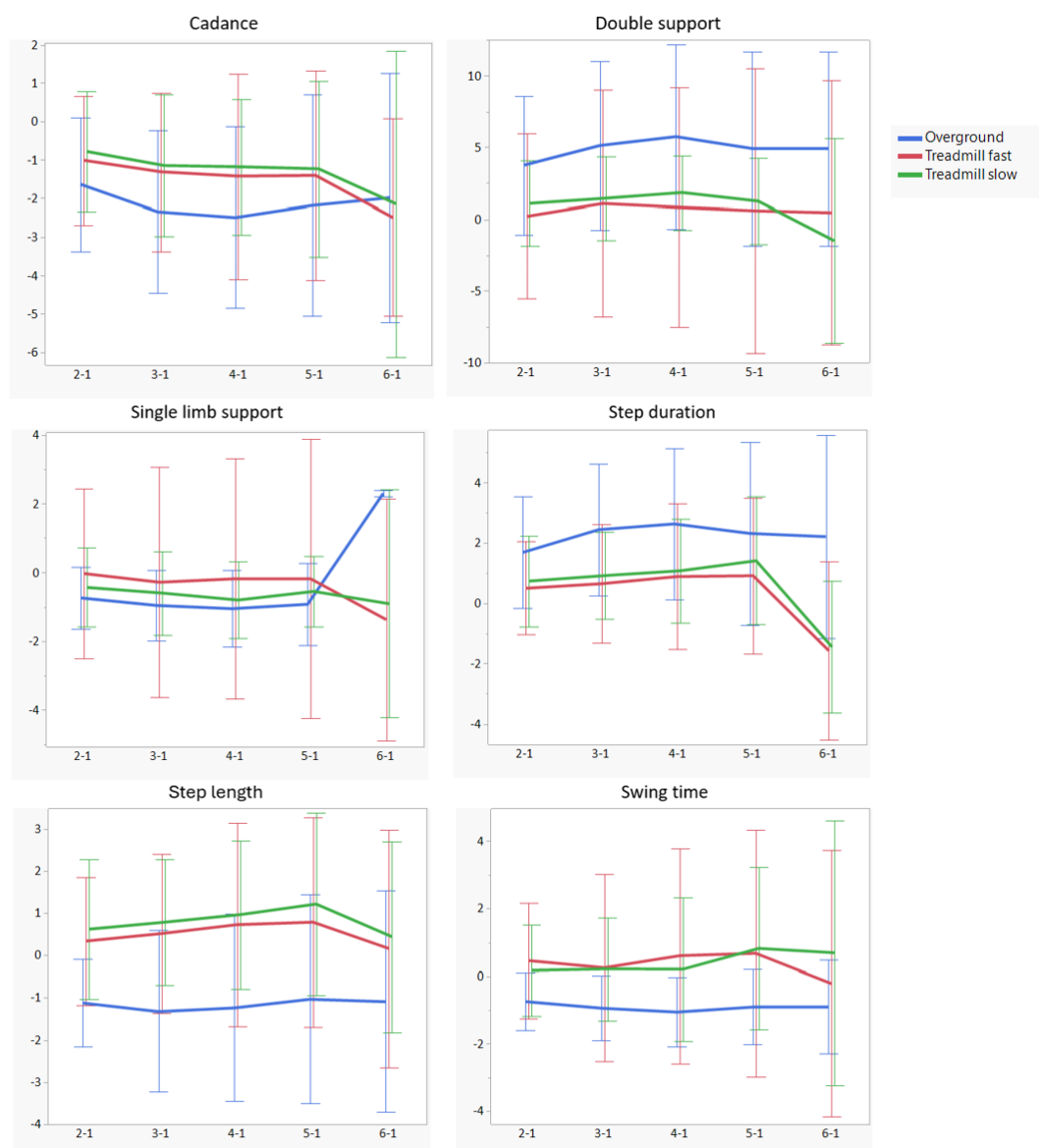


Note: Decision tree for selecting the statistical test for the primary and secondary outcome measures. The path in green was followed

Appendix C

Figure 7

Overview of the Percentage of Change of all Spatiotemporal Parameters During the Six-minute Walking Test Minute-by-minute



Note: Average of specific spatiotemporal gait characteristics through the 6MWT for pwMS in overground (blue), treadmill fast (red), and treadmill slow (green). 2-1: difference from minute 2 to 1, 3-1: difference from minute 3 to 1, 4-1: difference from minute 4 to 1, 5-1: difference from minute 5 to 1, and 6-1: difference from minute 6 to 1. The speed of the slow condition was 50%, and the fast condition was 80% of the mean speed measured by the T25FW. Error bars indicate standard deviation.