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**Clinical manifestation and perceived symptoms of walking-related performance fatigability in persons with MS.**

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## **Abstract**

**Introduction:** Fatigue and walking difficulties are common impairments and activity limitations in persons with Multiple Sclerosis (PwMS). Walking fatigability (WF) can be measured by a Distance Walked Index (DWI) and is defined as a decline in walking distance of 10% or more during the six-minute walking test (6MWT). However, the clinical manifestation and perceived symptoms related to fatigability is still not well documented.

**Materials and methods:** Forty-nine PwMS (EDSS  $\leq 6$ ) and 28 healthy controls (HC) performed a 6MWT. The perceived severity of 11 common symptoms was rated on a visual analogue scale of 0-10 before, immediately after, and 10, 20 and 30 minutes after the 6MWT by means of the symptom inventory. Short motor impairment screening tests at baseline together with other descriptive measures were performed..

**Results:** Twenty pwMS were categorized in the WF group and were more disabled (EDSS:  $4.16 \pm 1.41$ ) than the non-walking fatigability group (NWF) ( $n=29$ , EDSS:  $2.62 \pm 1.94$ ). PwMS showed exacerbations of several perceived symptoms in MS, where most symptoms returned to baseline within 10 minutes after the walking test. The WF group showed significantly more muscle weakness and gait impairment, together with balance problems, and experienced an increase in spasticity, pain and dizziness after 6MWT.

**Discussion and conclusions:** Our findings showed that perceived severity of symptoms are higher in pwMS presenting WF, and increase temporally after the 6MWT. Future research with quantitative measurement during and after walking is recommended.

**Search terms:** 1. Manifestation, 2. Fatigability 3. Walking 4. Clinical 5. Multiple Sclerosis

## 1. Introduction

Fatigue and walking impairment are highly prevalent in persons with Multiple Sclerosis (pwMS), and regarded as impactful and disabling symptoms due to the interference with many aspects of daily living [1-4]. Fatigue is classified into trait fatigue and state fatigue [3-5]. Trait fatigue is a general feeling or perspective of fatigue, that does not fluctuate strongly over time. It is therefore considered a characteristic and is examined by questionnaires related to fatigue over a bigger retrospective period in time, e.g. Modified Fatigue Impact Scale (MFIS) and Fatigue Severity Scale (FSS) [3-5]. State fatigue - also seen as fatigability - is seen as decline in task-related performance over time and therefore susceptible to changes. Fatigability has a performance (objective) and perceived (subjective) component. Perceived fatigability can be measured during or after a fatigable task using a subjective scale, such as visual analogue scale (VAS) [3-6]. The performance component can be measured objectively through a task at ICF body function level (e.g. Static or dynamic Fatigue Index for muscle strength) [6] or at ICF activity level during walking (e.g. Distance walked index (DWI)) [7, 8]. In the DWI, a distance decline of -10% between the first and the last minute of the six-minute walk test (6MWT) has been proposed as a cut-off value to determine walking fatigability [7]. However, the clinical manifestation in changes other than gait speed or spatiotemporal parameters [5, 9, 10] are still rarely documented, e.g. increased spasticity, pain, or perceptions of changed severity of symptoms. In addition, there is a lack of knowledge on the duration that perceived symptoms persist after a walking task, and how this differs between pwMS with and without manifestation of fatigability.

To answer these questions, the following will be investigated in PwMS with and without walking-related fatigability compared to healthy controls: 1) During every minute of the 6MWT, we will analyse the course of performance and perceived fatigability; 2) Investigate

the clinical manifestations and perceived severity of symptoms before and after completion of 6MWT, and repeatedly during 30 minutes afterwards.

## **2. Methods**

### **2.1. Participants**

Fifty-two PwMS and 32 age- and gender-matched Healthy Controls (HC) were included. All participants were recruited and tested at the rehabilitation research institute of Hasselt University, National MS Center Melsbroek or Rehabilitation and MS Center Overpelt in Belgium. The ethical committees approved the protocol and written informed consent was obtained from all participating subjects. The protocol was registered at [clinicaltrials.gov](https://clinicaltrials.gov) [NCT03860675]. This study was part (day 1 measures) of a larger study, investigating day to day reliability of walking fatigability measurements reported elsewhere [7]. Criteria for inclusion were adults up to 70 years, diagnosed with MS according to the McDonald criteria (24), an Expanded Disability Status Scale (EDSS)  $\leq 6$  and the ability to walk independently or with unilateral walking aid for six minutes without rest. Participants were excluded in case of a MS relapse within the past three months or in the presence of co-morbidities that could interfere with their walking capacity. This latter criterion also applied for HC (self-reported information).

### **2.2. Study design**

#### *2.2.1. descriptive characteristics*

Demographic and clinical characteristics were collected (i.e. age, gender, weight, length, MS phenotype, disease duration and EDSS). The Timed 25-Foot Walk (T25FW) was used to assess walking speed, and the Nine Hole Peg Test (NHPT) to assess manual dexterity.

The following short objective motor screening tests were performed: the Romberg test adapted to age categories was performed to evaluate balance [11]. Balance problems were assumed when participants were not able to keep their balance for at least 10 seconds (ordinal scale: 0). When participants could keep their balance for 30 seconds, no balance problem was assumed (ordinal scale: 3). Keeping balance between 10 and 30 seconds was considered as a danger zone (ordinal scale: 2). Maximal muscle strength was assessed for ankle dorsiflexion, knee extension and hip flexion using the Motricity Index (MI) for the lower limbs. Spasticity was evaluated by the Modified Ashworth Scale (MAS) (with fast passive movements) on the quadriceps, hamstrings and triceps surae, while seated on a chair.

#### *2.2.2 Questionnaires.*

PwMS were given the following questionnaires to complete at home and send back to the assessors within two weeks: Fatigue Scale for Motor and Cognitive functions (FSMC) to rate cognitive and motor fatigue; the severity of fatigue (Fatigue severity scale; FSS) and the impact of fatigue on physical, cognitive and psychosocial domains (Modified fatigue impact scale; MFIS). Perceived walking ability was assessed through the Multiple Sclerosis Walking scale (MSWS-12) and fear of falling was questioned via the Falls Efficacy Scale International (FES-I). Additionally, participants completed questionnaires evaluating sleep problems (Sleep condition indicator; SCI) and anxiety and depression (Hospital anxiety and depression scale: HADS).

#### *2.2.3 Experimental protocol*

##### *2.2.3.1 6MWT and VAS for Perceived and performance fatigability*

Participants performed the 6MWT and were instructed to walk as fast as possible along a 30m corridor [12] and were allowed to use their (unilateral) assistive device if necessary.

Participants were informed about each expired minute without further encouragement.

Every minute, the distance covered was noted and participants indicated their instant perceived fatigue on a scale ranging from 0-10. To differentiate between pwMS with and without walking fatigability, the distance walked index (DWI) was calculated using the following formula based on Leone et al. [8]:  $DWI_{(6-1)} = (\text{distance walked}(\text{min}6) - \text{distance walked}(\text{min}1)) / \text{distance walked}(\text{min}1) \times 100$ . Walking fatigability was determined with a cut-off value of -10% [7].

### 2.2.3.2 *The symptom inventory (SI)*

The SI is a standardized VAS questionnaire previously used by Moumdjian et al., Skjerbaek et al., [13, 14]; consisting of ten possible clinical symptoms commonly present in pwMS; General fatigue, motor fatigability, muscle weakness, gait pattern impairments, balance disturbance, spasticity, visual impairment, sensory disturbance, pain and dizziness. ‘Attention problems’ was added to the questionnaire to have an indication of the involvement of cognitive attentional problems that can be present [15, 16]. Participants were asked to rate the severity of these eleven symptoms on VAS ranging from zero to ten, where zero indicated that they did not experience the symptom, whereas ten indicated the most possible severity of the symptom. Right before (together with the descriptive testing) and after the test, participants were asked to fill in the SI, as also every ten minutes for a total duration of half an hour after the 6MWT (pre, post, post10, post20 and post30) as shown in Figure 1.

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**Insert near here. Figure 1. study design.** SI: symptom inventory, 6MWT: Six minute walking test

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## 2.3. Statistical analysis

Normality of the data was checked using the Shapiro-Wilk test for normality and evaluation of Q-Q plots. The course of perceived fatigability and performance fatigability across the minutes of the 6MWT was evaluated using repeated measures ANOVA. A Tukey’s post-hoc



test with Bonferroni correction was conducted on significant interactions. The same analysis was conducted in order to compare the differences among the time points of the SI (e.g. pre, post, post10, post20 and post30). Differences between all groups at baseline was seen as exploratory and therefore we evaluated every groups as equal by using three different zero hypotheses:  $H_{01}: WF=NWF$   $H_{11}: WF \neq NWF$ ,  $H_{02}: WF=HC$   $H_{12}: WF \neq HC$ ,  $H_{03}: NWF=HC$   $H_{13}: NWF \neq HC$ . Groups were therefore compared by using independent-samples t-tests if normality was assumed or Mann-Whitney U tests in case of no normal distribution. All testing was performed using SPSS (IBM SPSS® Statistics). For five pwMS, MS disease related data was incomplete (EDSS; n=5, MS type: n=3, disease duration: n=2). Other missing data comprised of less than 5% of the sample for descriptive measures (see an overview in supplementary table A1).

### 3. Results

#### 3.1 Participants

Forty-nine pwMS and 28 HC were included for data-analysis in this study [7]. According to the DWI cut-off of 10%, pwMS were subdivided into a walking fatigability (WF) and non-walking fatigability (NWF) group consisting of 20 and 29 patients respectively.

#### 3.2 Study results

##### 3.2.1 Descriptive characteristics

Demographic characteristics for all groups are presented in table 1. No significant differences were found between any groups for age, gender and height. Only weight differed significantly between HC and WF group of pwMS. Eighty-three percent of the RRMS was on immunomodulatory medication. Eight out of all pwMS used Fampyra (16%) and 14% took medication for spasticity and/or sleep disorders.

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**Insert near here Table 1: Demographic characteristics.** Data is represented as mean $\pm$ SD for total MS group, MS subgroups (WF and NWF) and HC.

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The clinical characteristics of the MS group(s) are presented in table 2. The EDSS was significantly higher in WF compared to NWF. WF exhibited a higher motor impairment than NWF, with a worse performance on the T25FW in the WF group. Significant higher scores were found in WF compared to NWF for the MSWS-12 and FES-I, indicating perception of reduced walking ability and higher fall risk in the WF group. The mean score of the MFIS was above the cut off value of 38 in WF, indicating a greater impact of fatigue. Other questionnaires and disease duration did not significantly differ between WF and NWF.

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**Insert near here Table 2: Clinical MS characteristics.** Data is represented as mean $\pm$ SD for total MS group and MS fatigability subgroups (WF and NWF).

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**Insert near here Table 3: Motor function measurements at baseline (i.e. before the 6MWT)** for the MAS, MI and Romberg in WF, NWF and HC. Data is represented as median (Q1-Q3).

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The motor tests at baseline are presented in table 3 for each group. Mann-Whitney U tests between HC and both NWF and WF showed a significant lower muscle strength in the WF and NWF group for ankle dorsiflexion, knee extension and hip flexion in both legs compared to HC. Comparisons between NWF and WF showed significant lower muscle strength in WF for all MIs of the right leg. The Romberg balance test at baseline showed significant differences between all groups (HC>NWF>WF). Significant differences in spasticity were found between HC and NWF for the right quadriceps (HC<NWF) and between HC and WF for all spasticity measurements (HC<WF), except for the left hamstrings. Between WF and NWF, spasticity was significantly higher in WF for the left triceps.

### 3.2.2. Course of walking fatigability during the 6MWT

The manifestation of fatigability during the 6MWT was evaluated in terms of distance and perceived fatigability every minute.

#### 3.2.2.1. *Performance walking fatigability*

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**Insert figure 2 near here**

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The course of the mean distance covered each minute of the 6MWT for WF, NWF and HC is shown in figure 2a. Significant differences were seen between all groups at every minute of the 6MWT. Sequential minute analysis showed only significant decreased distance between the first and the second minute in WF and HC. Data were normalized to minute 1 in order to investigate the decline in walking distance compared to the first minute (Figure 2b). The mean walking distance in WF significantly declined every minute compared to minute 1 to an averaged decline of 20%. In the NWF group, significant declines exhibited starting from minute 3 compared to minute 1. HC showed significant declines compared to minute 1 for all minutes, except for minute 4. Average decline of the HC and NWF groups at minute 6 were lower than 5%. Between groups, significant differences were observed between the WF on the one hand side, and the NWF and HC at the other hand side, starting from minute 3 and 4 respectively. No significant differences were found between HC and NWF.

#### 3.2.2.2. *Perceived walking fatigability*

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**Insert figure 3 near here**

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The course of the mean perceived fatigability (VAS score ranging from 0-10) before and during the 6MWT for WF, NWF and HC are shown in figure 3a. Significant differences were seen every minute between NWF and WF compared HC. Between WF and NWF, significant differences were only present starting from minute 3 to 5. Sequential minute analysis in the

WF group revealed significant increases in perceived fatigability between all timepoints. The NWF group also showed significant increases in perceived fatigability after the first minute (min 1-2) and all the following minutes, except for min 2 to 3. HC showed significant increases in perceived fatigability also after the first minute of walking and between the 5th and last minute of the 6MWT. After normalization of the perceived fatigability towards the baseline VAS scores, all groups showed a similar significant increase in perceived fatigability from the first minute (WF) or second minute (HC and NWF) compared to baseline (figure 3b), with consequently no significant differences between groups anymore.

### 3.2.3. Perceived severity of symptoms and its duration after the 6MWT

The course of the perceived severity of all the symptoms before and after the 6MWT for MS and HC are presented in figure 4.

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**Insert figure 4 near here**

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The VAS score of all the subjective symptoms at baseline was significantly greater in the MS subgroups for all symptoms compared to HC, except for dizziness between HC and NWF, according to the Mann-Whitney U tests. Comparisons between NWF and WF showed significant higher baseline VAS scores in WF for gait pattern impairments and muscle weakness.

General fatigue VAS scores increased significantly after the 6MWT in all groups. For the WF and NWF group, this lasted until post10 after 6MWT, unlike the HC, which showed no significant differences anymore at post10 compared to baseline. Motor fatigability VAS scores increased significantly after the 6MWT in HC and WF, but not in NWF.

The perceived severity of muscle weakness and gait pattern impairments increased significantly post 6MWT in only WF and NWF. For the NWF group, significant higher perceived severity with baseline scores were present until post20 for muscle weakness VAS scores.

Only the WF group showed higher VAS scores for spasticity, dizziness and pain after the 6MWT. Post10 there was no significant difference anymore with baseline VAS scores.

Sensitivity VAS scores did only significantly increase in the NWF group. The significant difference with baseline was present until post10. The VAS scores for attention problems, visual disturbance and balance problems did not show significant differences between or within all the groups at any timepoint.

## 4. Discussion

This study is, to our knowledge, the first to investigate the clinical manifestation of fatigability during and after a 6MWT by means of the symptom inventory before and up to 30 minutes after the 6MWT in pwMS with and without walking fatigability in comparison to healthy controls. At baseline, pwMS with WF showed higher disabilities, where also more disease-related symptoms were elevated after the 6MWT, compared to the NWF group and HC. However almost all increased symptoms return to baseline after 10 minutes.

### 4.1 Disability in MS subgroups

EDSS, motor function test (i.e. T25FW) and motor function questionnaires showed significantly higher disabilities in WF compared to NWF, confirming previous findings [8, 17]. Muscle strength was significantly lower at baseline in both MS groups compared to HC, and was mirrored by subjective rating of muscle weakness in the SI. However, objective lower muscle strength between WF and NWF was only present in one leg, which is similar to the findings by Ramari et al. [18] who also found a higher muscle weakness in the right leg. They found balance and muscle strength to be a predictor of WF, which is supported by our results, as the objective and subjective assessment of balance and falls did significantly differ between all groups (HC>NWF>WF). Spasticity at baseline was significantly more present in WF compared to NWF for the left triceps.

### 4.2. Course of walking fatigability during the 6MWT

#### 4.2.1. Performance walking fatigability

The study results confirmed the great decline of walking distance over time (on average 20%) found in Leone et al. and Phan-ba et al., once participants showed a  $DWI_{6-1} \leq -10\%$  [7, 8, 17]. Results show this is very different from HC, which show a stagnation after the second minute and accelerate again in the last minute, or other persons with MS that decline less than 10% in distance walked. The significant differences in decline in distance walked between NWF and

HC compared to WF were seen from the third and fourth minute (NWF and HC respectively) of the 6MWT. Therefore, our results confirm the value of using longer walking tests such as the 6WMT to differentiate between WF and NWF. Short walking tests such as the T25FW and 2MWT are not appropriate for diagnostic reasons of walking fatigability, as no clear deceleration can be measured, whereas longer walking tests longer than 6 minutes may give bias due to rest stops or drop out in the more disabled patients [5].

#### 4.2.2. Perceived walking fatigability

Perceived fatigability assessed before and throughout the 6MWT was found to be different between NWF and WF compared to HC. However, in order to differentiate between WF and NWF, three minutes of the walking test should be passed. This is again a confirmation for the need of long walking test for the patients to subjectively feel fatigued.

It is however important to note that, when normalizing the perceived fatigability VAS scores with the instant fatigue at baseline, no significant differences between all groups were found. The increase in perceived fatigability throughout the walking test showed a similar pattern in WF, NWF and HC as performance fatigability. Consequently, it can be concluded that de major differences in perceived fatigability are situated at baseline level, possibly more as trait fatigue, wherein the WF group experienced already more fatigue even before starting to walk. This elevated fatigue level in the WF group was confirmed by the MFIS scores which exceeded the cut off of 38. Also Loy et al. suggested that people reported a higher trait fatigue while having performance fatigability. However, the size of the relationship is stated not be large enough to suggest that fatigue and fatigability are the same construct (19).

#### 4.3. Perceived symptoms measured by the SI

The symptom inventory has been used in previous research investigating changes after performing a maximal cycling exercise test [13]. In this study in persons with a mild disability due to MS, an increase in general fatigue, muscle fatigue, balance, gait pattern, muscle weakness, and visual impairment, was found, with increases of 2 points considered to exceed minimal important change threshold [13, 19, 20]. In our study, the WF showed a significant increase in perceived general fatigue, motor fatigability, muscle weakness, spasticity, pain, dizziness and gait pattern impairments after the 6MWT. Most of the symptoms returned back to baseline within 10 minutes after the 6MWT, except for the general feeling of fatigue, which then returned to baseline after 20 minutes. Also the NWF group reported temporary increases in perceived symptoms, but for fewer symptoms. However, only in the WF did the mean increase of perceived severity of general fatigue and motor fatigability exceeded two points on the VAS, which can be considered as a minimal important change needed for clinical relevance [19, 20]. In the HC and NWF, mean significant differences did never exceed the minimal important change. The findings of our study suggest that the 6MWT is a safe test to measure walking capacity and walking fatigability, as most perceived symptoms return back towards baseline within 10 minutes. The presence of other symptoms when showing walking fatigability, supports that fatigability can be influenced by several internal and external related factors [5] and indicate that for future research, objective testing of these symptoms should be held within 10 minutes after the walking test and differentiate between WF and NWF.

### 4.4 Limitations

Limitations of the study are that participants executed testing at different research or rehabilitation centres. Possible interference of different settings and different testers might have biased our results. However, this bias was minimized by training every instructor and by following instruction booklets. Besides, a larger sample size and objective measures are still needed to confirm these results. Next, this study was seen as an exploratory study, where an



heterogeneous group of pwMS got included, with a wide range of EDSS and disease duration. Most pwMS took immunomodulatory medication, but we however do not think that immunomodulatory treatment would have impacted on the results of walking and perceived symptoms given chronic use of it. Next, a small sample took medication for spasticity and/or sleep disorders, which could have biased some results. Eight persons used Fampyra, where it is currently unknown to which extent it could have impacted the manifestation of walking fatigability. Future studies could look at the possible confounder of symptomatic treatment and investigate walking fatigability and perceived symptoms before and after intake. When reviewing the medication lists, we noticed that one participant reported to have COPD as co-morbidity. However, this did not seem to interfere with his walking endurance, as he did not show walking fatigability. As such, we do not think it importantly affected our results. Another consideration is the range of included subjects. The upper range of 70 years might have been too high given an expected impact on (perceived) balance and gait. Future studies could focus on lower and smaller limits of age, such as 40-55 years.

## **Conclusion**

This study showed that longer walking tests, such as the 6MWT, can detect and differentiate between persons with and without walking fatigability, and that certain perceived symptoms are exacerbated in pwMS presenting walking fatigability after the 6MWT. The perceived exacerbation of these different symptoms could be a possible explanation for the decline in walking distance over the 6MWT which is present in patients who experiences walking fatigability. Therefore, future studies should focus on measuring objective parameters related to MS symptoms before and after a walking protocol such as postural balance, gait pattern, muscle strength and pain sensitivity, as this could lead to new insights regarding the determinants of walking fatigability. The time course of perceived recovery of the symptoms

(within 10 minutes) may contribute to specify measures during future experimental studies design.

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## Tables

**Table 1: Demographic characteristics.** Data is represented as mean±SD for total MS group, MS subgroups (WF and NWF) and HC. Corresponding p-values indicate significant differences

	MS			HC (n=28)	p-value
	Total (n=49)	WF (n=20)	NWF (n=29)		
Demographics					
Age (years)	47.5±10.2	47.8±10.5	47.0±10.1	49.2±11.7	NS <sup>a</sup>
Gender: female (n; %)	36; 74%	15; 75%	21; 72%	21; 75%	NS <sup>d</sup>
Height (cm)	167.0±25.6	162.1±38.4	170.4±8.4	167.7±9.1	NS <sup>b</sup>
Weight (kg)	75.3±14.0	76.7±14.1	74.5±14.1	69.1±14.9	<0.05 <sup>c</sup>

MS: multiple sclerosis, WF: walking fatigability, NWF: non-walking fatigability, HC: healthy controls, SD: standard deviation, NS: not significant (p>0.05).

<sup>a</sup> Independent-samples t-test for all groups, <sup>b</sup> Mann-Whitney U test for all groups, except NWF-HC; Independent-samples t-test;

<sup>c</sup> Mann-Whitney U test for all groups, revealing significant differences between HC-MS and HC-WF, <sup>d</sup> Chi-square test between all groups

**Table 2: Clinical MS characteristics.** Data is represented as mean±SD for total MS group and MS fatigability subgroups (WF and NWF).

	MS			p-value
	Total (n=49)	WF (n=20)	NWF (n=29)	
MS characteristics				
	RRMS: 41	RRMS: 16	RRMS: 25	NS <sup>c</sup>
MS-type (n)	PPMS: 2	PPMS: 1	PPMS: 1	
	SPMS: 3	SPMS: 2	SPMS: 1	
Disease duration (years)	12.13±7.47	14.00±7.99	10.85±6.95	NS <sup>a</sup>
EDSS	3.28±1.88	4.16±1.41	2.62±1.94	<b>0.006<sup>a</sup></b>
Motor function				
T25FW (s)	5.26±1.68	5.78±1.82	4.90±1.52	<b>0.020<sup>b</sup></b>
NHPT dominant (s)	22.62±5.88	23.89±6.36	21.75±4.46	NS <sup>b</sup>
NHPT non-dominant (s)	24.38±7.50	26.91±9.85	22.64±4.79	NS <sup>b</sup>
Questionnaires				
MFIS total	38.31±18.01	41.7±18.51	35.89±17.58	NS <sup>a</sup>
MFIS physical	17.48±8.13	19.80±7.95	15.82±7.99	NS <sup>a</sup>
MFIS cognitive	17.69±9.33	18.15±9.59	17.36±9.31	NS <sup>a</sup>
MFIS psychosocial	3.29±2.05	3.75±2.22	2.96±1.90	NS <sup>b</sup>
FSMC total	63.58±18.59	67.63±16.33	60.82±19.79	NS <sup>b</sup>
FSMC physical	33.17±9.34	35.95±6.65	31.29±10.50	NS <sup>b</sup>
FSMC mental	30.40±10.02	31.68±10.48	29.54±9.79	NS <sup>b</sup>
HADS total	11.87±8.50	14.47±8.33	10.11±8.30	NS <sup>b</sup>
HADS anxiety	6.21±4.45	7.42±4.54	5.39±4.27	NS <sup>b</sup>
HADS depression	5.66±4.83	7.05±4.64	4.71±4.81	NS <sup>b</sup>
MSWS-12	34.92±14.48	41.5±12.47	30.21±14.17	<b>0.008<sup>b</sup></b>
FSS average	4.65±1.53	4.97±1.36	4.43±1.63	NS <sup>b</sup>

## Clinical manifestation walking fatigability

SCIa	20.39±8.26	17.89±6.94	22.00±8.75	NS <sup>a</sup>
FES-I	28.81±11.85	33.45±12.14	25.50±11.80	<b>0.008<sup>b</sup></b>

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a independent samples t-test, b Mann-Whitney U test, c Chi-square test

MS: multiple sclerosis, WF: walking fatigability, NWF: non-walking fatigability, RRMS: relapsing remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, EDSS: expanded disability system score, FS scores: functional system scores, T25FW: timed 25 foot walk, NHPT: nine hole peg test, MFIS: modified fatigue impact scale, FSMC: fatigue scale for motor and cognitive function, HADS: hospital anxiety and depression scale, MSWS-12: 12 item multiple sclerosis walking scale, FSS: fatigue severity scale, SCI: sleep condition indicator, FES-I: falls efficacy scale international, SD: standard deviation, NS: not significant ( $p>0.05$ ).



**Table 3:** Motor function measurements at baseline (i.e. before the 6MWT) for the MAS, MI and Romberg in WF, NWF and HC. Data is represented as median (Q1-Q3).

	MS		
	WF (n=20)	NWF (n=29)	HC (n=28)
<b>Baseline MI</b>			
Ankle dorsiflexion R	25 (19-33)	33 (33-33)	33 (33-33)
Ankle dorsiflexion L	33 (25-33)	33 (33-33)	33 (33-33)
Knee extension R	25 (25-33)	33 (33-33)	33 (33-33)
Knee extension L	33 (25-33)	33 (25-33)	33 (33-33)
Hip flexion R	25 (25-25)	33 (25-33)	33 (33-33)
Hip flexion L	33 (25-33)	33 (25-33)	33 (33-33)
<b>Baseline MAS</b>			
Quadriceps R	0 (0-1)	0 (0-0)	0 (0-0)
Quadriceps L	0 (0-1)	0 (0-0)	0 (0-0)
Hamstrings R	0 (0-1)	0 (0-0)	0 (0-0)
Hamstrings L	0 (0-0)	0 (0-0)	0 (0-0)
Triceps R	0 (0-0)	0 (0-0)	0 (0-0)
Triceps L	0 (0-1)	0 (0-0)	0 (0-0)
<b>Baseline Romberg</b>	<b>1 (1-2)</b>	<b>2 (1-3)</b>	<b>3 (2-3)</b>

6MWT: six-minute walking test, MS: multiple sclerosis, WF: walking fatigability, NWF: non-walking fatigability, HC: healthy controls, MAS: modified ashworth scale, MI: motricity index;; Q1-Q3: interquartile range.

**Supplementary table A.1:** Number of participants missing in the analysis for demographic data and questionnaires

Outcome measure	Missing WF (n)	Missing NWF (n)	Missing HC (n)	Missing total (n)
Age (years)	0/20	0/29	1/28	1/77
Gender	0/20	0/29	0/28	0/77
Length (cm)	0/20	1/29	1/28	2/77
Weight (kg)	0/20	1/29	1/28	2/77
MS Type	1/20	2/29	NA	3/49
Disease duration (years)	1/20	1/29	NA	2/49
EDSS	1/20	4/29	NA	5/49
MFIS	0/20	1/29	NA	1/49
FSMC	1/20	1/29	NA	2/49
HADS	1/20	1/29	NA	2/49
MSWS-12	0/20	1/29	NA	1/49
FSS	0/20	0/29	NA	0/49
SCI	2/20	1/29	NA	1/49
FES- I	0/20	1/29	NA	1/49

MS: Multiple Sclerosis, WF: Walking fatigability group, NWF: non walking fatigability group, HC: Healthy Control, NA: not applicable, FS: functional system scores, EDSS: expanded disability system score, MFIS: modified fatigue impact scale, FSMC: fatigue scale for motor and cognitive function, HADS: hospital anxiety and depression scale, MSWS-12: 12 item multiple sclerosis walking scale, FSS: fatigue severity scale, SCI: sleep condition indicator, FES-I: falls efficacy scale international

## Figure legend

**Figure 1. study design.** SI: symptom inventory, 6MWT: Six minute walking test

**Figure 2: A. Distance walked every minute of the 6MWT for both MS groups (WF and NWF) and HC. B. Normalized distance walked every minute of the 6MWT for both MS groups (WF and NWF) and HC.** Data is presented as mean $\pm$ 2SE. \* indicates  $p\leq 0.05$ . 6MWT: six-minute walking test, HC: healthy control, MS: multiple sclerosis, WF: walking fatigability, NWF: non-walking fatigability.

**Figure 3: A. Perceived fatigability (0-10 VAS) before (pre) and after every minute of the 6MWT for both MS groups (WF and NWF) and HC. B. Normalized perceived fatigability (0-10 VAS) before (pre) and after every minute of the 6MWT for both MS groups (WF and NWF) and HC** Data is presented as mean $\pm$ 2SE.

\* indicates  $p\leq 0.05$ . VAS: visual analogue scale, 6MWT: six-minute walking test, HC: healthy controls, MS: multiple sclerosis, WF: walking fatigability, NWF: non-walking fatigability.

**Figure 4: Mean VAS scores (range 0-10) for the symptoms inventory pre, post, 10, 20 and 30 minutes after the 6MWT for HC, WF and NWF.**\*Significant difference relative to VAS pre 6MWT ( $p\leq 0.05$ ). VAS: visual analogue scale, 6MWT: six-minute walking test, HC: healthy controls, WF: walking fatigability, NWF: non-walking fatigability.