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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

The relation between vascular function, physical activity and sedentary behaviour in persons with Multiple Sclerosis and healthy controls

Sven Van Campenhout

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :
Prof. dr. Bert OP 'T EIJNDE

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The relation between vascular function, physical activity and sedentary behaviour in persons with Multiple Sclerosis and healthy controls.

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**Activity and vascular function in Multiple sclerosis*

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Keywords: Physical activity, sedentary behavior, Cardiovascular risk, Multiple Sclerosis

ABSTRACT

Multiple Sclerosis (MS) is a chronic neurodegenerative autoimmune disorder of the central nervous system. Noticeably in MS is the heterogenicity in the clinical course which can partly be explained by the presence of cardiovascular diseases (CVD) like hypertension, heart disease, and peripheral vascular disease. Co-existence of CVD increases risk of ambulatory disability in persons with MS (PwMS) 1,5 fold at time of diagnosis. Furthermore, CVD prevalence is higher in PwMS compared to the general population. We hypothesize that this increased prevalence in PwMS is primarily due to the sedentary lifestyle and physical inactivity seen in PwMS. We therefore want to show that lifestyle could be a crucial factor in the progression of MS and cardiometabolic comorbidities. For this, sedentary PwMS and healthy controls were recruited. Groups were divided on 150 min of moderate-to-vigorous physical activity per week. Activity was tracked using activPAL3^{tm} monitor. Body composition was assessed using DXA scan and anthropometric measurements, vascular function was determined as arterial stiffness and endothelial function via non-invasive measurements using Spygmocore and EndoPat 2000 respectively. Our study showed no significant impact of physical activity levels and sedentary time on body composition or vascular function. Correlation analysis showed associations of sedentary behaviour with vascular function (i.e. pulse wave velocity). Physical activity was associated with fat percentage, VO_2peak and heart rate variability. To conclude, physical activity in sedentary PwMS tends to elucidate improvements on vascular function, be it direct or indirect. In the future, activity might be a promising therapy for PwMS.

INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory and neurodegenerative autoimmune disorder of the central nervous system, affecting more than 2.8 million people worldwide (1, 2). It is characterized by the destruction of myelin in the central nervous system leading to chronic inflammation and oxidative stress. This causes impairments throughout the body with symptoms ranging from visual and speech impairments to spasticity, paralysis, and walking difficulties.

Noticeably in MS is the heterogenicity in the clinical course (3). MS can affect different parts of the central nervous system, so the symptoms can vary depending on which areas are affected. Additionally, the severity of symptoms can vary depending on the individual and the stage of the disease. Importantly, previous research showed that the co-existence of cardiovascular diseases (CVD) is also an important explanatory factor for heterogenic disease course in persons with MS (PwMS) (4-6). A As indicated by Marrie et al. (6) PwMS reporting one or more vascular comorbidity

like diabetes, hypertension, heart disease, hypercholesterolemia, and peripheral vascular disease at diagnosis of MS, had a more than 1.5-fold increased risk of ambulatory disability. In literature, different indicators for cardiovascular risk have been studied in MS, such as atherosclerosis, arteriosclerosis and arterial stiffness. Atherosclerosis is one of the mechanism responsible for multiple cardiovascular events (7). Detection of subclinical atherosclerosis. and arteriosclerosis is possible through the evaluation of arterial stiffness, intima-media thickness and endothelial dysfunction, which can be measured using noninvasive and inexpensive techniques (8, 9). Next to that, arterial stiffness, a term that refers to the loss of arterial compliance and/or changes in vessel wall properties (10). Literature indicates this stiffness to be higher in PwMS compared to Healthy controls, and is identified as an early marker for clinical hypertension (11, 12).

Literature suggests an increased prevalence of vascular comorbidities in PwMS compared to a healthy population. According to current research available, the increased risk in PwMS is due to a combination of factors, including genetic overlap, inflammation and oxidative stress, side-effects of certain medication used to treat MS symptoms (such as glucocorticoids and antispastic/anticonvulsant drugs) and physical inactivity (4, 5, 13-15). In other clinical populations such as chronic obstructive pulmonary disease, schizophrenia and depression (16-20), physical inactivity has also been documented as an important risk factor for the development of vascular comorbidities (6). This indicates that, besides (epi)genetics, environmental, hormonal and medical risk factors in MS, lifestyle is a crucial determinant for the development of cardiovascular risk factors. Importantly, this also applies for a healthy population (21). Arterial stiffness is lower among individuals who regularly perform continued aerobic exercise, and an inverse association between physical activity and arterial stiffness has been observed (2, 22, 23).

Moreover, there is contrasting evidence on the increased CVD risk in PwMS in systematic reviews and prevalence studies (24, 25). This can be explained by differences in demographics (differences in medication intake for example) and clinical characteristics (hospital-based versus population-based studies, different cut-off values for CVD risk, etc.) between studies, but most of the studies on CVD risk factors in PwMS, also fail to account for PA and sedentary behaviour (SB). More specifically, the analysis of activity profiles indicates that PwMS are more sedentary and less active in the moderate-to-vigorous physical activity (MVPA) intensity domain than age-matched healthy controls (HC) (26). MVPA is typically defined as activity that requires a moderate amount of effort and causes a noticeable increase in heart rate and breathing, with at least 3 metabolic equivalents (METs), with 1 MET being the energy expenditure at rest (27). Examples of MVPA are cycling, brisk walking or running (28). SB is defined as any waking behaviour characterized by an energy expenditure of ≤1.5 METs while in a sitting, reclining or lying posture (29). Current international physical activity (PA) guidelines of 150 min of MVPA, or 75 min of vigorous activity, are not met by 23 % of the general population worldwide (30). Clinical populations display even higher percentages of inactivity due to disease-related barriers (31, 32). SB has been inversely associated with cardiovascular risk maskers such as high blood pressure, which has been associated with a variety of comorbidities (33), especially in physically inactive populations (34, 35). Greater amounts of sedentary time have been shown to be associated with an increased likelihood of developing cardiovascular disease. Furthermore, research indicates that greater sedentary time is related to indicators of physical activity. For example the percentage of the day spent in inactivity being negatively correlated with the average daily step count and average number of minutes being active (36, 37) The symptoms displayed by PwMS (e.g. spasticity, paralysis, walking difficulties) could explain the increased sedentary behaviour (SB) and decreased physical activity (PA) (38-40). The question then arises whether the CVD risk still differs when PwMS and HC are equally active/sedentary. Ranadive *et al.* (12) indicated worse vascular function in MS population *VS*. a healthy population. However, after statistical adjustment for physical activity, differences between both groups receded. Furthermore, Hubbard *et al.* found that SB was

significantly correlated with BP outcomes, independent of BMI (41, 42).

However, in the study of Ranadive *et al*. (12) groups were not matched on physical activity. The conclusion that the worse vascular function was due to differences is physical activity, was only based on a statistical correction. Furthermore, this study had a rather low sample size, limiting the power. Next to that, in the study of Hubbard et al. (41), subjective measurements were used to analyze SB, which could bias the results (43). Therefore, in the present study we aim to examine the association between objectively measured PA and SB and vascular function in PwMS and PA-matched sedentary healthy controls and compare vascular health between these groups. We hypothesize that in a PA-matched sedentary population of healthy controls and PwMS, sedentary behaviour is associated with cardiovascular health and cardiovascular risk is comparable between PwMS and healthy controls. When the vascular function is strongly correlated with PA in PwMS and similar to that of PA-matched HC, this would emphasize the importance of lifestyle interventions for PwMS to increase PA and reduce SB in a clinical setting.

EXPERIMENTAL PROCEDURES

Study design – cross-sectional study design was set up for this project (Figure s1 in the Appendix). Participants were invited for 3 study visits. During the first visit, eligibility of participants was checked based on participant demographics and a PA-assessment of 7 consecutive days after the first visit. Based on this, participants were categorized as active or inactive according to the WHO guidelines (> or ≤ 150 min MVPA/week respectively)(28). Eligible participants were invited for the second study visit. Here, an electrocardiogram was captured for approval by a cardiologist to perform a maximal cardiopulmonary exercise test (CPET) during the third study visit. Furthermore, the activity monitor was attached again for 7-14 consecutive days until the third study visit, where vascular function was assessed (cfr. '*Measures'* below).

Participants – Participants included were PwMS between the age of 25 and 60 years with the relapse-remitting phenotype, with a Kurtzke expanded disability status scale (EDSS) of less than 5. Sample size was calculated based on the difference in the augmentation index corrected for heart rate (AIx@75bpm) in a previous study where participants were not PA-matched (26,5 and 19,28 for PwMS and HC respectively (15)). Twenty-eight PwMS and 28 HC are needed to detect a similar 7,22 AIx@75bpm difference with 80% power, taking into account a drop-out rate of 10%. PwMS were matched with HC based on age, sex, body mass index (BMI), and PA levels. All participants were required to have >9 h SB/day, as it is associated with increased risk of developing CVD (44). Participants were excluded if they experienced an acute exacerbation within 6 months before the start of the study (in case of MS), had experimental drug use or medication changes in the last month, medical conditions precluding PA participation, alcohol abuse (>20 units/week), reported dietary habits or weight loss (>2kg) in the last month before the study, intention to start a new specific diet or start to follow an exercise intervention, blood donation in the past month, or a diagnosis of cardiometabolic diseases such as diabetes mellitus or heart and vascular diseases.

Primary outcome measures

Physical activity levels – SB and PA were quantified using the activPAL3^{tm} activity monitor (PAL Technologies Ltd, Glasgow, UK) attached to the anterior mid-thigh of the participant's dominant leg. PA included standing time, light intensity physical activity (LIPA), and moderate-to-vigorous physical activity (MVPA). Measures for SB included seated transport, secondary lying time, total sedentary time (in % of waking time per day [WT/day]) and sedentary time in bouts (5-60min; reported in hours). With uninterrupted SB referring to sedentary bouts of >60min in duration. In addition, sleeping and exercise times were self-reported by participants using paper diaries, and sleeping time was manually adjusted in the activPAL sleep algorithm as suggested by previous literature (45). The exercise diary included time, duration, exercise time, level of perceived exertion and average heart rate if measured.

Vascular function

Arterial stiffness - After an initial resting period of 10min with participants in a supine position in a quiet room with constant temperature (19-21°C), blood pressure (BP) and resting heart rate (HRrest) were measured until stable peripheral pressure was achieved by 3 similar measurements (SBP ± 1/DBP ± 1) intervals using an electronic sphygmomanometer (Omron®, Omron Healthcare, IL, USA) from the left arm and documented as the mean value of the final 3 measurements.

Arterial stiffness was determined by pulse wave analysis (PWA) and pulse wave velocity (PWV) using applanation tonometry (SphygmoCor v9; Atcor Medical, Sydney, Australia). Talking or sleeping was not allowed during the examination. Both vascular measurements measure pressure pulses by mean of mechanotransducers, which were applied to the skin at the left side of the body, blood pressure was the first measured using sphygmomanometer before measuring Mean arterial pressure (MAP) in the brachialis artery using the formula MAP = systolic BP + (2 x diastolic BP) / 3. The MAP allows for the algorithm to calculate different components of the pressure waveforms, including central (aortic) blood pressure, and absolute and heartrate-corrected augmentation index (AIx and AIx@75bpm). For PWV measurements, a probe was positioned at the site of the common carotid artery and the femoral artery site. cfPWV was determined according to recent guidelines (46). Pressure waveforms were determined at the right common carotid and right

common femoral arteries. Difference in the time of pulse arrival from the R-wave of the ECG between the 2 sites (transit time) was determined with the intersecting tangents algorithm. The pulse wave travel distance was calculated as 80% of the direct straight distance between the 2 arterial sites. PWA and PWV were performed in triplicate, calculating the average value as definite outcome.

Endothelial function - After the arterial stiffness measurements, endothelial function was assessed by non-invasive peripheral arterial tonometry using the EndoPATTM 2000 device (Itamar Medical Ltd, Caesarea, Israel), according to manufacturer's instructions. After the 5-min baseline period, a blood pressure cuff on the left lower arm was inflated to at least 60mmHg above baseline systolic blood pressure (minimally 200mmHg, maximal 300mmHg) for 5min. Occlusion of the pulsatile arterial flow, causing transitory arm ischemia, was confirmed by the reduction of the peripheral arterial tone (PAT) signal to zero. Upon cuff deflation changes in PAT signal was recorded in response to reactive hyperaemia, reflected by the reactive hyperaemia index (RHI) and calculated as the ratio of the average PAT signal in the post hyperemic phase to the baseline PAT signal in the occluded arm.

Secondary outcome measures

Heart rate variability (HRV) - Simultaneous with arterial stiffness, HRV was assessed. HRV is an important indicator of autonomic nervous system function and might be impaired in PwMS. Continuous beat-to-beat heart rate signal measurements were obtained for the duration of 20 minutes using the Polar V800 heart rate monitor (Polar Electro, Kempele, Finland) in combination with a Polar H10 chest strap heart rate sensor. Time domain analysis and frequency domain analysis were performed using the Kubios free HRV software (ver. 3.5) (KUBIOS OY, Kuopio, Finland) giving parameters that reflect autonomic system dynamics (autonomic balance) including parasympathetic nervous system activity and baroreflex activity (47).

Body composition and anthropometrics – body height was measured using a wall-mounted Harpenden stadiometer, with patients barefoot. Body weight (in underwear) was determined using a digital-balanced weighing scale to the nearest 0.1 kg. BMI was calculated from weight and height measurements (weight/height^2). Waist and hip circumference were measured to the nearest 0.1 kg using a flexible metric measuring tape with participants barefoot (in underwear) in standing position. Waist circumference was measured at the midpoint between the lower rib margin and the top of the iliac crest. Hip circumference was measured at the widest circumference of the hip at the level of the greater trochanter. Waist-to-hip ratio was calculated by dividing waist circumference (cm) by hip circumference (cm). Whole body fat, lean tissue mass and bone mineral density were evaluated using Dual Energy X-ray Absorptiometry (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium).

Cardiorespiratory fitness (CRF) – CRF was measured by peak oxygen consumption (VO_{2peak}) using a graded cardiopulmonary exercise test on a bicycle ergometer. VO2peak was measured using a gas exchange analyzer (Jaeger Oxycon®) until volitional exhaustion or failure to maintain a cadence above 60 repetitions per minute. Heart rate was measured with a Polar chest strap (Polar®, Oy, Finland). After 5 min of warming up with a light load (♂: 60 W, ♀: 40 W), participants performed a CPET based on a MS cycle protocol (48). Based on PA levels, participants either performed an inactive version of the protocol or the active protocol: (♂: 60 W + 15 W/min ♀: 40 W + 10 W/min) and (♂: 80 W + 15 W/min ♀: 60 W + 10 W/min) respectively. As described by Langeskov et al. (49), RER, rate of perceived exertion (RPE), maximal heart rate (HR) and blood lactate levels were evaluated to maximal effort (satisfaction of 2/4 criteria: RER >1.15 at the time of VO2peak; RPE ≥ 17; maximal HR ≥ predicted - 10 beats per minute (HRmax=208- 0.7*age) (50); post-lactate level > 8.0mmol/L).

Questionnaires – Participants were asked to fill in several questionnaires regarding stress, quality of life and physical ability. These were measured by Perceived Stress Scale (PSS), RAND-36, MS walking scale, and modified fatigue impact scale (MFIS) respectively.

Statistical analysis – *Statistical* analysis was performed with SPSS IBM SPSS® version 28.0.1.1 (IBM SPSS Statistics for Mac, Chicago, IL, USA). Data was be expressed as mean ± SD. A Shapiro-Wilk test was used to test normality of the data ($p<0.05$). Equal variance was analyzed using Levene's test. In case of normal distribution, ANOVA was used to compare means between both active and inactive PwMS, as well as HC and PwMS groups. Chi-square was used for categorical variables. Bonferroni post hoc was used for multiple Comparison. bivariate correlation was used to assess the correlates between PA, SB and vascular function. Pearson partial correlation was used to adjust for covariates. In case data was not normally distributed, Welch test was used instead of ANOVA and non-parametric spearman correlation was used.

Table 1: Participant demographics.

		Multiple Sclerosis		Healthy controls
Characteristics		Inactive (n=6)	Active (n=14)	Active (n=5)
Age (y)		36.8 ± 8.1	37.6 ± 6.9	36.4 ± 9.1
Gender (female, %)		5 (83.3%)	13 (92.9%)	5 (100%)
EDSS		2.8 ±1.2	1.7 ±0.8 *	/
MS duration (y)		7.7 ± 4.6	6.2 ± 4.5	/
Body fat (%)		37.1 ± 7.9	31.4 ± 9.7	36.2 ± 5.4
BMI (kg/m^2)		27.2 ± 3.1	25.5 ± 4.5	26.4 ± 5.7
Waist-to-hip ratio		0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Vo_2peak (ml/min/kg)		25.7 ± 6.3	33.5 ± 10.0	31.6 ± 3.8
MVPA (expressed in min/week)		64.2 ± 44.4	346.4 ± 141.4 **	386.7 ± 230.3 **
MVPA (expressed in % WT)		0.9 ± 0.6	5.1 ± 2.1 **	5.7 ± 3.3 **
Sedentary behaviour (expressed in hours/day)		12.4 ± 0.8	10.4 ± 1.6 *	10.4 ± 0.7°
Sedentary behaviour (expressed in % WT)		73.9 ± 5.8	64.1 ± 8.2 *	64.6 ± 3.4
LIPA (expressed in % WT)		5.99 ± 2.6	7.8 ± 2.0	8.5 ± 2.5
Smoking (smokers, %)		0	1 (7.1%)	0
MS Medication (%)	No medication	2 (33.3)	7 (50.0)	/
	First-line	0	2 (14.3)	
	Second-line	4 (66.7)	5 (35.7)	
Blood/heart medication (%)		1 (16.7)	0	0
Lipid medication (%)		1 (16.7)	0	0
Blood+lipid medication (%)		1 (16.7)	1 (7.1)	0
Thryoid medication (%)		1 (16.7)	0	0

Data expressed as mean (± SD). *denotes $p < 0.05$ vs. inactive PwMS, ** denotes $p < 0.01$ vs. inactive PwMS. ° denotes $p = 0.083$ vs. inactive PwMS.

EDSS; expanded disability status scale, MVPA; moderate-to-vigorous physical activity, LIPA; light intensity physical activity.

RESULTS

Participant demographics – In total 25 participants completed the study and were included. A summary is presented in Table 1, 20 PwMS were included. The MS group had an average age of 37.4 ± 7.1. The group consisted of 18 females (90 %) with a BMI of 26.0 ± 4.1. The average MS duration was 6.7 ± 4.5 years. The active PwMS (≥ 150min of MVPA/Week) consisted of 14 persons, the inactive group consisted of 6 persons. No demographical differences were noted between the active and inactive PwMS, except for EDDS (2.8 ± 1.2 VS. 1.7 ± 0.8, p = 0.034), physical activity expressed as both total time (min/week) and % of waking time (% of WT) in the MVPA intensity (p = 0.002, p = 0.002 respectively) and SB expressed as total time (hour/day) and % of WT (p = 0.022, p = 0.027 respectively). Healthy controls (HC's) consisted of 5 active persons, with an average age of 36.4 ± 9.1. Active HC's were significantly more active in the MVPA category regarding total MVPA and MVPA in % of WT compared to inactive PwMS (p = 0.005 and P = 0.004 respectively). Values of total sedentary time between active HC and inactive PwMS tended to be different, although not significant (p = 0.083).

Differences in vascular parameters between groups – To test whether PA had a significant impact on the vascular parameters in groups of sedentary PwMS and HC's, ANOVA was used to assess the differences in primary outcomes (AIX@75, cfPWV, RHI) and MAP, BP and aortic BP between the active PwMS, inactive PwMS and active HC's. No significant differences between the active and inactive PwMS were found (Table 2). Values of PwMS and active HC also remained comparable.

Correlation analysis – Although mean values might not differ between groups, there could still be an association of the amount of PA or SB with the primary outcomes. To assess what parameters may have influenced primary outcomes related to CVD risk, correlation analysis were performed. Initial bivariate correlations (Table 3) indicated that LIPA was significantly correlated with RHI (r = -0.477, p = 0.025), SB and MVPA were not correlated with RHI. SB was not correlated with any of the primary outcomes. MVPA tended to correlate with cfPWV (p = 0.084), yet this was accounted for by age and body fat % (r = -0.210, p = 0.419) (Table 4). Correlations of MVPA and SB with cfPWV are plotted in Figure 1A and B respectively. Partial correlations adjusted for age and body fat % do show LIPA and RHI to remain significantly correlated (r = - 0.518, p = 0.033). In contrast to initial bivariate correlation, SB was significantly correlated with cfPWV as shown in Table 4 (r = 0.482, p = 0.050). Next to that, SB showed significant negative correlations with brachial (peripheral) (r = -0.419, p = 0.037) and aortic SBP (-0.527, p = 0.007) (Table s2).

Table 2: Primary outcomes of vascular function. Comparison between the three groups active PwMS, inactive PwMS and active HC's.

	Multiple Sclerosis		Healthy controls
Outcomes	Inactive (n=6)	Active (n=13)	Active (n=5)
SBP (mmHg)	106.5 ± 6.0	113.5 ± 11.7	114.0 ± 10.6
DBP (mmHg)	66.2 ± 5.7	69.1 ± 10.5	75.0 ± 11.4
MAP (mmHg)	83.0 ± 4.9	86.6 ± 11.4	91.4 ± 12.6
Aortic SBP (mmHg)	97.5 ± 15.1	106.6 ± 13.3	111.5 ± 15.0
Aortic DBP (mmHg)	67.1 ± 5.7	69.9 ± 10.8	75.7 ± 11.6
AIx@75 (%)	14.6 ± 10.0	14.8 ± 10.3	12.3 ± 11.7
cfPWV (m/s)	6.7 ± 1.1	6.1 ± 0.6	6.2 ± 0.6
RHI	2.7 ± 0.6	2.4 ± 0.3	2.4 ± 0.4

Data expressed as mean ± SD. Parameters of BP, and vascular function compared between active and inactive PwmS, as well as PwMS – HC's. SBP: systolic blood pressure, DBP; diastolic blood pressure, AIx@75; augmentation index HR75, cfPWV; carotid femoral Pulse Wave Velocity, RHI; reactive hyperemia index, MAP; mean arterial pressure.

Similarly, LIPA was correlated with systolic blood pressure in both brachial (r = 0.438, p = 0.028) and aortic measures (r = 0.491, p = 0.013). After adjustment for age and body fat %, SB remained correlated with aortic, but not brachial systolic blood pressure (r = -0.478, p = 0.021) (Table 5). LIPA was not significantly correlated with any measurement of BP after adjustment for age and body fat %.

Table 3: Correlation analysis of primary outcomes with SB and PA.

		LIPA (expressed in % of WT)	MVPA (expressed in % of WT)	SB (expressed in % of WT)
AIx@75bpm (%)	Pearson Correlation	-0.078	-0.246	-0.051
cfPWV (m/s)	Pearson Correlation	0.053	-0.368°	0.289
RHI	Pearson Correlation	-0.477*	0.049	0.150

* denotes p < 0.05, ° denotes p = 0.084

AIx@75bpm; Augmentation index corrected to 75 bpm heart rate, cfPWV; carotid–femoral pulse wave velocity, RHI; reactive hyperemia index, LIPA; light intensity physical activity, MVPA, moderate-to-vigorous intensity physical activity, SB; Sedentary behaviour.

MVPA is correlated with secondary outcomes such as parameters of heart rate variability, anthropometrics, and cardiorespiratory fitness – After correlation analysis of primary outcomes, bivariate correlations were also performed on secondary outcomes to analyze possible indirect effects of PA and SB on cardiovascular health (Table s1). These show MVPA to be significantly correlated with parameters of HRV (i.e. Root mean square of successive differences in heart beats (RMSSD)). MVPA was significantly correlated with RMSSD at p = 0.005. Additionally, MVPA was also significantly correlated with waist circumference (- 0.541, p = 0.008), waist-to-hip ratio (-0.485, p = 0.014), fat percentage (-0.402, p = 0.046) and VO_2peak (0.592, p = 0.004). finally, EDSS and MS duration were analyzed with regard to CDV risk parameters. Table s3 shows correlation analysis between EDSS and MS duration, and primary outcomes AIx@75bpm, cfPWV and RHI. No correlations were seen except for AIx@75bpm and MS duration (r = 0.483, p = 0.042).

Table 4: Partial Correlation of primary outcomes with SB and PA, adjusted for covariates age & body fat %.

Control Variables			LIPA (expressed in % of WT)	MVPA (expressed in % of WT)	SB (expressed in % of WT)
Age (y) & Body fat (%)	AIx@75bpm (%)	Correlation	-0.316	-0.377	0.408
	cfPWV (m/s)	Correlation	-0.225	-0.210	0.482*
	RHI	Correlation	-0.518*	-0.082	0.184

*. denotes p < 0.05

AIx@75bpm; Augmentation index corrected to 75 bpm heart rate, cfPWV; carotid–femoral pulse wave velocity, RHI; reactive hyperemia index, LIPA; light intensity physical activity, MVPA, moderate-to-vigorous intensity physical activity, SB; Sedentary behaviour.

DISCUSSION

This study was performed to try and provide clarity on the relation between SB, PA and vascular function of persons with MS. Literature remains contradictory when it comes to what influences vascular function in PwMS, and whether PwMS have an increased risk of developing CVD independent of activity levels and time spent sedentary (51). Our novel findings suggest that independent of activity levels, percentage of waking time spent sedentary is associated with CVD risk factor (i.e.) cfPWV. Due to low post hoc power, more studies are required to verify this finding.

noting cfPWV values up to 7.7 ± 2.0. For AIx@75, this difference was even more noticeable, 14,8 % noted in the highest group (active PwMS) vs. $22,81 \pm 2,01$ and $26,5 \pm 10$ noted by Ranadive et al. and Boshra et al.(53), with an average age of $47 \pm 1,83$ and $32 \pm 8,47$ respectively. However, it important to mention that the papers of both Ranadive and Boshra included a higher number of participants (n=33 and n=50 respectively) compared to the 20 PwMS included in the current study. With reference values for the age category ≤ 39 being 23,4 in a normal population (54). However, the primary reason for the absence of significant results might be due to the low value

Table 5: SB and PA and blood pressure outcomes after adjustment for age and body fat %.

Control Variables			SB (expressed in % of WT)	MVPA (expressed in % of WT)	LIPA (expressed in % of WT)
Age (y) & body fat (%)	SBP (mmHg)	Correlation	-0.303	0.192	0.318
	DBP (mmHg)	Correlation	-0.039	0.103	0.084
	MAP (mmHg)	Correlation	-0.160	0.141	0.194
	Aortic SBP (mmHg)	Correlation	-0.478*	0.308	0.380
	Aortic DBP (mmHg)	Correlation	-0.002	0.076	0.068

*. denotes $p < 0.05$,

SB; sedentary behaviour, MVPA; moderate-to-vigorous physical activity, LIPA; light intensity physical activity, (aortic) SBP; (aortic) systolic blood pressure, (aortic) DBP; (aortic) diastolic blood pressure, MAP; mean arterial pressure

Physical activity did not significantly alter primary outcomes – While there are no significant differences in primary outcomes in sedentary HC vs. PwMS, which agrees with the hypothesis that in matched participants, there are no differences concerning CVD risk between HC's and PwMS, we also failed to demonstrate significant differences between active and inactive sedentary PwMS. There might be several reasons for the absence of changes in primary outcomes. We measured values well below what had been noted in literature. The highest cfPWV, measured in the inactive MS group, was 6.7 m/s at an average age of 36.8. Reference values for a normal population in that age range (30-39) is 6.32 (52). Literature in PwMS typically measures values above that, Ranadive et al. noted a cfPWV of 7.06 ± 0.25 (12), with others of post hoc power analysis of 0.12. Which is caused by lack of time to fulfill sample size requirements of 28 per group (HC VS. MS). Another explanation may reside in the activity patterns of participants, we noted highly active participants in the active category, with some having upwards of 600min of MVPA/week. Yet most of the inactive participants were only slightly below the cut off value of 150min of MVPA/week. This could have skewed into a more active median, indicating also the inactive participants were relatively active. With almost none below 100min of MVPA/week. In addition to this, activpal data was not adjusted for daily heart rate. It was noticed certain activities are not measured as being active by the activPAL3^{tm} monitor (e.g. kayaking being identified as seated transport).

Vascular risk factors are correlated with SB, and LIPA – Our initial bivariate correlation provided significant results between LIPA and RHI. Following our hypothesis, it was expected both SB and MVPA would correlate with either AIx@75bpm, cfPWV or RHI. Noticeable is that, according to the correlation analysis, higher levels of LIPA would decrease RHI. It is generally known that exercise, be it LIPA or other intensities, would increase vascular endothelial function, and therefore RHI (55-57). It is surprising that the correlation remained significant after adjustment for fat % and age. Literature regarding RHI previously suggested an association between RHI and body fat % (58). Since we show associations between body fat % and MVPA, the reason our study had results indicating a trend of decreased RHI might be because persons with a higher

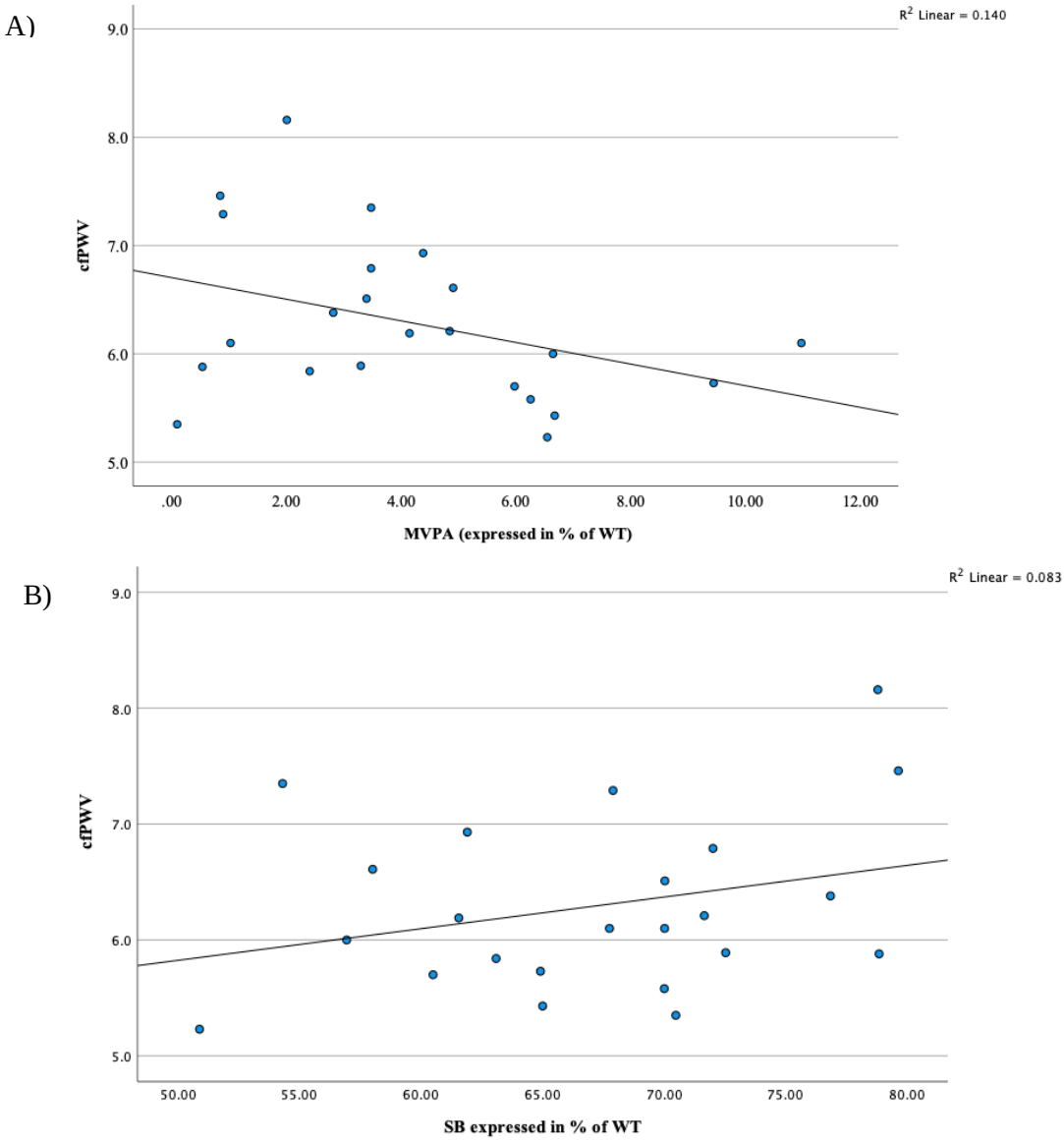

Figure 1 – A) Correlation between MVPA expressed as a percentage of waking time and primary outcome cfPWV. Graph shows a trend (p = 0.084) of decreased cfPWV when MVPA (% of WT) increases. B) Correlation between SB expressed as a percentage of waking and cfPWV (p = 0.183).

MVPA; moderate-to-vigorous physical activity, SB; sedentary behaviour, cfPWV; carotid-femoral pulse wave velocity. Correlation was performed using Pearson parametric correlation.

amount of time spent in LIPA, are therefore less active in the MVPA intensity, which could lead to those persons having a lower RHI value compared to the ones that are more active in the MVPA intensity. Following that logic, the reason MVPA did not affect RHI might be due to the low statistical power. A final reason resides in the fact RHI calculations by use of EndoPAT-2000 cannot provide any algorithm or statistic measures used to calculate RHI, but only provides a final value. This has previously also been indicated as an issue, as literature stated the EndoPAT software uses a fixed time frame during the hyperemic response to calculate the RHI, yet the maximal hyperemic response does not always occur in the same time period after occlusion (59).

Partial correlation of SB showed a significant correlation with cfPWV, which agrees our hypothesis that in all activity levels, SB has a significant influence on CVD risk parameters. This correlation was independent of MVPA, as additional adjustment for MVPA was performed. SB, although not significant, still tended to correlate with cfPWV at $p = 0.071$ (Table s4). Yet correlations might have lost significance due to a loss in power which is seen after correcting for covariates (60).

We demonstrate an inverse association between SB and aortic SBP. This is in direct contrast to general findings in literature, which indicate a positive correlation between SB and BP (41). Similar to the ANOVA, correlation analysis might suffer from low power. Additionally, values of BP seen in the inactive PwMS are well below 'normal' values of 120/80. Indicating the low number of participants in this group might have skewed result into less representative values for general inactive persons. Contrary, previous findings did not use an objective measure to assess SB, but assessed SB using self-reported sitting times by use of the International Physical Activity Questionnaire (IPAQ) (61). Additionally, meta–analysis show self-reported time spent in sedentary behaviors to be significantly associated with both SBP and DBP, whereas accelerometer-assessed time spent in sedentary behaviors failed to show an association (62). Current findings also failed to provide significant correlations between PA, either LIPA or MVPA, and BP. This is also contradictory to known literature that states endurance, dynamic resistance, and isometric resistance training lower SBP and DBP (63)

MVPA is correlated with secondary outcomes such as HRV, VO_2peak, fat percentage and WHR – Results show significant correlations with several secondary outcomes, including HRV parameter RMSSD, It is known HRV is improved upon frequent exercise in the general population(64, 65). Nonetheless in PwMS, no differences between HRV in PwMS VS. HC's have been previously found (66). Therefore, this might give new insight into the effect of exercise on HRV, which seems to elucidate similar effects in PwMS as it does in HC's. Furthermore, MVPA increased VO_2peak significantly. Cardiorespiratory fitness has previously been associated with disease severity (67). Herein VO_2peak was associated with EDSS score, adjusting for aging and differences between men and women. Suggesting disease progression might be exaggerated by a reduction in physical activity or sedentary lifestyle, thereby suggesting exercise therapy as a disease-modifying treatment. Which was partly supported by Motl et al. (68) who suggested a potential preventative function, in terms of disease progression, of PA in PwMS. Combining this with the significant correlation of MVPA and HRV, clinical relevance remains for PwMS to engage more in PA, as it could indirectly effect disease progression. However conclusive evidence on this matter cannot be provided, as this is a cross-sectional design and therefore progression over time related to PA or SB was not measured. Furthermore, low EDSS values due to the inclusion criteria of being ambulatory (EDSS<5) prevents us to generalize the possible preventive function of PA.

In contrast to previous studies, PWV was not correlated with MS duration ($r = 0.124$, $p = 0.623$) nor EDSS ($r = 0.120$, $p = 0.657$) (53). This agrees with our hypothesis that MS itself does not lead to increased CVD risk compared to HC's, but rather the decreased PA and sedentary lifestyle concomitant with MS. Literature in well-matched PwMS and HC's agrees with these findings, stating impaired vascular function, elevated inflammation and oxidative stress are not an obligatory accompaniment to MS (69).

Strengths – To our knowledge, this is the first study to analyze the effects of SB on CVD risk in objectively measured PA-matched populations of PwMS and HC's. The current study shows that, independent of MVPA, SB is correlated with risk factors for CVD (i.e. cfPWV).

Limitations – Our study came with several limitations. The first being the inability to reach sample size in the provided time. Group size of 28 PwMS and 28 HC's was not achieved, final analysis of results was performed with 20 PwMS and 5 HC's. Of the 20 PwMS, 14 were considered active, and 6 were considered inactive. This inequality can be the reason mean values were not significantly different between active and inactive PwMS. Next to that the activity levels were based purely on activPAL3® data, and individual heart rate has not been considered. This generates possible inaccuracies of the activity tracking, being that the activPAL3® monitor does not take into account intensive movements that do not require leg movement (e.g. upper body strength training, arm training or seated rowing exercises). However, activPAL3® monitor is still considered the gold standard for measuring SB and PA in free-living conditions (70). Next to that, we did not consider different distributions of PA and SB. For example, the differences between persons who do regular weekly exercises, VS. persons who combine all their activity into 1 or 2 days per week (so-called "weekend warriors"). Although no differences concerning CVD risk have been previously found between week warriors and regular exercisers, effects on secondary outcomes had not been previously investigated (71). Furthermore, all our study participants had the relapsing-remitting phenotype of MS and they only had mild to moderate disability, limiting generalizability of these findings to other phenotypes and non-ambulatory PwMS. Additionally, the HC group was small compared to the MS group, and we were unsuccessful to include inactive HC's. This led to another limitation, being the inability to use two-way ANOVA to examine the possible interaction between both PA and disease status, this was not possible due to lack of inactive HC participants. Hence, two-way ANOVA was replaced with one-way ANOVA followed by Bonferroni post hoc.

Future perspective – additional studies should be conducted to analyze CVD risk in PwMS while taking PA and SB into account. This study functions as an indication CVD risk in PwMS might be primarily affected by activity levels and sedentary lifestyle which often accompanies the MS disease course. Longitudinal studies could provide added deeper insight as to what degree PA and prevention of a sedentary lifestyle act as preventive therapy, and what the minimal amount of PA and maximal amount of SB would be to still gain from the benefits, while being achievable in a mobility disability population like MS populations. Furthermore, the effects of resistance training remains inconclusive in PwMS, as in the current study, resistance training was not accounted for.

CONCLUSION

The current study presented some data which supports the hypothesis that in PA-matched sedentary population of healthy controls and PwMS, sedentary behaviour is associated with cardiovascular health and cardiovascular risk is comparable between PwMS and healthy controls. This was supported by the significant correlation of SB with CVD risk factor cfPWV. In addition, MVPA is correlated to several secondary outcomes that may indirectly also impact CVD risk (e.g. VO_2peak, body fat %, and RMSSD). unfortunately, strong conclusive evidence cannot be provided as post hoc analysis yielded low statistical power (0.12). Nonetheless, current results hint at the relevance of implementing physical activity and sedentary lifestyle tracking in persons who suffer from MS.

REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med. 2000;343(13):938-52.
2. Tanaka H, DeSouza CA, Seals DR. Absence of age-related increase in central arterial stiffness in physically active women. Arteriosclerosis, thrombosis, and vascular biology. 1998;18(1):127-32.
3. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000;47(6):707-17.
4. Oliveira SR, Simão AN, Kallaur AP, de Almeida ER, Morimoto HK, Lopes J, et al. Disability in patients with multiple sclerosis: influence of insulin resistance, adiposity, and oxidative stress. Nutrition. 2014;30(3):268-73.
5. Weinstock-Guttman B, Zivadinov R, Mahfooz N, Carl E, Drake A, Schneider J, et al. Serum lipid profiles are associated with disability and MRI outcomes in multiple sclerosis. J Neuroinflammation. 2011;8:127.
6. Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. Neurology. 2010;74(13):1041-7.
7. Christiansen CF, Christensen S, Farkas DK, Miret M, Sørensen HT, Pedersen L. Risk of Arterial Cardiovascular Diseases in Patients with Multiple Sclerosis: A Population-Based Cohort Study. Neuroepidemiology. 2010;35(4):267-74.
8. Horta BL, Schaan BD, Bielemann RM, Vianna CÁ, Gigante DP, Barros FC, et al. Objectively measured physical activity and sedentary-time are associated with arterial stiffness in Brazilian young adults. Atherosclerosis. 2015;243(1):148-54.
9. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European heart journal. 2006;27(21):2588-605.
10. Laurent S, Boutouyrie P. Arterial Stiffness and Hypertension in the Elderly. Front Cardiovasc Med. 2020;7:544302.
11. Safar ME. Arterial stiffness as a risk factor for clinical hypertension. Nature Reviews Cardiology. 2018;15(2):97-105.
12. Ranadive SM, Yan H, Weikert M, Lane AD, Linden MA, Baynard T, et al. Vascular dysfunction and physical activity in multiple sclerosis. Med Sci Sports Exerc. 2012;44(2):238-43.
13. Wang Y, Bos SD, Harbo HF, Thompson WK, Schork AJ, Bettella F, et al. Genetic overlap between multiple sclerosis and several cardiovascular disease risk factors. Mult Scler. 2016;22(14):1783-93.
14. Motl RW, Fernhall B, McAuley E, Cutter G. Physical activity and self-reported cardiovascular comorbidities in persons with multiple sclerosis: evidence from a cross-sectional analysis. Neuroepidemiology. 2011;36(3):183-91.
15. Keményová P, Siarnik P, Sutovský S, Blaho A, Turcáni P, Kollár B. Impairment of endothelial function in patients with multiple sclerosis. Neuro Endocrinol Lett. 2015;36(1):67-71.
16. Garcia-Rio F, Rojo B, Casitas R, Lores V, Madero R, Romero D, et al. Prognostic value of the objective measurement of daily physical activity in patients with COPD. Chest. 2012;142(2):338-46.

17. Kandola A, Ashdown-Franks G, Hendrikse J, Sabiston CM, Stubbs B. Physical activity and depression: Towards understanding the antidepressant mechanisms of physical activity. Neurosci Biobehav Rev. 2019;107:525-39.
18. Keytsman C, Eijnde BO, Hansen D, Verboven K, Wens I. Elevated cardiovascular risk factors in multiple sclerosis. Multiple Sclerosis and Related Disorders. 2017;17:220-3.
19. Sin DD, Man SFP. Chronic obstructive pulmonary disease: a novel risk factor for cardiovascular disease. Canadian Journal of Physiology and Pharmacology. 2005;83(1):8-13.
20. Yogaratnam J, Biswas N, Vadivel R, Jacob R. Metabolic complications of schizophrenia and antipsychotic medications-an updated review. East Asian Archives of Psychiatry. 2013;23(1):21-8.
21. Gakidou E, Afshin A, Abajobir AA, Abate KH, Abbafati C, Abbas KM, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet. 2017;390(10100):1345-422.
22. Gomez-Marcos MA, Recio-Rodríguez JI, Patino-Alonso MC, Agudo-Conde C, Lasaosa-Medina L, Rodriguez-Sanchez E, et al. Relationship between objectively measured physical activity and vascular structure and function in adults. Atherosclerosis. 2014;234(2):366-72.
23. Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. Circulation. 2000;102(11):1270-5.
24. Tettey P, Simpson S, Taylor BV, van der Mei IAF. Vascular comorbidities in the onset and progression of multiple sclerosis. Journal of the Neurological Sciences. 2014;347(1):23-33.
25. Wens I, Dalgas U, Stenager E, Eijnde BO. Risk factors related to cardiovascular diseases and the metabolic syndrome in multiple sclerosis–a systematic review. Multiple Sclerosis Journal. 2013;19(12):1556-64.
26. Macdonald E, Buchan D, Cerexhe L, Renfrew L, Sculthorpe N. Accelerometer measured physical activity and sedentary time in individuals with multiple sclerosis versus age matched controls: A systematic review and meta-analysis. Multiple Sclerosis and Related Disorders. 2023;69:104462.
27. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc. 2007;39(8):1423-34.
28. WHO Guidelines Approved by the Guidelines Review Committee. Global Recommendations on Physical Activity for Health. Geneva: World Health Organization
Copyright © World Health Organization 2010.; 2010.
29. Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, et al. Sedentary Behavior Research Network (SBRN) – Terminology Consensus Project process and outcome. International Journal of Behavioral Nutrition and Physical Activity. 2017;14(1):75.
30. Organization WH. Global action plan on physical activity 2018-2030: more active people for a healthier world: World Health Organization; 2019.
31. Asano M, Duquette P, Andersen R, Lapierre Y, Mayo NE. Exercise barriers and preferences among women and men with multiple sclerosis. Disability and rehabilitation. 2013;35(5):353-61.
32. Veldhuijzen van Zanten JJ, Rouse PC, Hale ED, Ntoumanis N, Metsios GS, Duda JL, et al. Perceived barriers, facilitators and benefits for regular physical activity and exercise in patients with rheumatoid arthritis: a review of the literature. Sports medicine. 2015;45:1401-12.
33. UK G. UK Chief Medical Officers' physical activity guidelines. 2019.

34. Henson J, Yates T, Biddle SJ, Edwardson CL, Khunti K, Wilmot EG, et al. Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health. Diabetologia. 2013;56:1012-20.
35. Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. Preventive medicine. 2015;76:92-102.
36. Veldhuijzen van Zanten JJ, Pilutti LA, Duda JL, Motl RW. Sedentary behaviour in people with multiple sclerosis: Is it time to stand up against MS? Mult Scler. 2016;22(10):1250-6.
37. Cavanaugh JT, Gappmaier VO, Dibble LE, Gappmaier E. Ambulatory activity in individuals with multiple sclerosis. J Neurol Phys Ther. 2011;35(1):26-33.
38. Langeskov-Christensen M, Heine M, Kwakkel G, Dalgas U. Aerobic capacity in persons with multiple sclerosis: a systematic review and meta-analysis. Sports Med. 2015;45(6):905-23.
39. Ellis T, Motl RW. Physical Activity Behavior Change in Persons With Neurologic Disorders: Overview and Examples From Parkinson Disease and Multiple Sclerosis. Journal of Neurologic Physical Therapy. 2013;37(2):85-90.
40. Carroll CC, Gallagher PM, Seidle ME, Trappe SW. Skeletal muscle characteristics of people with multiple sclerosis. Archives of Physical Medicine and Rehabilitation. 2005;86(2):224-9.
41. Hubbard EA, Motl RW, Fernhall B. Sedentary Behavior and Blood Pressure in Patients with Multiple Sclerosis. Int J MS Care. 2018;20(1):1-8.
42. Wilmot EG, Edwardson CL, Achana FA, Davies MJ, Gorely T, Gray LJ, et al. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. Diabetologia. 2012;55(11):2895-905.
43. Blair SN, Cheng Y, Holder JS. Is physical activity or physical fitness more important in defining health benefits? Medicine & Science in Sports & Exercise. 2001;33(6):S379-S99.
44. Patterson R, McNamara E, Tainio M, de Sá TH, Smith AD, Sharp SJ, et al. Sedentary behaviour and risk of all-cause, cardiovascular and cancer mortality, and incident type 2 diabetes: a systematic review and dose response meta-analysis. Eur J Epidemiol. 2018;33(9):811-29.
45. Courtney JB, Nuss K, Lyden K, Harrall KK, Glueck DH, Villalobos A, et al. Comparing the activPAL software's Primary Time in Bed Algorithm against Self-Report and van der Berg's Algorithm. Meas Phys Educ Exerc Sci. 2021;25(3):212-26.
46. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank J, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. Journal of hypertension. 2012;30(3):445-8.
47. McCraty R, Shaffer F. Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk. Glob Adv Health Med. 2015;4(1):46-61.
48. Heine M, Hoogervorst EL, Hacking HG, Verschuren O, Kwakkel G. Validity of maximal exercise testing in people with multiple sclerosis and low to moderate levels of disability. Phys Ther. 2014;94(8):1168-75.
49. Langeskov-Christensen M, Langeskov-Christensen D, Overgaard K, Møller AB, Dalgas U. Validity and reliability of VO2-max measurements in persons with multiple sclerosis. Journal of the Neurological Sciences. 2014;342(1):79-87.
50. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology. 2001;37(1):153-6.

51. Wens I, Dalgas U, Stenager E, Eijnde BO. Risk factors related to cardiovascular diseases and the metabolic syndrome in multiple sclerosis – a systematic review. Multiple Sclerosis Journal. 2013;19(12):1556-64.
52. Díaz A, Galli C, Tringler M, Ramírez A, Cabrera Fischer EI. Reference values of pulse wave velocity in healthy people from an urban and rural argentinean population. Int J Hypertens. 2014;2014:653239.
53. Boshra H, Awad M, Hussein M, Elyamani E. Vascular dysfunction and dyslipidemia in multiple sclerosis: are they correlated with disease duration and disability status? Egypt Heart J. 2022;74(1):9.
54. Chung JW, Lee YS, Kim JH, Seong MJ, Kim SY, Lee JB, et al. Reference values for the augmentation index and pulse pressure in apparently healthy korean subjects. Korean Circ J. 2010;40(4):165-71.
55. Zhang H, Jiang L, Yang Y-J, Ge R-K, Zhou M, Hu H, et al. Aerobic exercise improves endothelial function and serum adropin levels in obese adolescents independent of body weight loss. Scientific Reports. 2017;7.
56. Gao J, Pan X, Li G, Chatterjee E, Xiao J. Physical Exercise Protects Against Endothelial Dysfunction in Cardiovascular and Metabolic Diseases. Journal of Cardiovascular Translational Research. 2022;15(3):604-20.
57. Moyna N, Thompson P. The effect of physical activity on endothelial function in man. Acta Physiologica Scandinavica. 2004;180(2):113-23.
58. Heijden D, Leeuwen M, Janssens G, Lenzen M, Ven P, Eringa E, et al. Body Mass Index Is Associated With Microvascular Endothelial Dysfunction in Patients With Treated Metabolic Risk Factors and Suspected Coronary Artery Disease. Journal of the American Heart Association. 2017;6:e006082.
59. Moerland M, Kales AJ, Schrier L, van Dongen MG, Bradnock D, Burggraaf J. Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. Int J Vasc Med. 2012;2012:904141.
60. Saccenti E, Hendriks MHWB, Smilde AK. Corruption of the Pearson correlation coefficient by measurement error and its estimation, bias, and correction under different error models. Scientific Reports. 2020;10(1):438.
61. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine & science in sports & exercise. 2003;35(8):1381-95.
62. Lee PH, Wong FKY. The Association Between Time Spent in Sedentary Behaviors and Blood Pressure: A Systematic Review and Meta-Analysis. Sports Medicine. 2015;45(6):867-80.
63. Cornelissen VA, Smart NA. Exercise training for blood pressure: a systematic review and meta-analysis. J Am Heart Assoc. 2013;2(1):e004473.
64. Routledge FS, Campbell TS, McFetridge-Durdle JA, Bacon SL. Improvements in heart rate variability with exercise therapy. Can J Cardiol. 2010;26(6):303-12.
65. Gutin B, Howe C, Johnson MH, Humphries MC, Snieder H, Barbeau P. Heart rate variability in adolescents: relations to physical activity, fitness, and adiposity. Med Sci Sports Exerc. 2005;37(11):1856-63.
66. Reynders T, Gidron Y, De Ville J, Bjerke M, Weets I, Van Remoortel A, et al. Relation between Heart Rate Variability and Disease Course in Multiple Sclerosis. J Clin Med. 2019;9(1).
67. Heine M, Wens I, Langeskov-Christensen M, Verschuren O, Eijnde BO, Kwakkel G, et al. Cardiopulmonary fitness is related to disease severity in multiple sclerosis. Multiple Sclerosis Journal. 2016;22(2):231-8.

68. Motl RW, Dlugonski D, Pilutti L, Sandroff B, McAuley E. Premorbid physical activity predicts disability progression in relapsing-remitting multiple sclerosis. J Neurol Sci. 2012;323(1-2):123-7.
69. Fjeldstad AS, McDaniel J, Witman MAH, Ives SJ, Zhao J, Rose JW, et al. Vascular function and multiple sclerosis. Journal of Neurology. 2011;258(11):2036-42.
70. Berendsen BA, Hendriks MR, Meijer K, Plasqui G, Schaper NC, Savelberg HH. Which activity monitor to use? Validity, reproducibility and user friendliness of three activity monitors. BMC Public Health. 2014;14:749.
71. Kunutsor SK, Jae SY, Laukkanen JA. 'Weekend warrior' and regularly active physical activity patterns confer similar cardiovascular and mortality benefits: a systematic meta-analysis. European Journal of Preventive Cardiology. 2022;30(3):e7-e10.

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Author contributions – IN and SVC conceived and designed the research. SVC performed experiments and data analysis, IN aided with both experiments and data analysis. SVC wrote the paper and IN provided feedback and guidance.

SUPPORTING INFORMATION

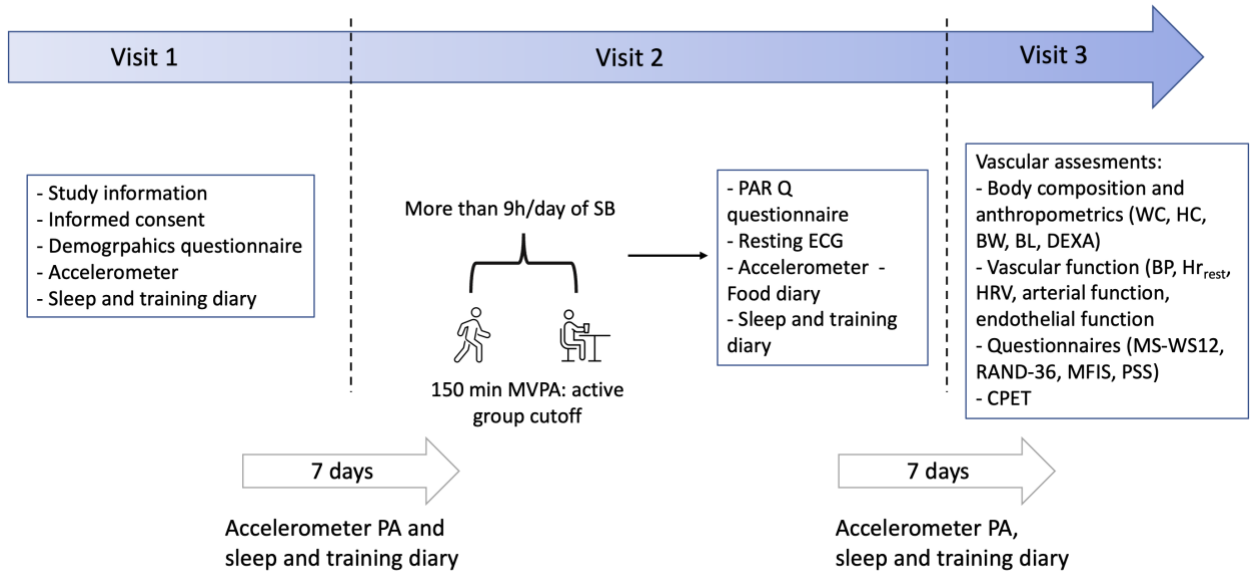

Figure s1: design schematic of the current study setup. The study included 3 visits of screening, eligibility, activity tracking (visit 2) and a test day where CVD risk parameters were analyzed (visit 3). PA; Physical activity, SB; Sedentary Behaviour, MVPA; moderate-to-vigorous activity, WC; waist circumference, HC; hip circumference, BW; body weight, BL; body length, DEXA; Dual Energy X-ray Absorptiometry, BP; blood pressure, HRrest; Heart rate in rest, HRV; heart rate variability, MS-WS12; Multiple Sclerosis 12-item walking scale, MFIS; modified fatigue impact scale, PSS; perceived stress scale, CPET; cardiopulmonary exercise test.

Table s1: Bivariate correlations of MVPA and secondary outcomes.

		MVPA (expressed in % of WT)	EDSS	Waist Circumference (cm)	Waist-to-hip ratio	Body fat (%)	Vo2 peak (ml/min/kg)	RMSSD (ms)
MVPA (expressed in % of WT)	Pearson Correlation	1	-0.361	- 0.541**	-0.485*	-0.402*	0.592**	0.547**
EDSS	Pearson Correlation	-0.361	1	0.478	0.507*	0.304	-0.288	- 0.29
Waist Circumference (cm)	Pearson Correlation	-0.541**	0.478	1	0.853**	0.485*	-0.649**	-0.296
Waist-to-hip ratio	Pearson Correlation	-0.485*	0.507*	0.853**	1	0.197	-0.454*	-0.367
Body fat (%)	Pearson Correlation	-0.402*	0.304	0.485*	0.197	1	-0.879**	-0.350
Vo2peak (ml/min/kg)	Pearson Correlation	0.592**	-0.288	-0.649**	-0.454*	0.879**	1	0.430*
RMSSD (ms)	Pearson Correlation	0.547**	-0.279	-0.296	-0.367	-0.350	0.430*	1

* denotes $p < 0.05$, ** denotes $p < 0.01$

EDSS. Expanded disability status scale, MVPA, moderate-to-vigorous intensity physical activity, RMSSD; Root mean square of successive differences between normal heartbeats.

Table s2: BP and independent variables MVPA, SB (expressed in % of WT and uninterrupted bouts of >60min), and LIPA.

		MVPA (expressed in % of WT)	SB (expressed in % of WT)	Uninterrupted bouts of SB (>60min)	LIPA (expressed in % of WT)
SBP (mmHg)	Pearson Correlation	0.007	-0.419*	-0.288	0.438*
DBP (mmHg)	Pearson Correlation	-0.174	-0.218	-0.166	0.296
MAP (mmHg)	Pearson Correlation	-0.160	-0.313	-0.214	0.380
Aortic SBP (mmHg)	Pearson Correlation	0.019	-0.527**	-0.328	0.491*
Aortic DBP (mmHg)	Pearson Correlation	-0.191	-0.194	-0.171	0.285

**. Denotes $p < 0.01$

*. Denotes $p < 0.05$

LIPA; light intensity physical activity, MVPA, moderate-to-vigorous intensity physical activity, SB; Sedentary behaviour. SBP; systolic blood pressure, DBP, diastolic blood pressure, Map; mean arterial pressure.

Table s3: primary outcomes and EDSS and MS duration

		AIx@75bpm	cfPWV	RHI
MS duration	Pearson Correlation	0.483*	0.124	-0.284
EDSS	Pearson Correlation	-0.228	0.120	-0.134

*. Denotes $p < 0.05$

AIx@75bpm; augmentation index corrected to 75 bpm, cfPWV; carotid-femoral pulse wave velocity, RHI; reactive hyperemia index, EDSS; expanded disability status scale.

Table s4: Correlations between SB and cfPWV corrected for body fat %, age and MVPA.

Control Variables			SB (expressed in % of WT)	cfPWV (m/s)
Body fat (%) & Age (y) & MVPA (expressed in % of WT)	Total SB/day_% of WT	Correlation	1.000	0.412
	Avg calculated PWV	Correlation	0.412°	1.000

° denotes p = 0.071

MVPA, moderate-to-vigorous intensity physical activity, SB; Sedentary behaviour. cfPWV; carotid-femoral pulser wave velocity.