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Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in Multiple Sclerosis

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HIGHLIGHTS

- MS patients have extremely low levels of fitness regardless of levels of disability.
- Poor cardiorespiratory fitness in MS associated with increased GABAergic intracortical inhibition.
- Increased GABAergic intracortical inhibition may explain exacerbated feelings of MS fatigue.

ABSTRACT

Objective: Poor fitness among people with Multiple Sclerosis (MS) aggravates disease symptoms. Whether low fitness levels accompany brain functioning changes is unknown.
Methods: MS patients (n = 82) completed a graded maximal exercise test, blood was drawn, and transcranial magnetic stimulation determined resting and active motor thresholds, motor evoked potential latency, and cortical silent period (CSP).
Results: Sixty-two percent of participants had fitness levels ranked below 10th percentile. Fitness was not associated with disability measured using the Expanded Disability Status Scale (EDSS). Regression analyses revealed that, cardiorespiratory fitness, when controlling for disease demographics, contributed 23.7% ($p < 0.001$) to the model explaining variance in CSP. Regression analysis using cardiorespiratory fitness and CSP as predictors showed that CSP alone explained 19.9% of variance in subjective fatigue ($p = 0.002$). Tumor necrosis factor was not associated with any variable.
Conclusion: Low fitness was associated with longer CSP in MS. Longer CSP was, in turn, related to greater MS fatigue.
Significance: MS patients had extremely low levels of cardiorespiratory fitness. Poor fitness predicted longer CSP, a marker of greater intracortical inhibition, which was linked to MS fatigue. Future research should examine whether aerobic training could shorten CSP and potentially lessen inhibition of cortical networks.

1. Introduction

Multiple Sclerosis (MS) is a neuroimmune-inflammatory disease of the central nervous system and the most common cause of neurological disability among young adults worldwide (Amankwah et al., 2017). In the relapsing remitting form of MS, unpredictable demyelination causes sudden loss of sensory, physical, and/or cognitive function, which may completely or partially recover as spontaneous remyelination occurs (Dutta and Trapp, 2014). In the progressive form of MS, functions progressively worsen with little remyelination (Dutta and Trapp, 2014). Disease-modifying therapies help reduce relapses, but presently, there is no cure for MS (Zheleznyakova et al., 2017).

The healthy brain adapts in response to stimuli and to do so, requires the ability to undergo synaptic plasticity, an element of neuroplasticity (Granger and Nicoll, 2014). Neuroplasticity may be useful following recovery from relapse and in resistance to MS progression (Stampanoni Bassi et al., 2017a). A robust body of research has confirmed that physical exercise promotes

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neuroplasticity (Ploughman, 2008; Austin et al., 2014; Ploughman et al., 2015), so, it is not surprising that exercise improves functional performance and strength (Donze et al., 2017; Edwards and Pilutti, 2017; Driehuis et al., 2018; Ozkul et al., 2018), fatigue (Edwards and Pilutti, 2017; Driehuis et al., 2018), and cognition (Motl and Sandroff, 2018) among people with MS. Unfortunately, several groups have reported that a large proportion of people with MS have low levels of fitness and are sedentary (Veldhuijzen van Zanten et al., 2016; Bollaert and Motl, 2017; Ploughman, 2017a; Casey et al., 2018; Hubbard et al., 2018; Sasaki et al., 2018), and thus do not obtain the beneficial effects of exercise. Recent research in physical activity, rehabilitation and self-management, suggests that increasing levels of physical activity is of low priority for both clinicians and MS patients (Kinnett-Hopkins et al., 2017). In fact, despite lack of convincing evidence, some MS patients are advised to rest and conserve energy to reduce fatigue; discouraging exercise because it could aggravate MS symptoms (Blikman et al., 2017). In fact, exercise is likely an essential component of MS management (Dalgas and Stenager, 2012; Casey et al., 2018), since higher fitness is associated with better cognitive function and preserved brain white and grey matter structure on magnetic resonance imaging (Prakash et al., 2010). Exercise also reduces cardiovascular risk factors which have been shown to accelerate MS progression (Marrie, 2017). Nonetheless, researchers suggest that more evidence is required to determine whether or not fitness modulates brain function in MS (Dalgas and Stenager, 2012). Current magnetic resonance imaging methods have failed to show enduring brain activation changes despite improvements in motor performance in MS patients after participating in physical exercise training (Tavazzi et al., 2018). Understanding the benefits of fitness on brain activation could help reveal important targets for rehabilitation and physical exercise interventions (Dalgas and Stenager, 2012; Ploughman, 2017b).

Fatigue is the most frequent and disabling symptom (Vucic et al., 2010) interfering with physical and cognitive activities of daily living among people with MS (Ayache and Chalah, 2017), and it has been proposed to be related to neuronal-connectivity disruption (Russo et al., 2017). Fatigue may also be related to poor cardiorespiratory fitness or to high levels of circulating cytokines, and such, exercise prescription has been suggested in order to counteract inflammation in order to improve fatigue (Dalgas and Stenager, 2012; Alvarenga-Filho et al., 2016; Barry et al., 2016; Kjolhede et al., 2016; Mokhtarzade et al., 2017). Tumor necrosis factor (TNF) is a circulating cytokine and its dysregulation has been implicated in inflammatory-mediated diseases including MS (McCoy and Tansey, 2008). For example, Deckx and group reported that a combined aerobic and resistance exercise program reduced TNF and other markers of inflammation in patients with MS (Deckx et al., 2016). Whether fitness, fatigue, brain function and levels of circulating TNF are linked is not known.

Transcranial magnetic stimulation (TMS) is a non-invasive tool that measures brain function by quantifying the excitability of the corticospinal tract (Rösler, 2001; Rossini et al., 2015). Using TMS, corticospinal excitability (CSE) is determined by measuring motor neuron excitability and nerve conduction speed; resting and active motor thresholds (RMT and AMT, respectively), and motor evoked potential (MEP) latency (Rossini et al., 2015). Also, TMS assesses levels of intracortical inhibition, by measuring the length of the cortical silent period (CSP), an interruption of background muscle activity after a TMS pulse (Epstein et al., 2012; Rossini et al., 2015). CSP is thought to be mediated by g-aminobutyric acid (GABA) inhibitory neurotransmission (Epstein et al., 2012; Rossini et al., 2015) believed to be involved in neuroplasticity by modulating long-term potentiation (LTP) (Mott and Lewis, 1994; Ziemann et al., 2004; Jurado-Parras et al., 2016). MS patients have several CSE abnormalities in comparison to the general population, including higher motor thresholds (Neva et al., 2016), delayed MEP latencies (Neva et al., 2016), and longer CSP (Tataroglu et al., 2003), which supports the usefulness of TMS as a biomarker of brain functioning in MS.

As our primary objective, we investigated whether cardiorespiratory fitness, when controlling for MS severity and type, disease duration, and age, predicted RMT, AMT, MEP latency, and CSP. As our secondary aim, we investigated whether the TMS variables associated with cardiorespiratory fitness were related to TNF or subjective fatigue. We hypothesized that having lower levels of cardiorespiratory fitness would negatively impact brain excitability, and that, fitness-related TMS variables would be associated with greater fatigue and higher levels of TNF.

2. Materials and methods

2.1. Participants

Eighty-two MS patients (58 females, 24 males) aged 47.40 ± 10.2 years (mean ± SD), consecutively recruited from an MS clinic, participated in the study. All participants' descriptive data are reported in Table 1. Participants met the following inclusion criteria: (1) able to walk indoors independently with or without aid; (2) able to provide consent; (3) 18 years old or older, and; (4) no relapses in the previous 3 months. Demographic data were collected, including age (years), sex, MS type (relapsing remitting (RRMS), secondary progressive (SPMS), or primary progressive (PPMS)), disease duration ((DD) years), and type of medications and MS disability level was quantified by a neurologist using Expanded Disability Status Scale (EDSS; 0.5 unit increment; 0 = normal neurological exam, 10 = death due to MS). Participants were screened for exercise safety using the PAR-Q (Bredin et al., 2013) and for TMS safety using a standardized form (Rossi et al., 2009). All participants consented to participate in the study, and all procedures were approved by the local health research ethics board (Memorial University of Newfoundland; reference number: 2015.103).

Table 1
Participants characteristics.

Female (n)	58
Male (n)	24
Age (years)	47.40 ± 10.2
MS Type	75 RRMS, 6 SPMS, 1 PPMS†
Disease Duration (years)	13.10 ± 8.0
MS Severity (EDSS 0–10)	2.04 ± 1.7
Levels of Fatigue (0–100 mm)	41.31 ± 32.5
Fitness Profile	
VO_{2max} (mL min^{-1} kg^{-1})	25.34 ± 7.0
HR_{max} (bpm)	164 ± 17
% of Predicted HR_{max}	93.46 ± 8.8
RER at VO_{2max} (VCO_2/VO_2)	1.07 ± 0.1
TMS Variables	
RMT (MSO% 0–100)	41 ± 11
AMT (MSO% 0–100)	36 ± 10
MEP Latency (ms; ms/$height_{cm}$)	24.45 ± 2.6; 0.14 ± 0.01
CSP (ms)†	149.90 ± 37.2

Note: Data presented as mean ± SD. AMT, active motor threshold; CSP, cortical silent period; EDSS, Expanded Disability Status Scale; HR_{max}, maximal heart rate; MEP, motor evoked potential; MS, Multiple Sclerosis; MSO%, maximal stimulator output percentage; RER, respiratory exchange ratio; RMT, resting motor threshold; RRMS, relapsing remitting MS; TMS, transcranial magnetic stimulation; SPMS, secondary progressive MS; PPMS, primary progressive MS; VO_2, volume of oxygen; VCO_2, volume of dioxide oxygen; VO_{2max}, maximal volume of oxygen intake (cardiorespiratory fitness).

† CSP was collected in a subsample of 48 MS patients. † PPMS patient (female, 52, EDSS 6) was removed from all analyses.

2.2. TNF

Peripheral venous blood (5 mL) was drawn from all study participants in plasma collection tubes. Blood was spun at 1200 rpm for 10 min and plasma was aliquoted and stored in liquid nitrogen for long-term storage. The concentration of TNF within the plasma was quantified using a human BD OptEIA™ TNF Enzyme-Linked Immunosorbent Assay kit (BD BioSciences) and performed according to manufacturer's instructions.

2.3. Transcranial magnetic stimulation

Motor evoked potentials (MEP) were elicited from both brain hemispheres using monophasic magnetic pulses from a BiStim 200^2 stimulator (Magstim Co. Whitland, UK) connected to a double 70 mm figure-of-eight coil (Magstim, Co.). To measure electromyography (EMG) activity and collect the MEPs, foam surface electrodes (Kendall 200 Coviden, Mansfield, MA) were placed on the belly of the first dorsal interosseous (FDI) muscle, and the ground and the reference electrodes were placed on the styloid process and the interphalangeal joint of the index finger, respectively. Both dominant and non-dominant hands were assessed. Dominance determination was self-reported. A neuronavigation device (Brainsight, Rogue Research Inc, Montreal, QC, Canada) guided coil position and collected the MEPs with its built-in EMG system. This system uses a 2500 V/V amplification and collects with a sampling rate of 3 kHz and a gain of 600 V/V with a bandwidth of 16–550 Hz. The Montreal Neurological Institute brain template was rendered into the BrainSight software and used as a 3-D stereotaxic template (Collins et al., 1994; Fonov et al., 2011).

With the participant seated, the TMS coil was maintained tangentially to the scalp with the handle pointing backward and laterally at an angle of 45° from the midline perpendicular to the central sulcus. This coil position delivers posterior-anterior directed pulses that are known to produce large MEPs resulting from the gradual recruitment of indirect waves (Di Lazzaro et al., 2001; Hannah and Rothwell, 2017). First, TMS suprathreshold stimulations were fired at different locations over the primary motor area and the site with the highest averaged FDI response (MEP peak-to-peak amplitude) was taken as the *hotspot*. Motor thresholds were determined as the minimum amount of intensity of the TMS necessary to elicit 5 out of 10 MEPs with a peak-to-peak amplitude of $\geq$50 μV during muscle relaxation and $\geq$200 μV during 10% of FDI's maximal voluntary contraction, known as RMT and AMT, respectively (Rossini et al., 2015). To measure CSP, 6 pulses at 155% of AMT were delivered with participants' performing a pinch grip at 10% of the maximal contraction measured (Rossini et al., 2015). A pinch dynamometer (B&L engineering, Santa Ana, CA) was used to measure the maximal pinch grip, collected before the TMS assessment, and to provide feedback on the level of muscle contraction during the TMS assessment.

AMT and RMT were recorded as the maximal stimulator output (0–100%). The time in milliseconds between the MEP onset until the EMG activity returned to ±2SD of the mean EMG background activity was taken as the CSP (Rossini et al., 2015). MEP latency was calculated from the valid MEPs recorded during the RMT assessment as the time in milliseconds from the TMS stimulus to the MEP onset. MEP onset was determined as the time-point where the MEP exceeded ±2SD from the EMG background activity (Rossini et al., 2015). Because MEP latency is influenced by height and limb length (Tobimatsu et al., 1998; Livingston et al., 2013; Matamala et al., 2013; Sollmann et al., 2017), the normalized MEP latency (ms/height$_{cm}$) was used for analysis. MEPs with preceding EMG background activity ±2SD from the mean were disregarded. Each MEP was visually inspected. MEPs were analyzed with Signal software v6.04 (Cambridge Electronic Design, Cambridge, UK).

2.4. Subjective fatigue

Prior to any physical or neurophysiological assessment, participants indicated on a 100 mm line their present level of MS-related fatigue, from worst (100 mm – severely fatigued) to best (0 mm – Not fatigued at all).

2.5. Cardiorespiratory fitness

Levels of cardiorespiratory fitness were determined by the maximal capacity of volume of oxygen uptake (VO_{2max}) during a graded exercise test using a total body recumbent stepper (NuStep, Ann, Arbor, MI) (Billinger et al., 2008). Throughout the test, an indirect calorimetry system (Moxus, AEI Technologies, Pittsburgh, PA) was used to collect volume of oxygen uptake (VO_2), volume of carbon dioxide production (VCO_2), and heart rate (HR) (H10, Polar Electro Inc., NY, USA). In brief, participants were required to maintain a speed of 80 strides per minute while the load (1–10; beginning at level 3) was increased by one unit every 2 min. If exhaustion was not reached after completed load level 10 (maximal load), the strides per minute were increased by 10 every 2 min. The criteria for terminating the test were: (i) volitional exhaustion, (ii) no increase in VO_2 or HR despite increases in workload, (iii) inability to maintain workload, or; (iv) signs of excessive fatigue. Achievement of VO_{2max} was assessed based on attainment of at least two of the following criteria: (i) a plateau in VO_2 (<80 mL min^{-1}) despite increasing workload; (ii) respiratory exchange ratio (VCO_2/VO_2) $\geq$ 1.1; and/or (iii) HR_{max} ± 10 bpm of predicted maximum HR, calculated as $206.9 - (0.67 \times age)$ or $164 - (0.7 \times age)$ if prescribed beta-blockers (Ferguson, 2014). The breath-by-breath collected data was smoothed using a moving average of 10 data points. From the smoothed data, the absolute VO_{2max} was identified as the highest VO_2 uptake from participants' and further divided by their weight, to obtain participants' relative VO_{2max} (VO_{2max} = mL min^{-1} kg^{-1}).

3. Statistical analysis

TMS variables (RMT, AMT, MEP latency, and CSP) differences were investigated between dominant and non-dominant hands using paired t-tests. Differences in TMS variables between patients prescribed disease-modifying drugs versus those that were not were tested with Independent t-tests. Among treated patients, differences between oral versus injectable disease-modifying drugs was further investigated with Independent t-tests. Parametric or non-parametric t-tests were performed depending on the normality of the data assessed with Shapiro-Wilk, kurtosis and skewness tests (Mohd Razali and Yap, 2011). For non-parametric independent and paired t-tests, statistics were reported using Mann-Whitney U (Z-value) and Wilcoxon Signed-Ranks Test (Z-value), respectively, whereas for parametric independent and paired t-tests, *t statistic* with degrees of freedom (e.g. $t_{(dof)}$) was reported. In the case of differences or no differences between dominant and non-dominant hands, the TMS values were analyzed separately or collapsed, respectively.

To explore the relationship between levels of cardiorespiratory fitness and levels of physical disability, Pearson's correlation were performed with the absolute relative VO_{2max} and EDSS, as well as between EDSS and participants' VO_{2max} when normalized for age and sex (Ferguson, 2014). Pearson's correlations were also performed to explore the relationship between TNF and VO_{2max}, fatigue, and TMS measures.

Hierarchical linear regression analyses were performed to examine the degree to which cardiorespiratory fitness predicted the TMS variables when controlling for MS patients' demographics. In the first block, MS severity (EDSS), MS type, disease duration, and age, were included. In the second block, VO_{2max} was added and its contribution (ΔR^2) to the final model was calculated. Separate hierarchical regressions were performed for each TMS variable (RMT, AMT, MEP latency, and CSP).

In order to better understand the relationship between the TMS variables that were associated with cardiorespiratory fitness and fatigue, stepwise linear regression analyses were performed with VO_{2max} and TMS variables predicting level of fatigue.

Acceptable collinearity between the predictors was identified using tolerance levels (>0.1) and the variance inflation factor (<5.0) (Vatcheva et al., 2016). Outliers were identified with residuals plots (±3SD) and Cook's distance (>4/sample size), and removed from the regression analyses to avoid the influence of this data point on the results (Bollen et al., 1985). Due to the presence of random missing data, pairwise case exclusion was selected during the regressions (Peugh et al., 2004).

Significance was set at an alpha level of <0.05. Data are reported as Mean ± SD. All data were analyzed on SPSS v.24 (IBM Corporation, Armonk, New York). Graphs were created with GraphPad Software v.6 (La Jolla, California, USA).

4. Results

4.1. Transcranial magnetic stimulation

All participants had recordable MEPs in at least one of the FDIs. RMT could not be measured in 11 dominant and 8 non-dominant hands, and AMT could not be measured in 7 dominant, and 5 non-dominant hands, because: (i) TMS overheated; (ii) maximal levels of the stimulator output did not elicit MEPs, or; (iii) during RMT assessment, higher levels of EMG background activity preceding MEPs (i.e. participants unable to rest). The patient diagnosed with PPMS (female, 52 year-old, EDSS 6) was excluded from the analysis due to the different pathophysiology of this type of MS. Participants with RRMS and SPMS were analyzed together because: (1) ~80% of RRMS will likely develop SPMS, and the distinction between inflammatory (RRMS) and neurodegenerative phase (SPMS) in MS is not clear (Dutta and Trapp, 2014), and (2) levels of fitness may impact the RRMS-SPMS transition, as it has been proposed that superior fitness may delay onset of progressive phase of MS (Dalgas and Stenager, 2012).

There were no differences between dominant and non-dominant hands for RMT ($Z = -0.1.33$, $p = 0.183$), AMT ($t_{(70)} = -1.88$, $p = 0.064$), or MEP latency ($Z = -0.67$, $p = 0.504$). CSP was collected in a subsample of 48 MS patients, and for the same abovementioned reasons (i.e. i and ii), CSP could not be collected in 3 dominant hands, and 8 non-dominant hands. There was no difference between dominant and non-dominant hands for CSP ($t_{(36)} = -1.41$, $p = 0.168$). All TMS variables, were averaged between hemispheres for further analyses (see Table 1 for descriptive TMS values). Forty-eight patients were being treated with disease-modifying drugs including teriflunomide (3), interferon β-1a (4), glatiramer-acetate (6), fingolimod (5), dimethyl fumarate (29), and natalizumab (1). There were no differences between treated and untreated MS patients for any TMS variable ($Z < -0.54$, $p > 0.593$; $t < -0.39$, $p > 0.699$), with exception of MEP latency, in which patients prescribed disease-modifying drugs had faster nerve conduction speed (untreated vs treated: 0.15 ± 0.01 vs 0.14 ± 0.02 ms/height$_{cm}$; $Z = -2.03$, $p = 0.042$). There were no differences between treated patients taking oral (n = 37, dimethyl fumarate, teriflunomide, and fingolimod) versus injectable (n = 11, interferon β-1a, glatiramer-acetate, natalizumab) disease modifying drugs for any TMS measure ($t < -1.45$, $p > 0.155$, $Z < -0.69$, $p > 0.489$).

4.2. Levels of cardiorespiratory fitness were not associated with physical disability

Fitness data is provided in Table 1. Levels of cardiorespiratory fitness among participants ranged from *very poor* (n = 31) to *excellent* (n = 1) (Ferguson, 2014) and were irrespective of their levels of physical disability measured using EDSS. Fig. 1 shows the levels of physical disability and cardiorespiratory fitness normalized by age and sex as recommended by the American College Sports of Medicine (Ferguson, 2014) ($r = -0.032$, $p = 0.792$). There was also no relationship between the non-normalized levels of cardiorespiratory fitness (mL min^{-1} kg^{-1}) and EDSS ($r = -0.173$, $p = 0.146$). It was notable that 91% of participants had values below the 50th percentile, and 63% had values below the 10th percentile of normative values. Also, seventeen participants across all levels of disability (EDSS 0–6) scored below the *very poor* cut off. We could not obtain VO_{2max} values from 9 MS patients due to participants' inability to wear a mask during the test. Sixteen participants did not meet the pre-determined criteria for achieving a VO_{2max} (e.g. reached a respiratory exchange ratio of ≤1.1 and/or achieved at least 90% of predicted HR_{max} values (Ferguson, 2014)).

4.3. No association between cytokine levels and fitness, fatigue or TMS measures

From the total sample, 71 participants (87%) were tested for TNF (mean ± SD: 6.88 ± 10.6 pg/mL). TNF did not correlate with any physical (fitness and fatigue) or neurophysiological (AMT, RMT, CSP, and latency) measure ($p > 0.05$).

4.4. Low cardiorespiratory fitness predicted greater intracortical inhibition

The results of the hierarchical regressions are summarized in Table 2. In the first block, MS demographics explained significant variance in and MEP latency ($R^2 = 0.186$, $F_{(4, 61)} = 3.49$, $p < 0.012$), RMT ($R^2 = 0.158$, $F_{(4, 58)} = 2.71$, $p < 0.039$), and AMT ($R^2 = 0.167$, $F_{(4, 57)} = 2.85$, $p < 0.032$), but not CSP. The addition of cardiorespiratory fitness in the second block contributed significantly ($p < 0.001$), adding 23.7% to the final model explaining variance

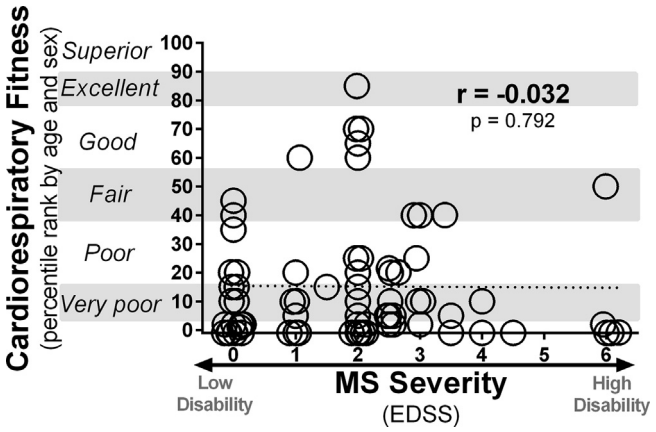

Fig. 1. Cardiorespiratory fitness and severity of MS-related disability. 73 MS patients were ranked against the percentiles of normative values for cardiorespiratory fitness with reference to age and sex as 1–15% (*very poor*), 20–35% (*poor*), 40–55% (*fair*), 60–75% (*good*), and 90–99% (*superior*) levels of fitness (Ferguson, 2014). EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis.

Table 2
Predictors of corticospinal excitability.

Block 1 – MS demographics					Block 2 – Cardiorespiratory Fitness				Final Model		
Controlling Variables:	*Outcome Variables:*	R^2	$F_{statistic}$	Sig.		ΔR^2	$F_{statistic}$	Sig.	R^2	$F_{statistic}$	sig.
EDSS, MS Type, Age, DD	**RMT**	0.158	2.71	0.039*	VO_{2nax}	+0.005	0.33	0.567	0.162	2.21	0.066
	AMT	0.167	2.85	0.032*		+0.054	3.85	0.055	0.220	3.17	0.014*
	MEP Latency	0.186	3.49	0.012*		+0.008	0.62	0.434	0.195	2.90	0.021*
	CSP	0.093	0.93	0.460		+0.237	12.41	<0.001†	0.331	3.46	0.012*

Note: AMT, active motor threshold; CSP, cortical silent period; DD, disease duration; EDSS, Expanded Disability Status Scale; MEP, motor evoked potential; MS, Multiple Sclerosis; RMT, resting motor threshold; VO_{2max}, maximal volume of oxygen intake (cardiorespiratory fitness, mL min^{-1} kg^{-1}). Sig, p-value.
* Model significantly predicted the outcome variable ($p < 0.05$); ΔR^2, R^2 change (amount of contribution of VO_{2max} to the final model).
† VO_{2max} significantly contributed to the model ($p < 0.01$).

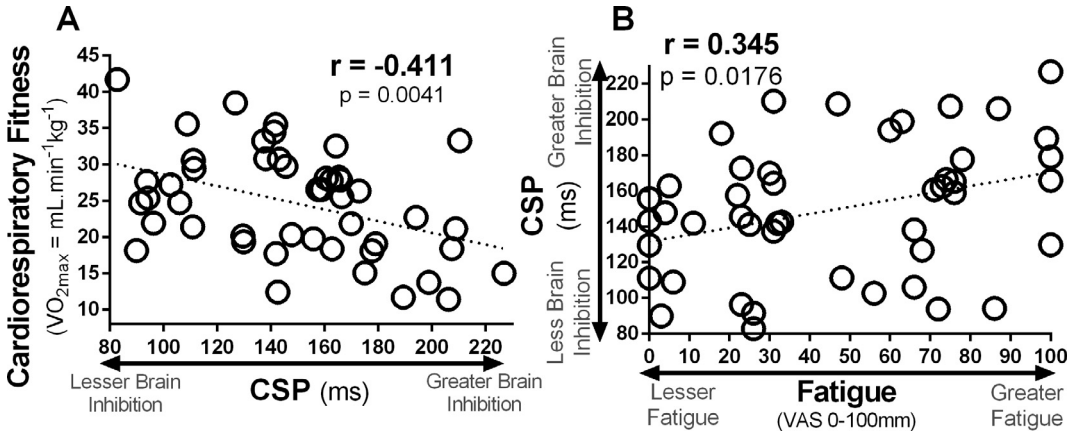

Fig. 2. Associations between cardiorespiratory fitness and cortical silent period (CSP), and CSP with fatigue. (A) Low cardiorespiratory fitness predicted greater brain inhibition; (B) Greater inhibition predicted greater fatigue: MS patients with longer CSP reported greater levels of fatigue on a visual analog scale (0–100 mm).

in CSP ($R^2 = 0.331$, $F_{(5, 35)} = 3.46$, $p = 0.012$), whereby higher levels of fitness predicted less intracortical inhibition (shorter CSP). Fig. 2A shows the association between CSP and VO_{2max} ($r = -0.411$, $p < 0.004$). Fig. 3 shows representative data from three MS subjects, their levels of cardiorespiratory fitness and CSP. Cardiorespiratory fitness did not contribute to variance in RMT or MEP latency. Addition of cardiorespiratory fitness, although not significant ($\Delta R^2 = 5.4\%$, $p = 0.055$), improved the significance to the final model explaining variance in AMT ($R^2 = 0.220$, $F_{(5, 56)} = 3.17$, $p = 0.014$).

4.5. Higher intracortical inhibition predicted greater fatigue

CSP predicted 19.9% of the variance in fatigue ($F_{(1, 43)} = 10.66$, $p = 0.002$) while VO_{2max} was excluded from the model. In other words, increased GABAergic-related intracortical inhibition (longer CSP) predicted worsened fatigue in MS. Fig. 2B shows the associations between CSP and levels of fatigue ($r = 0.345$, $p = 0.018$).

5. Discussion

We undertook this study to examine the link between cardiorespiratory fitness, brain excitability, circulating TNF, and subjective symptoms of fatigue in MS. In this clinic sample, we demonstrated that MS patients indeed had very low levels of cardiorespiratory fitness suggesting that participation in any exercise was very unlikely. Using TMS, we demonstrated that, when controlling for MS disease demographics, cardiorespiratory fitness predicted levels of intracortical inhibition measured with CSP. More specifically, MS patients with poor levels of cardiorespiratory fitness had longer CSP. Moreover, longer CSP predicted worsened fatigue. Cytokine levels (TNF) were not associated with any other measure collected in this study.

5.1. Levels of cardiorespiratory fitness and physical disability in MS

In a meta-analysis involving 40 studies and a total of 1137 MS patients, Langeskov-Christensen et al. (2015) reported weak to moderate ($r = -0.250$–0.580) associations between poor levels of fitness and higher levels of disability (EDSS). We did not detect such an association. When values were converted to percentile ranks (Ferguson, 2014), as previously proposed (Langeskov-Christensen et al., 2015), there was no significant relationship as well. This difference in results may be equipment-related, since previous authors employed bicycle ergometer during fitness testing (Romberg et al., 2004; Motl and Goldman, 2011; Heine et al., 2014; Langeskov-Christensen et al., 2014), which restricts the workload to the legs. Since degree of disability correlates with the severity of lower limb impairment (Devasahayam et al., 2017), it is likely that participants with greater leg weakness would not be able to fully achieve their maximal values on a leg ergometer. Ponichtera-Mulcare et al. (1995) confirmed that MS patients were only able to achieve their predicted maximal fitness values when using both upper and lower body, but not when using only the arms or the legs (Ponichtera-Mulcare et al., 1995). In our study, we employed a recumbent stepper which permitted the workload to be distributed between the upper and lower body. It is also important to note that, our participants were recruited consecutively from an MS clinic and therefore they likely represent a typical clinic cohort. Other studies examining fitness levels among MS patients typically report baseline characteristics of people volunteering for exercise trials (Langeskov-Christensen et al., 2015), which could inflate fitness values due to recruitment bias.

Over 60% of our participants had cardiorespiratory fitness levels below the cut off for high risk of all-cause mortality (<27 mL min^{-1} kg^{-1}) (Langeskov-Christensen et al., 2015), and 28% had insufficient cardiorespiratory fitness to comfortably

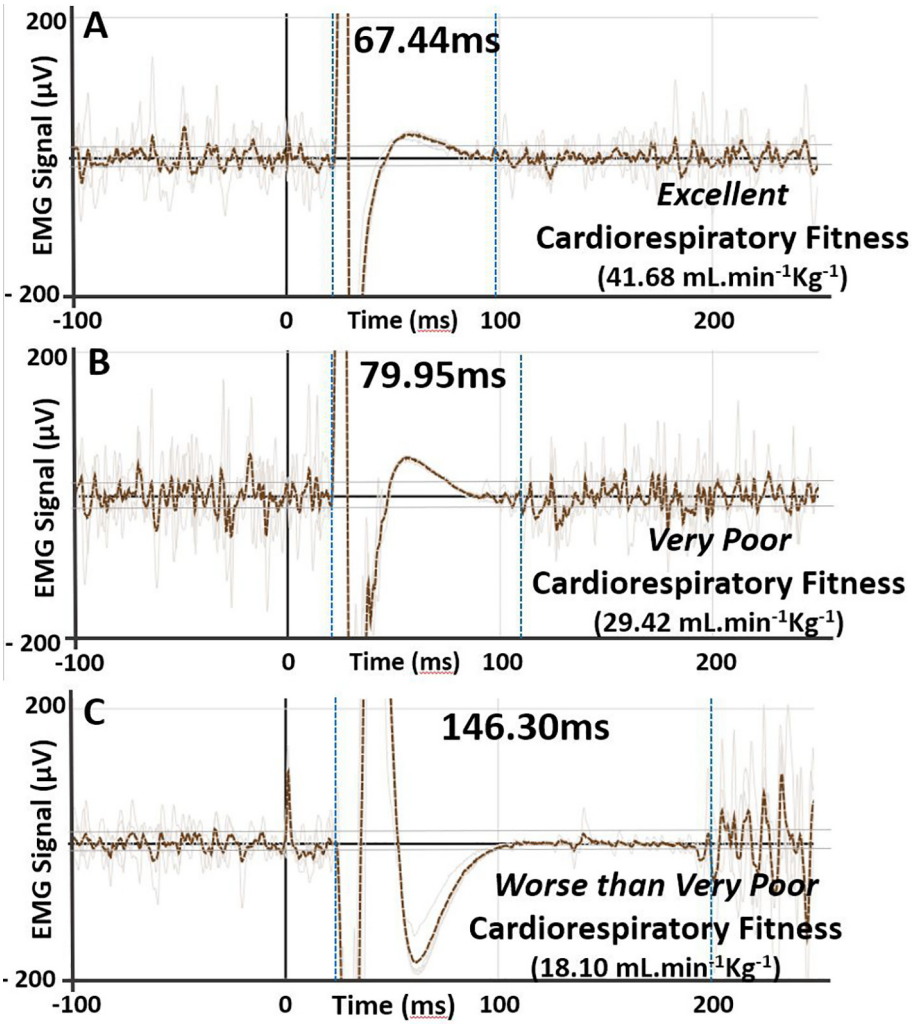

Fig. 3. MS patients with poorer cardiorespiratory fitness had greater brain inhibition. Representative MEP outputs showing cortical silent period. (A) 33-year-old female, RRMS, EDSS 2.0; (B) 23-year-old female, RRMS, EDSS 1.0, and; (C) female 40-year-old, RRMS, EDSS 2.0. Ranked fitness was normalized by age and sex according to American College Sports of Medicine (Ferguson, 2014). The vertical dotted lines indicate the CSP time in milliseconds, (time-point where the MEP leaves ±2SD from the EMG background activity until the EMG activity returned to ±2SD of the mean EMG background activity).

carry-out activities of daily living (<20 mL min^{-1} kg^{-1}) (Cress et al., 2003). Considering that MS patients require more energy to perform activities of daily living (e.g. walking) due to physical impairments (Kempen et al., 2012), poor fitness will likely impact independence (Rosalie Driehuis et al., 2018). Also, in an event of a relapse whereby physical function decreases considerably, the cardiorespiratory fitness reserve would not be sufficient to maintain and to optimally regain function during recovery. It is reasonable to consider therefore, that exercise therapies should be implemented at the time of first MS symptoms (Riemenschneider et al., 2018). Improving fitness during this "window of opportunity" may postpone diagnosis of clinical definite MS, preserve neurological reserve (i.e. brain volume and functionality), and reduce manifestation and progression of disability (Riemenschneider et al., 2018). It was notable that all (100%) of our asymptomatic MS patients (n = 19, EDSS 0) had poor fitness that was below the 50%, and alarmingly, 65% of them were below the 1% of normative value (Ferguson, 2014). Clearly, there is a need for both health care professionals and people with MS to increase focus on fitness.

5.2. Cardiorespiratory fitness as a target to foster plasticity

We demonstrated that, when controlling for MS demographics, higher levels of cardiorespiratory fitness predicted shorter CSP in MS, a measure of the strength of GABAergic-mediated intracortical inhibition (Epstein et al., 2012; Rossini et al., 2015). The long-lasting intracortical inhibition seen in the CSP is from both spinal and cortical origins whereby longer CSP represents increased intracortical inhibition (Epstein et al., 2012; Rossini et al., 2015). CSP is mediated by both ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors (Mott and Lewis, 1994; Rossini et al., 2015) as well as by glutamatergic activity (Tremblay et al., 2013; Dyke et al., 2017). Increased activity of both $GABA_A$ and $GABA_B$ suppresses neuronal depolarization and undermines LTP formation (Mott and Lewis, 1994; Jurado-Parras et al., 2016). Accordingly, in healthy individuals, less GABAergic inhibition measured as shorter CSP predicts enhanced LTP response (Sale et al., 2007) assessed with paired-associative stimulation (Meunier et al., 2007). Although in MS, reduced GABAergic activity (less inhibition) has been associated with greater disability (Cawley et al., 2015), it is unknown whether this phenomenon contributes to MS progression or is a compensatory mechanism to enhance LTP, protect the brain and maintain optimal brain function (Stampanoni Bassi et al., 2017b; Chaves et al., 2019; Wirsching et al., 2018). Lengthening of CSP has been reported in MS patients with motor dysfunction (Tataroglu et al., 2003), and in MS patients post-relapse, with longer CSP associated with larger brain lesions and poorer upper extremity function (Tataroglu et al., 2003; Nantes et al.,

2016b). Longer CSPs are indicative of exaggerated intracortical inhibition, greater disability, and poor motor function in other clinical populations such as Huntington's (Priori et al., 1994) and stroke (Classen et al., 1997; Gray et al., 2017).

It could be argued that the correlation between CSP and fitness would be predictable, considering that CSP is modulated by stimulus intensity and more disabled patients with higher MTs would have prolonged CSP. In fact, we employed two methods to examine the singular effects of fitness on CSP. First of all, we tested all participants at the same relative MSO% (i.e. same MSO% to elicit a 200 μV amplitude MEP during 10% of MVC – AMT). Secondly, we controlled for disease-related variables and those variables that we controlled for, (Disability (EDSS), MS type, age, and disease duration) did not predict CSP, which suggests that CSP length is likely not being affected by disability in this cohort of MS patients.

Our results align with others suggesting that participation in physical exercise among healthy individuals decreases intracortical inhibition (shortens CSP) (Carroll et al., 2011; Kidgell et al., 2017) and may be a stimulant to foster LTP (Ziemann et al., 2004). For example, Cirillo et al. (2009) showed that following regular structured exercise (e.g. running, cycling), participants had superior LTP, measured using paired-associative stimulation, in comparison to their sedentary peers (Cirillo et al., 2009). Sale et al. (2007) demonstrated that shorter CSP, but not other TMS measures, predicted enhanced LTP (Sale et al., 2007), which points to the importance of reduced GABAergic activity (short CSP) to foster neuroplasticity. Interestingly, when comparing our CSP lengths to previously reported values (e.g. 100–300 ms) (Rossini et al., 2015), our CSPs were shorter especially among the fitter MS patients (e.g. Fitness level *excellent,* 82 ms, and *good*, 125 ms). The reasons for this discrepancy are not clear, however, emerging evidence supports that decreased GABAergic activity may serve as a protective mechanism in MS (Mori et al., 2016; Stampanoni Bassi et al., 2017b; Chaves et al., 2019; Mango et al., 2018; Wirsching et al., 2018) which could potentially shorten CSP. Furthermore, some investigators have proposed that increased levels of interleukins (IL) (e.g. IL-1β) in MS may create brain hyperexcitability, thereby enhancing LTP which may serve to protect the brain from relapses and progression of MS (Stampanoni Bassi et al., 2017b; Chaves et al., 2019; Mango et al., 2018; Wirsching et al., 2018). This concept of *LTP-reserve* was recently supported by Zeller, D. and group, in which, after an inhibitory-inducing stimulation protocol, MS patients demonstrated paradoxical LTP rather than LTD (Wirsching et al., 2018). Our findings support that poor fitness in MS patients may impact levels of GABAergic intracortical inhibition as measured by longer CSP, which may hinder neuroplasticity by diminishing LTP-reserve. Longitudinal studies investigating physical, cognitive, and fitness changes over time should consider the investigation of CSP and its implications during learning, function, and neuroplasticity in MS.

5.3. Fatigue and CSP

We showed that CSP, but not fitness or TNF, predicted fatigue, which suggests that improving fitness could mitigate fatigue by decreasing GABAergic-mediated intracortical inhibition (shortening CSP). In MS, previous studies support that physical disability and fatigue can be lessened by as little as 10 weeks of structured exercise training (Coote et al., 2017). Using diffusor tensor imaging, Russo et al. (2017) demonstrated disruption of thalamo-frontal connections in MS patients with higher levels of subjective fatigue (Russo et al., 2017). Interestingly, using TMS, these authors also demonstrated that fatigued MS patients had reduced intracortical facilitation (Russo et al., 2017), concluding that reduced corticospinal output due microstructural damage in cortico-subcortical white matter tracts may explain subjective and central fatigue (Russo et al., 2015). Our findings align with Russo et al. (2017), since we demonstrated that greater intracortical inhibition predicted subjective fatigue in MS. Therefore, improving fitness may act through reduction in intracortical inhibition to reduce some of the central fatigue experienced by people with MS.

5.4. No link between cardiorespiratory fitness and motor thresholds or nerve conduction speed

In MS, prolonged MEP latency is a biomarker of degree of demyelination and disease progression (Zeller et al., 2012; Nantes et al., 2016b; Neva et al., 2016) that is associated with decrements in motor function (Zeller et al., 2012; Nantes et al., 2016a). The role of motor thresholds, RMT and AMT, in MS, however, remains ambiguous. For example, although lower CSE assessed by higher motor thresholds has been shown in MS patients recovering from relapses (Caramia et al., 2004) and in highly disabled MS patients (Fierro et al., 2002), there is an enormous variability among study results. For instance, when compared to healthy individuals, some authors report higher motor thresholds in MS (Zeller et al., 2012; Neva et al., 2016) while others report no differences (Nantes et al., 2016a, 2016b). Nonetheless, we confirmed that MS demographics (age, disease duration, EDSS, and type of MS) predicted variability in RMT, AMT, and MEP latency, suggesting that these biomarkers of damage and repair are being negatively affected by MS. It was interesting to note that the use of disease-modifying drugs was related to faster conduction speed (MEP latency). This could be due to the neuroprotective effects of the prescribed drugs or the fact that drugs are prescribed earlier in the disease. Ayache et al. (2015) have previously demonstrated that disease-modifying drugs preserve CSE among MS patients (Ayache et al., 2015).

In comparison to physically active, sedentary or individuals with lower levels of physical activity have lowered CSE, as measured by decreased MEP amplitude and LTP responses (Cirillo et al., 2009; Lulic et al., 2017), higher motor thresholds (Jensen et al., 2005; Monda et al., 2017), and lengthened MEP latencies (Monda et al., 2017). We expected therefore, that higher levels of cardiorespiratory fitness would be associated with lower motor thresholds and faster MEP latencies in our cohort of MS patients. Contrary to our expectations, our results showed that cardiorespiratory fitness did not contribute significantly to RMT or MEP latency and its addition contributed only 5.4% to the final model explaining variance in AMT. This lack of association between cardiorespiratory fitness, motor thresholds, and nerve conduction speed (MEP latency) could be related to the severe deconditioning among our participants. Previous research supports that while there are significant differences in CSE measures between trained athletes and non-athletes (Pearce et al., 2000; Jensen et al., 2005; Monda et al., 2017), there are no differences between physically active and sedentary healthy subjects (Cirillo et al., 2009; Lulic et al., 2017), which suggests that long-term intense structured exercise training may be necessary in order to modulate brain mechanisms that enhance CSE. In support of this theory, Naghibzadeh et al. (2018), in an animal model of MS, showed that long term high intensity interval training protected against demyelination and loss of motor function, and increased neurotrophic factors more than moderate intensity continuous exercise (Naghibzadeh et al., 2018). Future research should examine whether long-term exercise, especially using higher-intensity type of training, could improve motor thresholds and MEP latency in MS.

5.5. Cytokines, fitness, and fatigue

Some studies propose that strength or aerobic training may have anti-inflammatory benefits in MS patients because of reduced

cytokine levels (e.g. interleukin (IL)-22, TNF, and IL-17F) (Alvarenga-Filho et al., 2016; Deckx et al., 2016; Kjolhede et al., 2016). We therefore hypothesized that MS patients with higher levels of fitness would have lower levels of TNF, a pro-inflammatory cytokine that is thought to be related to MS disease activity and demyelination (McCoy and Tansey, 2008; Dalgas and Stenager, 2012). However, we did not detect such association. This could be, again, due to the fact that our MS patients had very poor levels of fitness, too low to measure any anti-inflammatory benefit. Dalgas and Stenager (2012) and Negaresh et al., (2018) reported that the effects of exercise on cytokines levels in MS, including TNF, is equivocal, with some studies reporting increase, decrease or no change in cytokines after chronic or acute exercise (Dalgas and Stenager, 2012; Negaresh et al., 2018). These authors concluded that, due to the large methodological differences between studies and small sample sizes, there was no clear pattern of cytokine responses to exercise. Therefore, it remains unknown how fitness and exercise affect cytokine levels in MS, and, its relationships with CSE awaits future research.

Higher levels of TNF have been previously linked to fatigue in MS (Heesen et al., 2006). Therefore, we also hypothesized that levels of TNF would correlate with fatigue, however, no association was found. Similarly, in MS, Malekzadeh et al (2015) measured several anti- and pro-inflammatory cytokines, including TNF. With the exception of IL-6, there was no association between anti- or pro-inflammatory cytokines and fatigue (Malekzadeh et al., 2015). Whether levels of cytokines within the CNS (rather than the systemic circulation) are associated with fitness, fatigue or CSE is an area for future research. Inclusion of a wider range of pro- and anti-inflammatory cytokines (e.g. RANTES, IL-1β, IL-6, and TNF) are warranted in future studies investigating fitness, CSE, and MS symptoms.

6. Limitations

Although this study is the first to describe the relationship between fitness and CSE in MS, there are some limitations. First of all, we can not determine causality from this cross-sectional study. Longitudinal and interventional studies which measure fitness levels, CSE changes, and symptoms are necessary. The sample was one of convenience including consecutive patients recruited from an MS clinic, therefore generalizability cannot be assured. Furthermore, we attempted to recruit patients with a wide disability range, but those with EDSS > 4 were underrepresented. Although we showed that fitness predicted short CSP, the functionality of having short CSP in MS needs to be elucidated. For example, studies investigating fitness and CSP change, in response to paired-associative stimulation and learning are needed to determine the role of fitness and CSP on neuroplasticity. Also, prolonged CSP only accounted for 19.9% of the variance in fatigue suggesting that other factors besides intracortical inhibition might explain fatigue. Moreover, levels of fatigue were measured subjectively, and although it correlates with fatigability, measuring fatigue objectively could have better helped decipher the mechanisms underlying fatigue and its relationship to CSP. We used a visual analogue scale to measure the experience of fatigue at the time of testing as opposed to validated questionnaires such as the modified Fatigue Impact Scale (Fisk et al., 1994). Also, although fatigability of the FDI muscle during CSP assessment was unlikely (e.g. low level of voluntary contraction (10% MVC) and only 6 trials collected), it cannot be excluded. Therefore, it is possible that MS patients experiencing higher levels of hand fatigue during testing could have increased CSP. Moreover, the addition of other TMS measures (e.g. short and long intracortical inhibition) would have better helped elucidate the findings since, physiologically, CSP is modulated by both $GABA_A$ and $GABA_B$ receptors, and is from both spinal and cortical origins. The relationship between CSE and impact of fatigue in MS has yet to be elucidated. Also, MEPs could not be collected bilaterally from some participants because the TMS unit overheated or the stimulator output was not sufficient to elicit a MEP. Especially because of the latter, data from MS patients with greater unilateral CST damage could have been missed. Lastly, we attempted to use TNF as a biomarker of neuroinflammation, and possibly demonstrate that this biomarker would be decreased in MS patients with higher levels of fitness, decreased fatigue, or increased CSE. The susceptibility of this cytokine to daytime variations (Zhou et al., 2010; Altara et al., 2015), and gender-related differences (Nguyen et al., 2003; Luchetti et al., 2014), may explain these lack of associations between TNF and other values, especially in a cross-sectional design. Future research should take into consideration gender and time of the day when analysing TNF.

7. Conclusion

MS patients had extremely low levels of cardiorespiratory fitness; 60% were in the high risk category of all-cause mortality (<27 mL min^{-1} kg^{-1}) (Langeskov-Christensen et al., 2015), and 28% had cardiorespiratory fitness too low to comfortably carry-out activities of daily living (<20 mL min^{-1} kg^{-1}) (Cress et al., 2003), which may also worsen MS symptoms (Dalgas and Stenager, 2012; Riemenschneider et al., 2018). Evidence in healthy populations supports that long-term training and higher fitness improves LTP, lowers motor thresholds and quickens nerve conduction speed (shorter MEP latency) (Fulton et al., 2002; Cirillo et al., 2009; Lulic et al., 2017; Monda et al., 2017). However, we did not detect an association between cardiorespiratory fitness and RMT, AMT, or MEP latency in this cohort of MS patients, which could be a result of their extremely low levels of cardiorespiratory fitness. Importantly, CSP, a measure of GABAergic-mediated intracortical inhibition, was a more sensitive biomarker, with poor cardiorespiratory fitness predicting greater intracortical inhibition (prolonged CSP). Prolonged CSP is indicative of neurological impairment (Tataroglu et al., 2003) and diminished neuroplastic capacity (Sale et al., 2007). Moreover, greater intracortical inhibition, but not lower cardiorespiratory fitness, predicted fatigue, which may suggest that this mechanism of $GABA_B$-mediated type of intracortical inhibition may explain some of the central fatigue experienced by MS patients. Levels of the pro-inflammatory cytokine TNF was not associated with any physical (fitness and fatigue) or TMS measure. Our findings support that poor cardiorespiratory fitness in MS patients may negatively impact brain mechanisms that are important for neuroplasticity to occur. Therapists should encourage physical exercise strategies such as aerobic exercise in order to improve cardiorespiratory fitness in MS patients. Assessing the effects of long-term exercise on brain excitability in MS is worthy of future research.

Disclosures

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Author contributions

AC designed the experiment, collected, cleaned, analyzed the data, interpreted the findings, wrote, edited and submitted the manuscript. LK designed the experiment, collected data and edited

the manuscript. CM performed analysis of cytokines and edited the manuscript. MS assessed and screened patients. MP conceived of and designed the experiment, screened subjects, and edited manuscript.

Declarations of interest

None.

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