

Forearm muscle mitochondrial capacity and resting oxygen uptake: Relationship to symptomatic fatigue in persons with multiple sclerosis

*Multiple Sclerosis Journal—
Experimental, Translational
and Clinical*

April–June 2021, 1–10

DOI: 10.1177/
20552173211028875© The Author(s), 2021.
Article reuse guidelines:
sagepub.com/journals-
permissionsElizabeth M DePauw* , Mitra Rouhani* , Aidan M Flanagan and Alexander V Ng 

Abstract

Background: Mitochondrial dysfunction has been implicated in the pathogenesis of multiple sclerosis (MS). Whether mitochondrial alterations are a function of ambulatory dysfunction or are of a non-ambulatory systemic nature is unclear.

Objective: To compare oxidative capacity, and rest muscle oxygen consumption ($m\dot{V}O_2$) in the upper limb of persons with multiple sclerosis (PwMS) to a control group (CON), whereby an upper limb would be comparatively independent of ambulation or deconditioning.

Methods: Near infra-red spectroscopy was used to measure oxidative capacity of the wrist flexors in PwMS ($n = 16$) and CON ($n = 13$). Oxidative capacity was indicated by the time constant (TC) of $m\dot{V}O_2$ recovery following brief wrist flexion contractions. Measurements included well-being, depression, symptomatic fatigue, disability, handgrip strength, cognition, and functional endurance. Analysis was by T-tests and Pearson correlations with $p \leq 0.05$. Data are mean (SD).

Results: TC of $m\dot{V}O_2$ recovery was slower in PwMS (MS = 47(14) sec, CON = 36(11) sec; $p = 0.03$). No significant correlations were found between oxidative capacity and any other measures. Rest $m\dot{V}O_2$ was not different between groups, but correlated with symptomatic fatigue ($r = 0.694$, $p = 0.003$) and strength (0.585, $p = 0.017$) in PwMS.

Conclusion: Oxidative capacity was lower in the wrist flexors of PwMS, possibly indicating a systemic component of the disease. Within PwMS, rest $m\dot{V}O_2$ was associated with symptomatic fatigue.

Keywords: Multiple sclerosis, fatigue, gait, upper extremity, muscle oxidative capacity

Date received: 19 March 2021; accepted: 10 June 2021

Introduction

Multiple Sclerosis (MS) is a progressive inflammatory disease of the central nervous system.¹ Subjective self-reported symptomatic fatigue is common in persons with MS (PwMS),^{1,2} as is increased muscle fatigability.^{3,4} Muscle fatigability refers to the susceptibility of a muscle to fatigue, whereby muscle fatigue can be defined as a decrease in muscle force generating capacity.⁵ The association between symptomatic fatigue and muscle fatigability is unclear.^{3,4,6,7} Furthermore, PwMS are characterized with walking impairment and decreased physical activity which, in turn, may result in deconditioning and further loss of function.⁸

Increased muscle fatigability can originate from central impairments or peripheral impairments secondary to deconditioning,^{3,4,9} such as impaired oxidative or mitochondrial function. Decreased muscle oxidative capacity has been reported in lower limbs of PwMS compared with healthy populations, and could contribute to ambulation and gait impairments.^{9–11} Whether decreased mitochondrial oxidative capacity is secondary to impaired walking ability or is associated with the inflammatory state of the disease is unclear. Mitochondrial dysfunction can be associated with systemic inflammation which can lead to excessive generation of reactive oxygen species,^{12,13} and mitochondrial dysfunction and oxidative stress can be

*These authors share first authorship.

Correspondence to:
Alexander V Ng,
Alexander V Ng, Program in
Exercise Science,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI
53201-1881, USA.
alexander.ng@marquette.
edu

Elizabeth M DePauw*,
Program in Exercise Science,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI,
USA



Mitra Rouhani*,
Exercise and Rehabilitation
Science program,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI,
USA

Aidan M Flanagan,
Program in Exercise Science,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI,
USA

Alexander V Ng,
Program in Exercise Science,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI,
USA
Exercise and Rehabilitation
Science program,
Department of Physical
Therapy, Marquette
University, Milwaukee, WI,
USA

involved in MS.^{14–16} Therefore, investigating mitochondrial dysfunction, independent of ambulation could be important for PwMS. Surprisingly, there are no studies, that we are aware of, that report mitochondrial capacity in PwMS from an upper limb, which would be relatively independent of bipedal ambulation. Furthermore, evidence for altered rest muscle oxygen consumption (mVO_2) in PwMS is conflicting,^{11,17} and rest mVO_2 could also reflect skeletal muscle metabolic alterations, which have been suggested to indicate disease progression.¹⁷

Several methodologies are available for the assessment of muscle mitochondrial capacity. Muscle biopsy and ³¹P-magnetic resonance spectroscopy (³¹P-MRS) are both valid techniques to assess mitochondrial function in-vitro and in-vivo, respectively; however, several limitations such as the invasive nature of biopsies or the expense and availability of ³¹P-MRS have restricted their application in research.¹⁸ Near-infrared spectroscopy (NIRS) is another in-vivo approach to evaluate tissue oxygenation.^{19,20} Under ischemic conditions, NIRS can indicate mVO_2 , and the rate or time constant (TC) of recovery of mVO_2 back to rest levels after a series of contractions. The latter has been used as an indication of muscle mitochondrial capacity.^{21,22} This NIRS oxygen uptake recovery technique has been validated against phosphocreatine (PCr) recovery kinetics with ³¹P-MRS.²¹ Advantages of NIRS are that it is non-invasive, can obtain valid real-time measurements, can have low signal-noise during movement and has been used previously to indicate mitochondrial oxidative capacity in health and disease including in PwMS.^{23,24}

The objectives of this study were to compare mitochondrial oxidative capacity and mVO_2 in an upper limb of PwMS with healthy age-matched control participants (CON), evaluate the association of these measures with ambulatory measures, and evaluate the association of mitochondrial oxidative measures with non-ambulatory possibly systemic outcomes. We hypothesized that in an upper limb: 1) mitochondrial oxidative capacity is decreased in PwMS, 2) mitochondrial function is not associated with ambulatory measures, and 3) mitochondrial function is associated with non-ambulatory measures in PwMS.

Materials and methods

Participants

PwMS were recruited from the greater Milwaukee metropolitan area with the aid of the Wisconsin

Chapter of the National Multiple Sclerosis Society, local postings, and lists of past research participants. Participants must have been 20–66 years of age and diagnosed with MS for at least a year with no exacerbations or steroid use in the previous 3 months. Similarly aged-matched healthy adults without MS were also recruited from the same sources to serve as a control group. Exclusion criteria included any chronic conditions that could influence results including cardiovascular disease, metabolic diseases, mitochondrial myopathies, other co-existing neurologic conditions, or adipose tissue thickness (ATT) greater than 20 mm over the wrist flexor muscle belly. Signed informed consent, approved by the Marquette University Institutional Review Board, was obtained from all participants.

Protocol

Anthropometric and clinical information was gathered initially followed by a NIRS serial occlusion procedure,²² to indicate mitochondrial oxidative capacity in the wrist flexors of the non-dominant arm. Participants then completed cognitive and physical function assessments, and questionnaires. All testing took place in a single session.

NIRS device

Mitochondrial oxidative capacity was indicated by recovery of mVO_2 as measured with a NIRS oximeter.²² The NIRS measurements were obtained using a continuous wave oximeter (Portamon, Artinis Medical Systems, The Netherlands) which measures the relative changes in oxyhemoglobin/myoglobin and deoxyhemoglobin/myoglobin. The oximeter penetration depth was up to 20 mm. To ensure that adipose tissue did not exceed the oximeter penetration depth, ATT was measured on the muscle belly of the wrist flexors using ultrasound imaging (GE Vivid-E) and used as inclusion criteria.

Mitochondrial oxidative capacity assessment

During NIRS evaluation, participants rested supine with their non-dominant arm abducted to 90 degree, supported at heart level with an inflation cuff wrapped around their upper arm. The cuff was attached to a Hokanson E20 Rapid Cuff Inflator connected to a commercial air compressor (10020 C, California Air Tools, San Diego). Cuff pressures were maintained at 250 mmHg for all occlusion procedures. The NIRS oximeter was secured with double-sided tape on the middle of the wrist flexor muscle belly, and then the limb and oximeter were covered with a black elastic limb sleeve. Finally, all was wrapped loosely with a black cloth to block ambient light.

Rest mVO_2 was assessed during 30 s arterial occlusions, averaged over three trials each separated by 60 seconds. The slope of the change in oxygen saturation, during cuff inflation was used to indicate rest mVO_2 . All NIRS measurements were recorded using OxySoft (ver. 3.0.97.1, Artinis Medical Systems, The Netherlands).

An ischemic and hyperemic period was used to provide a physiological calibration for the NIRS, as described previously.²² After a 2-minute rest period, participants performed dynamic wrist flexion exercise 10 times at 1–2 Hz while holding a 1-kilogram (kg) weight to activate the wrist flexor muscle group, after which the cuff was inflated for 5–7 minutes until the NIRS oxy- and deoxyhemoglobin levels stabilized with no further change in oxygen saturation. This represented 0% muscle oxygenation at the site being measured. The cuff was then released causing a hyperemic response in which the maximum overshoot of the NIRS signal was used to represent 100% of muscle oxygenation.

Mitochondrial oxidative capacity was quantified as the rate of recovery of activated wrist flexor mVO_2 back to the rest mVO_2 after a series of brief contractions. For this procedure, participants were handed a 1 kg weight and performed dynamic wrist flexion exercise for 10–15 repetitions at 1–2 Hz until the oxyhemoglobin signal decreased to about 30% but no more than 50% of the original signal.²⁵ Immediately following these contractions, a series of repeated cuff inflations was performed to indicate the recovery of mVO_2 after exercise. Inflations were administered as follows: inflations 1–7 were 5 s on and 5 s off, inflations 8–14 were 7 s on and 7 s off, and inflations 15–21 were 10 s on and 10 s off. Cuff inflation and deflation was controlled by a custom program in Spike 2 (version 6.18, Cambridge Electronic Design, Cambridge). During each cuff inflation, slopes of mVO_2 were obtained similar to the rest mVO_2 measurement. These methods have been described in more detail previously.²² A second series of contractions and occlusions followed for a total of two trials.

Analysis of NIRS data

NIRS data was analyzed using a custom-written MATLAB program whereby exercise recovery slopes were fit to a monoexponential curve according to $y = \text{End} - \Delta \times e^{-t/TC}$.²² In this equation, y represents mVO_2 , End is the mVO_2 at the end of dynamic wrist flexion exercise, Δ is the difference in mVO_2 from rest to End and TC is the fitting

time constant.²² A shorter time constant would indicate a quicker recovery time.

Cognitive and functional assessment

After the NIRS procedures, participants completed the Multiple Sclerosis Functional Composite Measure (MSFC) consisting of a Timed 25-Foot Walk (T25FW), 3-second Paced Auditory Serial Addition Test (PASAT-3"), and 9-hole peg test (9HPT) to indicate disease status.²⁶ The individual tests were also used as measures of functional ability. The Symbol Digit Modality Test (SDMT) was administered as an additional cognitive measure.²⁷ Strength was indicated by handgrip maximal voluntary contraction (MVC) of 3–5 sec using an isometric handgrip dynamometer (JAMAR 5030J1, Sammons Preston, Chicago, IL). Handgrip strength is considered a valid measure of whole-limb strength.²⁸ Functional endurance was indicated by the 6-minute walk test (6MWT).²⁹

Following the cognitive and functional measurements, each participant completed the following questionnaires: Patient Determined Disease Steps (PDDS) to indicate the level of disability,³⁰ Centers for Epidemiological Studies Depression Scale (CES-D) to indicate depressive symptomology,³¹ the Fatigue Impact Scale (FIS) to measure symptomatic self-reported fatigue,³² and the Patient-Reported Outcomes Measurement Information System (PROMIS) Global Well-Being (PROMIS-GWB) to indicate well-being.³³

Statistical analysis

Statistical analyses were performed using SPSS 24.0 (IBM, Armonk, NY). Group comparisons between PwMS and CON were performed with independent samples T-tests. Bivariate Pearson correlation was also used to investigate associations between variables of interest. Significance was selected as $p \leq 0.05$. Data are presented as mean (SD).

Results

Baseline characteristics

Sixteen PwMS (M = 3, F = 13) and thirteen CON (M = 5, F = 8) participated in the study. Average age, height, and weight of PwMS and CON were similar (Table 1). PwMS varied in diagnosis: 11 relapsing-remitting, 3 secondary-progressive, and 2 primary-progressive. Disease durations ranged from 2–29 years. Level of disability as determined by the PDDS could be considered mild-moderate.

Table 1. Anthropometric and physical characteristics of PwMS and CON. ATT= adipose tissue thickness. Values are Mean (SD).

	MS	Control	p-value
n	16	13	–
Sex (M/F)	3/13	5/8	–
Age (years)	51 (9)	49 (10)	0.56
Height (cm)	167 (7)	169 (10)	0.51
Weight (kg)	70 (14)	78 (19)	0.20
ATT (mm)	2.1 (0.9)	2.0 (0.5)	0.72

Clinical and functional outcome measures

Outcome measures for PwMS and CON are in Table 2. Rest mVO_2 was similar in PwMS and CON but mitochondrial capacity was lower in PwMS as indicated by the longer TC of mVO_2 recovery ($p=0.03$), whereby PwMS had slower mVO_2 recovery to rest levels than CON (Figure 1).

PwMS performed or reported significantly worse MSFC total scores ($p < 0.01$), 9HPT ($p < 0.01$), cognition ($p < 0.01$), handgrip strength ($p < 0.02$), 6MWT ($p = 0.01$), depression ($p < 0.01$), symptomatic fatigue ($p < 0.01$) and well-being ($p < 0.01$).

Bivariate Pearson correlations were calculated among PwMS for the mitochondrial related measures, for all variables that were different than CON, or that were otherwise associated with such variables (Table 3). Rest mVO_2 of the wrist flexors was related to symptomatic fatigue whereby the higher the rate of rest mVO_2 , the greater the reported symptomatic fatigue (Figure 2). Rest mVO_2 was also significantly associated with strength of the wrist flexors, whereby the higher the rate of rest mVO_2 , the greater the reported strength (Figure 3). No significant correlations were noted between the mitochondrial capacity and any other measures, including grip-strength and 9HPTC. Though not associated with muscle mitochondrial capacity, symptomatic fatigue was correlated with depression ($r=0.584$, $p=0.018$), and inversely correlated to well-being ($r=-0.653$, $p=0.006$). Further, MSFC was correlated with 6MWT ($r=0.585$, $p=0.028$). PDDS was also associated with T25FW ($r=0.674$, $p=0.008$) and inversely associated with 6MWT ($r=-0.609$, $p=0.730$).

To give context to our results, we also performed similar bivariate correlations in CON. For CON, there were no significant correlations ($p > 0.05$) between any variable and mitochondrial capacity

(TC) including 9HPT ($r=-0.27$, $p=0.38$) and grip strength (MVC, $r=0.06$, $p=0.86$). Similarly, there were no significant correlations between any variable and rest mVO_2 including symptomatic fatigue (FIS, $r=-0.12$, $p=0.71$) and grip strength (MVC, $r=-.282$, $p=0.35$). Although both depressive symptomatology and symptomatic fatigue were significantly lower in CON compared to MS, depression was nevertheless related to symptomatic fatigue in a similar fashion and magnitude as in PwMS ($r=0.56$, $p=0.05$).

Discussion

To the best of our knowledge, this is the first study to analyze mitochondrial oxidative capacity in PwMS in a muscle comparatively independent of upright locomotion and thus ambulation status. As such, we considered this upper limb measure of mitochondrial capacity to indicate an ambulatory independent measure, possibly of systemic origin. Our primary result was that PwMS had lower mitochondrial oxidative capacity compared to CON, as indicated by longer TC of mVO_2 recovery (Figure 4). In addition, we made the novel observation that rest mVO_2 in PwMS was associated with self-reported symptomatic fatigue.

Mitochondrial oxidative capacity in upper limb of PwMS

Impaired muscle mitochondrial oxidative capacity in PwMS has been reported previously in lower limb muscles. Kent-Braun and colleagues examined the rate of phosphocreatine (PCr) recovery in the tibialis anterior muscle of a group of mildly-moderately disabled but mobile PwMS compared to control participants with ^{31}P -MRS.¹⁰ They reported slower PCr recovery (i.e., lower mitochondrial oxidative capacity) following a brief contraction in PwMS, which was attributed to reduced physical activity and deconditioning although physical activity was not measured in this study. Harp and colleagues also reported lower mitochondrial oxidative capacity from lower limb of a group of mobile PwMS compared to control participants using a similar NIRS protocol as used in our study.¹¹ In this later study, the authors did not find significant associations between calf mitochondrial capacity and walking speed. However, a tendency towards lower calf muscle mitochondrial capacity was noted in those PwMS who used an assistive device. Physical activity was measured subjectively by questionnaire and no differences were found between the groups studied, although the MS group tended to report greater physical activity.¹¹ Thus, the question of whether

Table 2. Comparison of PwMS and CON clinical and functional outcomes.

	MS	Control	p-value
Rest mVO ₂ (%/s)	0.0032 (.0010)	0.0033 (.0020)	0.87
Time Constant (s)	47 (14)	36 (11)	0.03*
MSFC	0.73 (0.44)	1.73 (0.67)	<0.01*
T25FW (s)	5.1 (1.4)	4.2 (0.6)	0.08
PASAT-3	47 (11)	52 (7)	0.13
9HPT (s)	21.6 (1.6)	18.4 (1.8)	<0.01*
SDMT	57 (11)	71 (10)	<0.01*
MVC (kg)	33 (8)	40 (7)	<0.02*
6MWT (m)	536 (97)	633 (62)	0.01*
PDDS	2 (2)	—	—
CES-D	11 (7)	3 (3)	<0.01*
FIS	45 (34)	4 (5)	<0.01*
PROMIS-GWB	35 (5)	46 (3)	<0.01*

Rest mVO₂: muscle oxygen consumption at rest; Time Constant: time constant of mVO₂ recovery; MSFC: Multiple Sclerosis Functional Composite Measure; T25FW: Timed 25-foot Walk Test; PASAT: Paced Auditory Serial Addition Test; 9HPT: Nine Hole Peg Test; SDMT: Symbol Digit Modalities Test; MVC: Maximal Voluntary Contraction of the wrist flexors; 6MWT: Six Minute Walk Test; PDDS: Patient Determined Disease Steps; CES-D: Centers for Epidemiological Studies-Depression Scale; FIS: Fatigue Impact Scale; PROMIS-GWB: Patient-Reported Outcomes Measurement Information System Global Well-Being.

Note: See text for details. Values are mean (SD). significance was accepted as $p \leq 0.05$ and indicated by *.

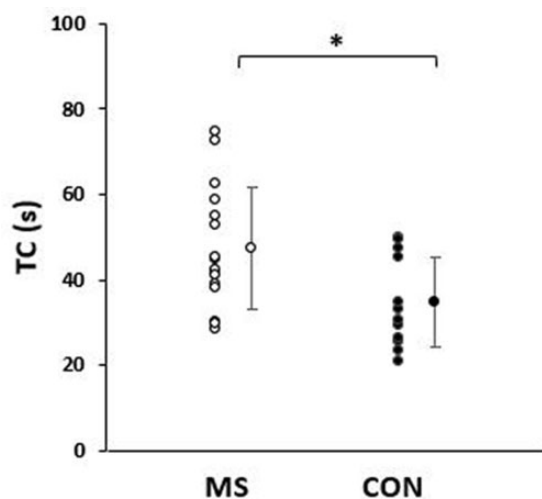


Figure 1. Time constant of mVO₂ recovery of the wrist flexors in persons with MS (MS) and control (CON). Open circles represent MS and solid circles represent CON. Mean and SD values for each group are indicated on the right side of the individual values. Asterisk denotes $p \leq 0.05$.

locomotor muscle mitochondrial capacity is a function of muscle use in PwMS remains unclear. Overall, lower muscle mitochondrial capacity in an upper body limb is consistent with what has been reported from lower limbs. In these previous

studies,^{10,11} lower mitochondrial oxidative capacity was discussed to be related to decreased physical activity or ambulation status. Our results would then suggest that lower muscle mitochondrial capacity in PwMS in locomotor muscles could be due to at least in part a systemic non-ambulatory component, possibly in addition to any effect of muscle deconditioning.

In accordance with our hypotheses, we did not find correlations between mitochondrial capacity and any of the ambulatory measures including the T25FW and 6MWT. Thus, the lower mitochondrial capacity of the wrist flexors was seemingly independent of ambulatory status. These results were also consistent with the T25FW results reported by Harp and colleagues.¹¹ However, Hansen and colleagues reported a significant relationship between muscle oxidative capacity by calculation of exercise-onset oxygen uptake kinetics during a cycling exercise and the distance walked in 6MWT.³⁴ Compared to the measurement of muscle oxidative capacity using NIRS, measuring the onset kinetics during exercise may not be considered a muscle-specific measurement; as it can be affected by central factors such as cardiovascular capacity.³⁵ Unlike the whole-body measures of oxidative capacity by Hansen and colleagues,³⁴ Willingham and colleagues showed that gastrocnemius muscle oxidative capacity was significantly

Table 3. Pearson bivariate correlations.

	Rest mVO ₂	TC	MSFC	T25FW	9HPT	SDMT	MVC	6MWT	PDDS	CES-D	FIS	PROMIS-GWB
Rest mVO ₂		-0.256	-0.130	0.057	-0.152	0.037	0.585	-0.070	0.066	0.330	0.694	-0.469
TC	0.338		0.659	0.843	0.588	0.893	0.017*	0.803	0.809	0.211	0.003*	0.067
MSFC				-0.101	0.094	0.062	0.060	-0.157	0.169	0.011	-0.339	0.020
T25FW			0.439		0.739	0.819	0.825	0.576	0.530	0.967	0.200	0.942
9HPT				-0.429		-0.036	-0.233	0.585	-0.317	-0.123	-0.317	0.414
SDMT				0.126	0.023*	0.904	0.422	0.028*	0.270	0.675	0.270	0.141
MVC					0.092	0.373	-0.423	-0.785	0.674	-0.221	0.109	0.305
6MWT					0.755	0.189	0.131	0.001*	0.008*	0.448	0.711	0.288
PDDS						-0.157	-0.003	0.494	0.358	0.283	0.017	-0.225
CES-D						0.576	0.990	0.072	0.190	0.307	0.952	0.420
FIS							0.039	-0.086	-0.302	-0.372	0.009	-0.049
PROMIS-GWB							0.886	0.760	0.255	0.156	0.972	0.857
								0.237	-0.230	0.208	0.275	-0.149
								0.394	0.392	0.439	0.303	0.583
									-0.609	0.168	-0.097	0.453
									0.730	0.549	0.730	0.090
										0.259	0.133	-0.177
										0.332	0.622	0.513
											0.584	-0.420
											0.018*	0.105
												-0.653
												0.006*

BMI: body mass index; Rest mVO₂: Rest muscle oxygen uptake; TC: Time Constant of mVO₂ recovery; MSFC: Multiple Sclerosis Functional Composite Measure; T25FW: Timed 25-foot Walk; 9HPT: Nine Hole Peg Test; SDMT: Symbol Digit Modalities Test; MVC: Maximal Voluntary Contraction of the wrist flexors; 6MWT = Six Minute Walk Test; PDDS: Patient Determined Disease Steps; CES-D: Centers for Epidemiological Studies-Depression Scale; FIS: Fatigue Impact Scale; PROMIS-GWB: Patient-Reported Outcomes Measurement Information System Global Well-Being.
 Note: See text for details. Values are correlation on the top and p-value on the bottom. significance was accepted as $p \leq 0.05$.

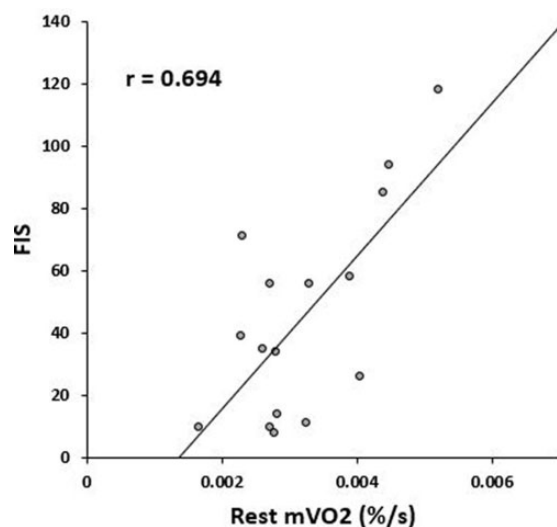


Figure 2. Pearson correlation between Fatigue Impact Scale and rest mVO_2 in persons with MS.

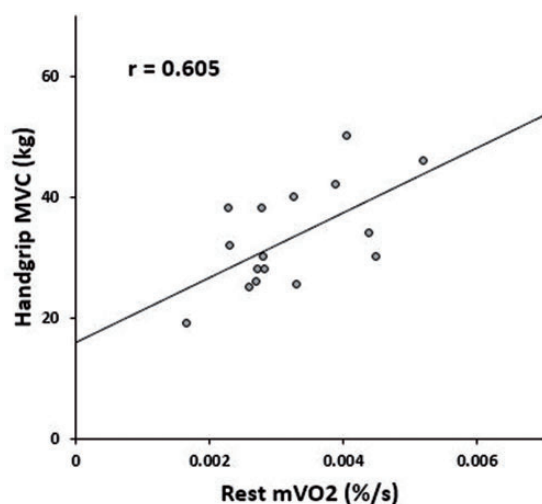


Figure 3. Pearson correlation between handgrip strength and rest mVO_2 in persons with MS.

associated with walking speed measured by T25FW and walking endurance measured by 6MWT in women with MS.³⁵ In this same study, oxidative capacity, walking speed and walking endurance was significantly lower in women with moderate-severe disability compared to women with mild disability.³⁵

Upper body function as indicated by handgrip strength (MVC) and motor control (9HPT), was affected in PwMS, and while pyramidal weakness or motor control could have contributed to decreased mitochondrial capacity compared to control, we failed to find correlations between forearm

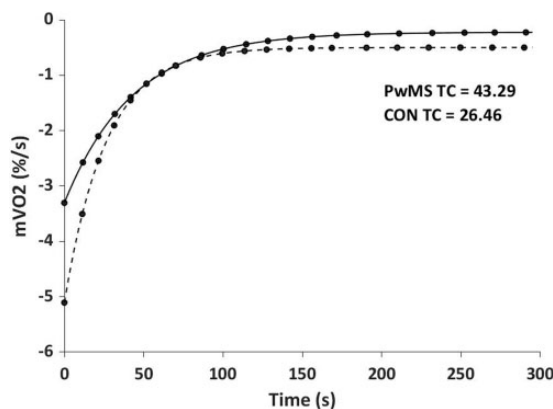


Figure 4. Representative fitted curves with time constant of mVO_2 recovery. Slopes of the mVO_2 recovery were fit to a monoexponential curve for a person with MS (PwMS) and a control participant. Solid line is from PwMS and dashed line is from a control participant. Solid circles represent the slopes of the mVO_2 recovery. TC= time constant of fitted curve.

mitochondrial capacity and these upper body physical function measures. These findings are consistent with decreased forearm oxidative measures as being independent of ambulation and not upper body physical function per se.

Contrary to our hypotheses was the lack of correlation between forearm mitochondrial capacity and any of the not specifically ambulatory or physical measures including well-being, depression, symptomatic fatigue, disease status, disability, or cognition. Thus, the functional or clinical implications of decreased mitochondrial capacity in the wrist flexors are unclear.

We speculate that impaired systemic mitochondrial function could be characteristic of MS. PwMS can have diminished mitochondrial capacity in circulating peripheral blood mononuclear cells (PBMC), e.g., lymphocytes,³⁶ and thus, systemic mitochondrial function might be characteristic of MS, presumably mediated through inflammation and the production of reactive oxygen or nitrogen species. Further, there is evidence that impaired mitochondrial function in PBMC is associated with altered mitochondrial function in skeletal or cardiac muscle.³⁷ However, although systemic impaired mitochondrial function in PwMS is an attractive hypothesis, we have no evidence to support this.

Resting muscle oxygen uptake

Our finding of similar rest mVO_2 in PwMS and CON agrees with a previous report in the calf muscles,¹¹ but are contrary to the greater mVO_2 ,

also reported in the calf.¹⁷ We can only speculate that differences noted in the later could have been due some sample specific characteristics such as a greater range of disability. The novel finding that rest mVO_2 was associated with symptomatic fatigue in PwMS was unexpected, given the similarity of local rest mVO_2 in PwMS and CON. Because mitochondrial oxidative capacity was lower in PwMS, it could be that relative to maximal capacity, PwMS were using a higher percent of their maximal level at any absolute level of oxygen consumption at rest resulting in higher relative mVO_2 at rest. Thus, those PwMS with higher rest mVO_2 percent of maximum, might translate to greater perceived fatigue. Previous studies did not compare rest mVO_2 to functional measures. Correlations do not imply causality, and the associations we observed with rest mVO_2 could be result from other factors not measured. Thus, the role of resting metabolism in MS is unclear and should be the subject of further investigation.

Based on our results, PwMS were significantly weaker than CON as indicated by handgrip strength, an indication of whole-body strength.²⁸ Nevertheless, the direct association of rest mVO_2 to handgrip strength was unexpected. We can only question whether stronger persons may have greater muscle mass within the field of measurement of the oximeter.

Along with rest mVO_2 , symptomatic fatigue was associated with depression and inversely related to well-being, as expected. Also, PDDS was correlated with T25FW. These correlations all attest to the internal validity of our measurement procedures. In addition, mVO_2 was not significantly associated with depression, which suggests that depression and mVO_2 are related to symptomatic fatigue through different pathways.

Future studies should identify possible roles and mechanisms for decreased mitochondrial oxidative capacity and both ambulatory and non-ambulatory measures. Also, whether mitochondrial capacity is altered proportionally throughout the body remained unanswered. Comparing oxidative capacity between upper and lower limbs in a same group of PwMS would provide a better understanding of the effect of the disease on mitochondrial function.

Limitations

A primary limitation just mentioned is that because of the pilot nature of our studies, we did not obtain NIRS measurements from both upper and lower

body limbs. Further, our participants were mildly-moderately affected by MS, and our sample size was comparatively small; both of which affect power to find correlations as well as generalization and application of this result to other groups of PwMS with greater disability.

The PwMS in our study differed significantly from CON as evidenced by clinical and functional measures. We used PDDS instead of the Expanded Disability Status Scale (EDSS), which is often used for the assessment of functional disability in PwMS.³⁸ However, the PDDS is a simple, valid, and reproducible tool for measuring primarily gait disability in PwMS and is highly correlated with EDSS.³⁰ Finally, a limitation of the NIRS measurements is excluding people with ATT greater than 20 mm. This subcutaneous fat criteria limited our sample and the generalizability of our results.

Conclusion

Based upon the finding of slower mVO_2 recovery in upper limb muscles of PwMS compared to CON, we concluded that PwMS have lower muscle mitochondrial oxidative capacity that is independent of ambulation. Further, based on correlation analysis we also conclude that rest mVO_2 is related to symptomatic fatigue in PwMS, and that this finding may be related by unknown mechanisms to decreased muscle mitochondrial capacity. These results are novel and could be clinically significant and might benefit neurologists and clinicians in terms of assessment and rehabilitative approaches; based on our results, impaired oxidative capacity might not be fully explained by deconditioning but might indicate a systemic component of the disease, possibly resulting from primary immune system dysfunction.

Acknowledgements

The authors would like to thank all the participants of this study, and Dr. Kevin K. McCully for his help with the methodology and analysis.

Conflict of Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.


Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially funded by Marquette University's Regular Research Grant Program.

ORCID iDs

Elizabeth M DePauw  <https://orcid.org/0000-0003-0805-4081>

Mitra Rouhani  <https://orcid.org/0000-0002-9066-4403>

Alexander V Ng  <https://orcid.org/0000-0002-0742-9825>

References

- Compston A and Coles A. Multiple sclerosis. *Lancet* 2008; 372: 1502–1517.
- Kos D, Kerckhofs E, Nagels G, et al. Origin of fatigue in multiple sclerosis: review of the literature. *Neurorehabil Neural Repair* 2008; 22: 91–100.
- Sheean GL, Murray NM, Rothwell JC, et al. An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain* 1997; 120: 299–315.
- Zijdewind I, Prak RF and Wolkorte R. Fatigue and fatigability in persons with multiple sclerosis. *Exerc Sport Sci Rev* 2016; 44: 123–128.
- Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 2001; 81: 1725–1789.
- Sharma KR, Kent-Braun J, Mynhier MA, et al. Evidence of an abnormal intramuscular component of fatigue in multiple sclerosis. *Muscle Nerve* 1995; 18: 1403–1411.
- Steens A, de Vries A, Hemmen J, et al. Fatigue perceived by multiple sclerosis patients is associated with muscle fatigue. *Neurorehabil Neural Repair* 2012; 26: 48–57.
- Motl RW, Goldman MD and Benedict RH. Walking impairment in patients with multiple sclerosis: exercise training as a treatment option. *Neuropsychiatr Dis Treat* 2010; 6: 767–774.
- Kent-Braun JA, Ng AV, Castro M, et al. Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis. *J Appl Physiol* 1997; 83: 1998–2004.
- Kent-Braun JA, Sharma KR, Miller RG, et al. Postexercise phosphocreatine resynthesis is slowed in multiple sclerosis. *Muscle Nerve* 1994; 17: 835–841.
- Harp MA, McCully KK, Moldavskiy M, et al. Skeletal muscle mitochondrial capacity in people with multiple sclerosis. *Mult Scler J Exp Transl Clin* 2016; 2: 1–7.
- Chacko BK, Kramer PA, Ravi S, et al. The bioenergetic health index: a new concept in mitochondrial translational research. *Clin Sci (Lond)* 2014; 127: 367–373.
- Kalman B and Leist TP. A mitochondrial component of neurodegeneration in multiple sclerosis. *Neuromolecular Med* 2003; 3: 147–157.
- Mirshafiey A and Mohsenzadegan M. Antioxidant therapy in multiple sclerosis. *Immunopharmacol Immunotoxicol* 2009; 31: 13–29.
- Hansen D, Wens I, Vandenebeele F, et al. Altered signaling for mitochondrial and myofibrillar biogenesis in skeletal muscles of patients with multiple sclerosis. *Transl Res* 2015; 166: 70–79.
- Mao P and Reddy PH. Is multiple sclerosis a mitochondrial disease? *Biochim Biophys Acta* 2010; 1802: 66–79.
- Malagoni AM, Felisatti M, Lamberti N, et al. Muscle oxygen consumption by NIRS and mobility in multiple sclerosis patients. *BMC Neurol* 2013; 13: 5.
- Lanza IR, Bhagra S, Nair KS, et al. Measurement of human skeletal muscle oxidative capacity by ³¹P-MR spectroscopy: a cross-validation with in vitro measurements. *J Magn Reson Imaging* 2011; 34: 1143–1150.
- Boushel R and Piantadosi CA. Near-infrared spectroscopy for monitoring muscle oxygenation. *Acta Physiol Scand* 2000; 168: 615–622.
- Hamaoka T and McCully KK. Review of early development of near-infrared spectroscopy and recent advancement of studies on muscle oxygenation and oxidative metabolism. *J Physiol Sci* 2019; 69: 799–811.
- Ryan TE, Southern WM, Reynolds MA, et al. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *J Appl Physiol* 2013; 115: 1757–1766.
- Ryan TE, Erickson ML, Brizendine JT, et al. Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. *J Appl Physiol (1985)* 2012; 113: 175–183.
- Boushel R, Langberg H, Olesen J, et al. Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. *Scand J Med Sci Sports* 2001; 11: 213–222.
- Willingham TB and McCully KK. In vivo assessment of mitochondrial dysfunction in clinical populations using near-infrared spectroscopy. *Front Physiol* 2017; 8: 689.
- Ryan TE, Brizendine JT and McCully KK. A comparison of exercise type and intensity on the noninvasive assessment of skeletal muscle mitochondrial function using near-infrared spectroscopy. *J Appl Physiol* 2013; 114: 230–237.
- Fischer JS, Rudick RA, Cutter GR, et al. The multiple sclerosis functional composite measure (MSFC): an integrated approach to MS clinical outcome assessment. *Mult Scler* 1999; 5: 244–250.
- Benedict RH, DeLuca J, Phillips G, et al.; Multiple Sclerosis Outcome Assessments Consortium. Validity of the symbol digit modalities test as a cognition performance outcome measure for multiple sclerosis. *Mult Scler* 2017; 23: 721–733.
- Bohannon RW, Magasi SR, Bubela DJ, et al. Grip and knee extension muscle strength reflect a common construct among adults. *Muscle Nerve* 2012; 46: 555–558.
- Enright PL. The six-minute walk test. *Respir Care* 2003; 48: 783–785.
- Learmonth YC, Motl RW, Sandroff BM, et al. Validation of patient determined disease steps

- (PDDS) scale scores in persons with multiple sclerosis. *BMC Neurol* 2013; 13: 37.
31. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1977; 1: 385–401.
 32. Fisk JD, Pontefract A, Ritvo PG, et al. The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci* 1994; 21: 9–14.
 33. Hays RD, Bjorner JB, Revicki DA, et al. Development of physical and mental health summary scores from the patient-reported outcomes measurement information system (PROMIS) global items. *Qual Life Res* 2009; 18: 873–880.
 34. Hansen D, Feys P, Wens I, et al. Is walking capacity in subjects with multiple sclerosis primarily related to muscle oxidative capacity or maximal muscle strength? A pilot study. *Mult Scler Int* 2014; 2014: 1–7.
 35. Willingham TB, Backus D and McCully KK. Muscle dysfunction and walking impairment in women with multiple sclerosis. *Int J MS Care* 2019; 21: 249–256.
 36. La Rocca C, Carbone F, De Rosa V, et al. Immunometabolic profiling of T cells from patients with relapsing-remitting multiple sclerosis reveals an impairment in glycolysis and mitochondrial respiration. *Metabolism* 2017; 77: 39–46.
 37. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, et al. Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol* 2016; 10: 65–77.
 38. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983; 33: 1444–1452.