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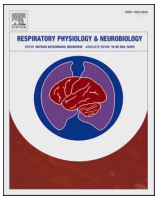

Inspiratory muscle training improves autonomic function in myalgic encephalomyelitis/chronic fatigue syndrome and post-acute sequelae of SARS-CoV-2: A pilot study

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ABSTRACT

Post-acute sequelae of SARS-CoV-2 (PASC), or Long COVID, and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) are debilitating post-viral conditions with many symptomatic overlaps, including exercise intolerance and autonomic dysfunction. Both conditions are growing in prevalence, and effective safe treatment strategies must be investigated. We hypothesized that inspiratory muscle training (IMT) could be used in PASC and mild to moderate ME/CFS to mitigate symptoms, improve exercise capacity, and improve autonomic function. We recruited healthy controls (n=12; 10 women), people with PASC (n=9; 8 women), and people with mild to moderate ME/CFS (n=12; 10 women) to complete 8 weeks of IMT. This project was registered as a clinical trial (NCT05196529) with clinicaltrials.gov. After completion of IMT, all groups experienced improvements in inspiratory muscle pressure ($p<0.001$), 6-minute walk distance ($p=0.002$), resting heart rate ($p=0.037$), heart rate variability ($p<0.05$), and symptoms related to sleep ($p=0.009$). In the ME/CFS group only, after completion of IMT, there were additional improvements with regard to vascular function ($p=0.001$), secretomotor function ($p=0.023$), the total weighted score ($p=0.005$) of the COMPASS 31 autonomic questionnaire, and symptoms related to pain ($p=0.016$). We found that after 8 weeks of IMT, people with PASC and/or ME/CFS could see some overall improvements in their autonomic function and symptomology.

1. Introduction

The Canadian Consensus Criteria define myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) as 1) new onset, unexplained, persistent physical and mental fatigue that substantially reduced activity, 2) post-exertional malaise or fatigue with pathologically slow recovery, 3) sleep dysfunction, 4) pain in muscles and joints, 5) neurological/cognitive manifestations, 6) illness persisting for at least 6 months, and 7) at least 1 symptom within: i) autonomic dysfunction such as orthostatic intolerance, ii) neuroendocrine, iii) immune manifestations (Carruthers et al., 2003). ME/CFS is often triggered by a viral infection (Hanson, 2023). Recently, the COVID-19 pandemic has resulted in the potential development of Post-Acute Sequelae of SARS-CoV-2 (PASC), or "Long COVID'' defined by the World Health Organization (WHO) as the continuation or development of COVID-19 symptoms for >3 months after the initial infection, with these symptoms lasting for >2 months with no other explanation. The WHO states that 10–20 % of people infected with COVID-19 have developed overt PASC, and in a fatigued cohort of 134 PASC patients, 43 % developed ME/CFS as defined by the Institute of Medicine (IOM) criteria (Bonilla et al., 2023). Similarly, a recent meta-analysis found a similar prevalence of PASC after COVID-19 infection of 43 % after synthesizing data from 50 studies including over 1.5 million individuals (Chen et al., 2022). Therefore, millions of people worldwide have developed PASC and/or ME/CFS over the course of the pandemic to date.

Both ME/CFS and PASC patients often have chronic orthostatic

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intolerance, leading to periods of dizziness, faintness, cognitive dysfunction, and near-syncope when upright, relieved by recumbent posture. Indeed, postural orthostatic tachycardia syndrome (POTS) is the primary vascular instability of ME/CFS, and there is growing evidence that many COVID survivors are developing POTS (Johansson et al., 2021; Kanjwal et al., 2020; Miglis et al., 2020; Raj et al., 2021). It has recently been found that PASC and ME/CFS have similar incidence of orthostatic intolerance and have similar hemodynamic and brain blood responses to upright tilt (van Campen and Visser, 2022). Many of the persistent symptoms in PASC could be attributed to autonomic dysfunction (e.g. fatigue, trouble breathing, excessive sweating), further suggesting that orthostatic intolerance may be a concern for individuals with PASC. Indeed, Joseph et al. recently suggested that neurovascular dysfunction could contribute to exercise intolerance in ME/CFS (Joseph et al., 2022). Further, SARS-CoV-2 is known to use angiotensin-converting enzyme 2 (ACE2) to enter cells (Hoffmann et al., 2020), and ACE2 is present in the brainstem, where it could influence hemodynamic control and autonomic function (Lukiw et al., 2022; Yamazato et al., 2007).

We suggest that inspiratory muscle training (IMT) will improve autonomic health and symptomology in ME/CFS and PASC (both post-viral conditions), particularly through improvements in the respiratory metaboreflex (as observed previously in healthy participants) (Chan et al., 2023) and thus autonomic balance. Notably, IMT has been shown to reduce blood pressure and heart rate responses to inspiratory muscle force generation and to improve vagal tone and whole-body exercise performance in healthy subjects (Rodrigues et al., 2018, 2021; Witt et al., 2007). Further, IMT has been shown to attenuate the ventilatory response to exercise, improve oxygen consumption, and improve the 6-minute walk distance (6MWD) in heart failure patients (Baral et al., 2020; Cahalin et al., 2013; Mello et al., 2012; Rodrigues et al., 2018). The 6MWD is a measure of functional capacity often used clinically in populations with movement impairment such as lung or heart disease (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002.). It is also important to note that hyperventilation has been noted in many patients with COVID-19 (Baratto et al., 2021; Crisafulli et al., 2021) and in patients with ME/CFS (Medow et al., 2014); the concurrent hypocapnia increases mortality risk (Hu et al., 2020). Further, hyperventilation due to impaired autonomic control has been suggested as a possible cause of exercise intolerance in COVID-19 survivors (Motiejunaite et al., 2020).

The two purposes of this study are first to investigate the autonomic function (primary) and symptomatology (secondary) of PASC and ME/CFS in comparison to each other and to a healthy control group. We hypothesize that both groups will have impaired autonomic function and more symptoms compared to the healthy control group. Secondly, we hypothesize that the use of an 8-week IMT protocol will be effective in improving autonomic function, exercise tolerance and symptoms of PASC and ME/CFS.

2. Materials and methods

2.1. Study design

This study was a pilot interventional open label single group assignment study of three groups (controls, ME/CFS, PASC) participating in an IMT protocol with measurements obtained before and after the intervention. As this was not a randomized trial, no researchers or participants were blinded to the study condition. All participants provided written informed consent approved by the Research Ethics Board of York University, Toronto, Ontario. This project was registered as a clinical trial (NCT05196529) and participants were recruited between February 1, 2022 and December 15, 2022. Testing was completed by February 1, 2023.

2.2. Participants

Healthy controls (Men, n=2; Women, n=10), people with PASC who had a COVID-19 infection and had ongoing symptoms for at least 3 months (Men, n=1; Women, n=8) and people with mild to moderate ME/CFS (Men, n=2; Women, n=10) were recruited to participate in the study (Fig. 1). Classification as “mild to moderate” was based on self-report and we excluded “severe” cases (mainly house or bed bound) as a precaution. As this was a pilot study, the sample size was determined by feasibility and funding. Participants were recruited in the Greater Toronto Area from collaborative clinics or public advertisements and must have been clinically diagnosed with PASC or ME/CFS. PASC patients were specifically asked to describe their novel symptoms after their COVID-19 infection, and ME/CFS patients had never been infected with COVID-19. Communication for recruitment was via phone or e-mail. It is important to note that all participants were fully informed about the requirements of testing, including the 6-minute walk test and inspiratory muscle training (described below) and only participated if they felt confident that they could do so bearing their own health and capabilities in mind to pro-actively avoid inducing post-exertional malaise and as such, participants were fully encouraged to communicate with the researchers about their ongoing capacity to continue in the study and were not pressured in any way to continue if they felt the need to stop. Two to three participants in each group could not complete the study after participating in baseline data collection (Fig. 1). For the healthy controls (n=3 dropouts, all women), reasons included vacation, COVID-19 infection, and lack of response during follow-up. For the PASC group (n=2 dropouts, all women), reasons included scheduling conflicts and lack of response during follow-up. For the ME/CFS group (n=3 dropouts, all women), the reasons included an accident resulting in hospitalization, a temporal mandibular joint disorder with pain during IMT, and post-exertional malaise. Only data with both baseline and follow-up visits completed were included in the analysis.

2.3. Protocols

2.3.1. Inspiratory muscle training (IMT)

The maximal inspiratory muscle pressure (MIP) of each participant was measured at York University (Respiratory Force Pressure Gauge, Vacumed, USA), a simple non-invasive procedure for determining inspiratory muscle strength. IMT consisted of 8 weeks of breath training done at home (described below) using either a Medium Resistance Trainer (POWERbreathe Plus IMT, UK) or Threshold Inspiratory Muscle Trainer (Vacumed, USA) training device according to their individual capacity. Due to some participants having higher MIP than anticipated prior to starting the study (meaning less impairment), 8/12 controls, 6/9 PASC, and 6/12 ME/CFS participants used the POWERbreathe device.

The IMT devices were set at 80 % of each individual’s MIP for the duration of the study. The IMT protocol was conducted 3 times per week (specific days were at the discretion of the participants) where each session consisted of 6 sets of 6 repetitions (total of 108 breaths/week) with the goal of progressively reducing the rest time between sets from 60 s for the first 2 weeks to 10 s for the last week. This protocol has been shown to be effective in increasing pulmonary function and work capacity (Enright and Unnithan, 2011). We chose this protocol with an effort to minimize the development of post-exertional malaise and to work with each participant’s current functional capacity. The time interval was reduced in consultation with the participants through follow-up by telephone or e-mail every week and after considering the participant’s health status and comfort with the breathing exercises. On week 1, 2 PASC patients described the presence of lightheadedness, and on week 6, there was 1 PASC patient who described dizziness. There were no other self-declared symptoms or complaints throughout the 8-week protocol. Adherence to the protocol was determined using log-sheets and continual communication between participants and researchers via phone/e-mail.

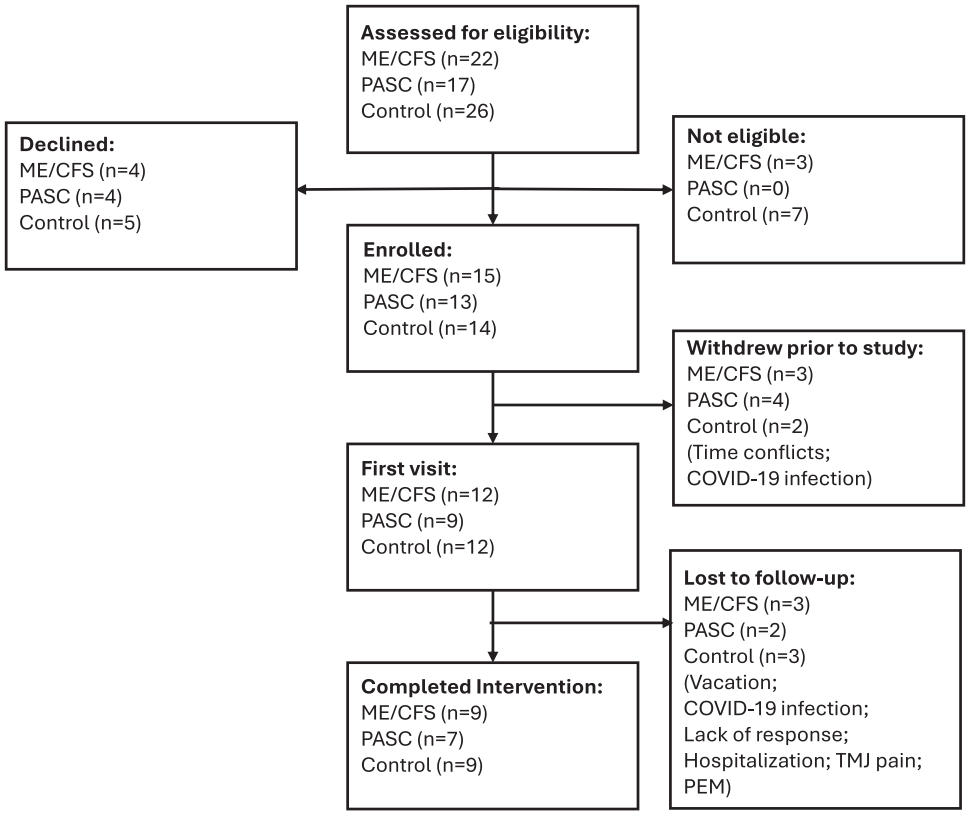

Fig. 1. : CONSORT Diagram for recruitment process. ME/CFS is myalgic encephalomyelitis/chronic fatigue syndrome; PASC is post-acute sequelae of COVID-19; PEM is post-exertional malaise; TMJ is temporomandibular joint.

2.3.2. *Questionnaires*

The De Paul Symptom Questionnaire (original version, DSQ-1) and Composite Autonomic Symptom Score 31 (COMPASS 31) questionnaires were completed online at home by patients using RedCap software to characterize the symptoms and autonomic function of the ME/CFS and PASC groups approximately 2–3 days prior to their participation in the study (the control group also completed the survey). Similarly, both questionnaires were also completed 2–3 days after completion of the training protocol and the in-lab protocols (see below) to determine any changes in symptomatology. Participants were able to save their results and return at any time to minimize any cognitive fatigue from answering long questionnaires.

The DSQ has very good psychometric properties including test-retest reliability, sensitivity/specificity, predictive and discriminant validity (Jason and Sunnquist, 2018). For the DSQ-1, participants were asked about the frequency and intensity of 54 common symptoms of ME/CFS on a scale from 0 to 4. The self-reported frequency and intensity scores for each symptom were averaged and multiplied by 25 for final scoring which normalizes them to range from 0 to 100. To minimize the number of statistical comparisons conducted, results from questions 13–18 were averaged as "Fatigue", results from questions 19–24 were averaged as "Sleep", questions 25–31 were averaged as "Pain", questions 32–44 were averaged as "Neurological and Cognitive", questions 45–51 were averaged as "Autonomic", questions 52–61 were averaged as "Neuroendocrine" and questions 62–66 were averaged as "Immune". The COMPASS 31 was scored as per Sletten et al. (Sletten et al., 2012). by combining questions 1–4 for "Orthostatic Intolerance", questions 5–7 for "Vasomotor", questions 8–11 for "Secretomotor", questions 12–23 for "Gastrointestinal", questions 24–26 for "Bladder", questions 27–31 for "Pupillomotor", and a final weighted score was calculated as:

Orthostatic Intolerance*4 + Vasomotor*(0.833) + Secretomotor*(2.14) + Gastrointestinal*(0.89) + Bladder*(1.11) + Pupilomotor*(0.33)

2.3.3. *Heart rate variability (HRV)*

After completing informed consent, answering medical history questions, and measuring height and weight on site at York University, participants rested in a supine position for at least 10 minutes prior to measuring heart rate variability (HRV) using 5 minutes of continuous data from a single lead electrocardiogram. Using the HRV module of LabChart Pro software (ADInstruments, USA), HRV was analyzed according to published guidelines using both time and frequency domain analysis (Heart rate variability, 1996). Briefly, a Fast Fourier Transform was conducted using a Hann (Cosine-Bell) data window with 50 % overlap and a transform size of 1024. Range of 0.04–0.15 Hz and 0.15–0.45 Hz were used for the low frequency (LF) and high frequency (HF) spectrums, respectively. Outcome variables included the standard deviation between R-R intervals (SDRR), total spectral power, low frequency (LF) power, high frequency (HF) power, LF/HF ratio, root mean square of successive differences between normal heartbeats (RMSSD), and percentage of differences higher than 50 msec in R-R intervals (pRR50).

2.3.4. *Six-minute walk distance (6MWD)*

Participants walked at their own pace for 6 minutes along a 40 m University hallway marked with 2 m increments. Participants were not verbally encouraged to walk further or faster and were followed with a rollator chair so that they could sit at any time. All participants were informed that they did not need to participate and/or could stop prior to the 6-minute endpoint without repercussion. One ME/CFS patient opted out of completing the 6MWD test at both timepoints, and one PASC patient opted out at baseline. Before and immediately after the 6MWD was completed, a standard automated brachial blood pressure was taken concurrently with heart rate and peripheral blood oxygen saturation using a portable pulse oximeter while in the seated position to evaluate sympathetic withdrawal and vagal reactivation after cessation of exercise. Ratings of Perceived Exertion (RPE) were taken using a standard

Borg scale (range 6–20).

2.3.5. Data analysis and statistics

For weight, MIP, 6MWD, HRV, and questionnaire data, a two-way mixed model analysis of variance (ANOVA) was completed using group (non-repeated measure) and IMT (repeated measure) as factors. Holm-Sidak post hoc tests were used for comparisons. Only data with both baseline and follow-up visits completed were included in the analysis. For age and height, Kruskal-Wallis ANOVA on Ranks or one-way ANOVA were used, respectively (Sigmaplot 15.0). Data are presented as mean±SD. Significance was accepted at $p<0.05$ and all p-values and statistical markers presented are from significant post hoc testing.

3. Results

For all participants, the average adherence to the 108 breaths/week for each week of the protocol was 99.3 ± 3.3 %, 99.7 ± 1.1 %, 98.8 ± 5 %.9, 99.1 ± 3.1 %, 94.1 ± 20 %.6, 97.6 ± 8.2 %, 98.8 ± 5.9 %, and 89.5 ± 28.3 % as determined by questionnaire. The interim time between sets of breaths was 60.0 ± 0 s, 60.0 ± 0 s, 50.0 ± 0 s, 40.4 ± 4.5 s, 32.9 ± 5.5 s, 22.4 ± 5.2 s, 14.0 ± 6.5 s, 11.3 ± 3.4 s per week for the 8-week protocol.

There were no differences in age or height between groups (p=0.097, p=0.311; Table 1). The ME/CFS group had a higher body mass than the PASC group (p=0.042) but not the control group (p=0.230; Table 1). There was no change in body mass with IMT (p=0.150; Table 1). The IMT protocol increased maximal inspiratory pressure in all groups (p<0.001) with no differences between groups (p=0.296; Table 1).

The rating of perceived exertion after completing the 6MWD was higher in ME/CFS (p=0.001) and PASC (p=0.003) compared to controls with no change after IMT (p=0.109; Table 1). The 6MWD was higher in the control group compared to ME/CFS (p=0.005) and PASC (p=0.037), and there was an improvement in 6MWD in all groups with IMT (p=0.002; Table 1). There were no differences between groups or in response to IMT with regards to systolic blood pressure, diastolic blood pressure, or peripheral oxygen saturation before completing the 6MWD (all p>0.128); however, resting HR was lower after IMT (p=0.037) with no differences between groups (p=0.716; Table 1). There was no influence of group or IMT on systolic blood pressure, diastolic blood pressure, HR, or peripheral oxygen saturation after completion of the 6MWD (all p>0.185; Table 1).

Table 1
Anthropometrics and 6MWD responses to Inspiratory Muscle Training (IMT).

	Control (n=9)		PASC (n=7)		ME/CFS (n=9)	
Age (years)	32 ± 18		47 ± 14		44 ± 12	
Height (cm)	165 ± 8		161 ± 6		167 ± 5	
	Baseline	Post-IMT	Baseline	Post-IMT	Baseline	Post-IMT
Weight (kg)	69 ± 11	68 ± 12	63 ± 9†	62 ± 8†	76 ± 9	76 ± 9
MIP (mmHg)	59 ± 16	**82 ± 19***	49 ± 12	**64 ± 27***	61 ± 24	**80 ± 25***
6MWD (m)	461 ± 62+	**502 ± 83+***	396 ± 86	**398 ± 111***	343 ± 65	**372 ± 60***
SBP-Pre 6MWD (mmHg)	121 ± 17	122 ± 15	118 ± 23	112 ± 22	119 ± 16	116 ± 16
DBP-Pre 6MWD (mmHg)	79 ± 9	81 ± 7	80 ± 9	76 ± 7	82 ± 10	79 ± 9
HR-Pre 6MWD (bpm)	70 ± 11	**69 ± 11***	75 ± 12	**68 ± 7***	69 ± 10	**66 ± 7***
O_2-Pre 6MWD (%)	98 ± 1	98 ± 1	98 ± 1	98 ± 3	96 ± 2	97 ± 2
SBP-Post 6MWD (mmHg)	117 ± 12	115 ± 12	118 ± 22	117 ± 26	119 ± 17	117 ± 13
DBP-Post 6MWD (mmHg)	77 ± 6	76 ± 6	80 ± 8	75 ± 6	78 ± 8	78 ± 9
HR-Post 6MWD (bpm)	94 ± 23	89 ± 23	100 ± 32	92 ± 16	80 ± 14	84 ± 17
O_2-Post 6MWD (%)	97 ± 2	97 ± 3	95 ± 6	94 ± 4	96 ± 3	96 ± 2
RPE	7 ± 2+	8 ± 2+	11 ± 3	12 ± 2	11 ± 2	12 ± 1

6MWD – 6-minute walk distance, DBP – diastolic blood pressure, HR – heart rate, IMT – inspiratory muscle training, ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome, MIP – maximal inspiratory pressure, O_2 – oxygen saturation, PASC – post-acute sequelae of COVID-19, RPE – rating of perceived exertion, SBP – systolic blood pressure; *indicates a main effect of IMT (bold), †indicates a group effect different than ME/CFS only, +indicates a group effect different than both ME/CFS and PASC. Data are mean ± SD

3.1. De Paul Questionnaire

The PASC patients had impaired Fatigue, Sleep, and Neurological and Cognitive scores compared to the healthy control group (all p≤0.003; Table 2). The ME/CFS group had impaired Fatigue, Sleep, Pain (at baseline only), Neurological and Cognitive, Autonomic and Immune scores compared to the healthy control group (a11 p≤0.031; Table 2). There were no differences in the symptom scores between PASC and ME/CFS (all p≥0.12; Table 2). The IMT protocol improved Sleep in all participants (p=0.009) and improved Pain in the ME/CFS group (p=0.016; Table 2).

3.2. COMPASS 31

As described in Tables 3, 1) both PASC and ME/CFS had greater scores than the control group for pupillomotor function (p=0.029 and p=<0.001) and the final weighted score (p=0.037 and p=0.007), 2) ME/CFS had greater scores than the control group for orthostatic intolerance (p=0.006), vasomotor function (p=0.004), secretomotor function (p=0.012), and gastrointestinal function (p=0.047), and 3) ME/CFS had greater scores than PASC for vasomotor function (p=0.036). We observed that the IMT protocol improved vasomotor function (p=0.001), secretomotor function (p=0.023) and weighted score (p=0.005) in the ME/CFS group.

3.3. Heart rate variability

Before completion of the IMT protocol, the control group had higher SDRR (p=0.005), RMSSD (p=0.008) and pRR50 (p<0.03) compared to both PASC and ME/CFS (Table 4). After completing IMT, the control group increased their total power (p=0.002), such that they had higher values than both PASC and ME/CFS (p<0.001; Table 4). In response to IMT, we observed that all groups decreased LF power (p=0.035), increased HF power (p=0.034), increased RMSSD (p=0.034) and increased pRR50 (p=0.035; Table 4).

4. Discussion

Our results indicated that compared to the control group PASC patients exhibited impairments in fatigue, sleep, and neurological and cognitive function whereas the ME/CFS group exhibited impairments in fatigue, sleep, pain, neurological and cognitive function, autonomic function and immune symptoms. Both PASC and ME/CFS exhibited impaired pupillomotor and total weighted scores compared to the control group, and the ME/CFS group exhibited worsened symptoms of orthostatic intolerance, vascular function, secretomotor function, and gastrointestinal function compared to the control group with greater impairment than PASC for vasomotor function. When cardiac autonomic

Table 2
De Paul Symptom Questionnaire responses to Inspiratory Muscle Training (IMT).

	Control (n=8)		PASC (n=7)		ME/CFS (n=9)	
	Baseline	Post-IMT	Baseline	Post-IMT	Baseline	Post-IMT
Fatigue	3.1 (0–17.2)+	3.1 (0–17.7)+	60.4 (56.3–79.2)	43.8 (38.5–79.2)	62.5 (58.3–70.8)	58.3 (47.9–64.6)
Sleep	14.6 ± 13.5+	**9.6 ± 10.0+***	39.3 ± 16.9	**36.3 ± 21.1***	43.1 ± 10.2	**32.6 ± 11.7***
Pain	11.4 ± 13.6†	16.3 ± 21.8	29.6 ± 20.6	25.3 ± 15.9	45.6 ± 21.3	**35.5 ± 17.0***
Neurological and Cognitive	7.1 ± 9.1+	11.8 ± 17.2+	39.6 ± 19.8	33.4 ± 14.9	54.5 ± 10.6	46.8 ± 12.5
Autonomic	6.9 ± 9.1†	9.2 ± 15.4†	25.8 ± 19.7	27.0 ± 20.9	33.5 ± 13.0	29.8 ± 17.4
Neuroendocrine	4.5 ± 5.8	13.4 ± 21.2	21.3 ± 17.8	21.3 ± 17.4	26.3 ± 13.2	24.6 ± 13.7
Immune	1.3 ± 2.3†	8.4 ± 12.0†	25.0 ± 23.4	20.0 ± 19.1	23.9 ± 10.8	22.5 ± 13.4

IMT – inspiratory muscle training, ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome, PASC – post-acute sequelae of COVID-19; +indicates group effect different than PASC and ME/CFS within timepoint; †indicates group effect different than ME/CFS within timepoint; # indicates group effect different than PASC within timepoint; *indicates effect of IMT (bold) within group; Data are mean ± SD or mean (IQR). Note: A higher score denotes more symptoms.

Table 3 –
COMPASS 31 responses to Inspiratory Muscle Training (IMT).

	Control (n=8)		PASC (n=7)		ME/CFS (n=7)	
	Pre-IMT	Post-IMT	Pre-IMT	Post-IMT	Pre-IMT	Post-IMT
Orthostatic Intolerance	1.0 ± 1.9†	1.5 ± 2.1†	3.6 ± 3.6	2.8 ± 2.5	5.9 ± 1.3	5.0 ± 2.7
Vasomotor	0†	0	0.6 ± 1.5†	0.4 ± 1.1	2.0 ± 1.4	**0.9 ± 1.2***
Secretomotor	0.5 ± 0.8†	1.1 ± 1.4	2.1 ± 2.0	2.7 ± 1.5	2.9 ± 1.6	**1.9 ± 1.3***
Gastrointestinal	2.5 ± 2.5†	4.0 ± 4.0†	8.7 ± 5.0	9.6 ± 5.6	10.3 ± 6.7	9.4 ± 5.3
Bladder	0.4 ± 0.7	0.3 ± 0.5	1.3 ± 2.0	1.4 ± 2.0	1.6 ± 1.1	1.6 ± 1.4
Pupillomotor	1.4 ± 1.5+	1.3 ± 1.4+	5.4 ± 3.7	5.4 ± 3.7	8.9 ± 3.5	8.9 ± 3.3
Weighted Score	8.2 ± 9.9+	12.7 ± 13.2†	30.4 ± 20.9	29.5 ± 16.3	45.1 ± 15.2	**37.8 ± 19.4***

IMT – inspiratory muscle training, ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome, PASC – post-acute sequelae of COVID-19; +indicates group effect different than PASC and ME/CFS within timepoint; †indicates group effect different than ME/CFS within timepoint; *indicates effect of IMT (bold) within group; Data are mean ± SD. Note: A higher score denotes more symptoms.

Table 4
Heart Rate Variability responses to Inspiratory Muscle Training (IMT).

	Control (n=9)		PASC (n=7)		ME/CFS (n=9)	
	Baseline	Post-IMT	Baseline	Post-IMT	Baseline	Post-IMT
Total Power (ms^2)	4162 ± 1863	**8342 ± 6034*+**	2210 ± 2118	2443 ± 2428	2667 ± 2424	1912 ± 990
LF (nu)	54.8 ± 25.4	**43.0 ± 22.4***	72.0 ± 21.1	**63.0 ± 24.0***	59.4 ± 22.8	**55.4 ± 21.4***
HF (nu)	45.0 ± 25.0	**55.7 ± 22.2***	28.5 ± 21.2	**38.2 ± 23.8***	40.1 ± 22.8	**43.7 ± 20.4***
LF/HF	2.3 ± 2.5	1.2 ± 1.1	5.0 ± 4.7	2.8 ± 2.6	2.6 ± 2.4	2.8 ± 4.3
SDRR (ms)	70.1 ± 15.4+	86.8 ± 31.6+	44.2 ± 18.6	45.1 ± 22.7	48.7 ± 22.4	41.5 ± 18.0
RMSSD (ms)	54.2 ± 23.0+	**75.7 ± 37.8*+**	27.6 ± 14.3	**36.5 ± 18.0***	33.7 ± 19.3	**30.8 ± 11.1***
pRR50 (%)	30.5 ± 19.6+	**39.0 ± 21.6*+**	10.0 ± 11.4	**17.5 ± 18.3***	11.4 ± 13.2	**11.7 ± 11.6***

HF – high frequency, IMT – inspiratory muscle training, LF – low frequency, ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome, PASC – post-acute sequelae of COVID-19, pRR50 - percentage of differences higher than 50msec in R-R intervals, RMSSD - root mean square of successive differences between normal heartbeats, SDRR – standard deviation of R-R intervals; +indicates group different than PASC and ME/CFS within timepoint; †indicates group different than ME/CFS within timepoint; *indicates main effect of IMT (bold); Data are mean ± SD

function was quantitatively measured with HRV, both PASC and ME/CFS exhibited equal impairments compared to the controls. Most notably, the IMT protocol 1) improved autonomic function in ME/CFS (vasomotor, secretomotor, weighted score), 2) improved sleep symptoms in all groups and improved pain in ME/CFS, and 3) improved HRV (LF, HF, RMSSD, pRR50), resting heart rate, maximal inspiratory pressure and 6MWD in all participants.

As expected, we found much baseline symptom overlap between ME/CFS and PASC including greater fatigue, impaired sleep and impaired cognitive function compared to the control group. There were, however, certain symptoms that were greater in ME/CFS versus PASC implying greater impairment of the autonomic nervous system including pain, immune function, orthostatic tolerance, vasomotor function, secretomotor function and gastrointestinal function. Indeed, we observed impaired heart rate variability in both PASC and ME/CFS compared to the control group implying reduced parasympathetic control of heart rate (i.e. vagal tone). As reduced parasympathetic activity has previously been shown to be associated with many of the symptoms including, but not limited to, pain (Koenig et al., 2014), gastrointestinal function (Ali and Chen, 2023), sleep (Lins-Filho et al., 2023), and cognitive function (Clark et al., 1999) we suggest that impaired parasympathetic function is a driving force behind many symptoms in PASC and ME/CFS.

We found improvement in MIP in all participants after IMT, indicating increased strength of inspiratory muscles and that the training protocol was successful. These improvements were similar to those previously observed in a healthy group using a different IMT protocol, 4 weeks of 50 % MIP adjusted weekly for improving MIP rather than shortening the time interval between breaths (Rodrigues et al., 2018). Further, we also found improvement in the 6MWD in all participants, which suggests a possible improvement in exercise capacity. However, as we did not measure physical activity levels nor any other indices of strength, we cannot firmly state that whole-body strength or fitness improvements were achieved. Recent meta-analyses found that IMT improved both MIP and 6MWD in patients with pulmonary hypertension (Luo et al., 2022) yet only improved MIP in heart failure (Li et al., 2022), indicating pathophysiological specificity.

In all participants, the 8-week IMT protocol improved heart rate

variability and sleep while further improving pain and autonomic symptoms in ME/CFS. The improvements in heart rate variability parameters suggest increased parasympathetic control of heart rate after the IMT coinciding with the results of Mello et al., who found that the HF component of HRV was improved after IMT in patients with heart failure (Mello et al., 2012). We also observed a reduction in the LF component of HRV in all participants which likely reflects reduced baroreflex sensitivity (Moak et al., 2007; Rahman et al., 2011). Mello et al. similarly observed a reduced LF component alongside a reduction in muscle sympathetic nerve activity (Mello et al., 2012). We speculate that the IMT protocol could have improved the respiratory metaboreflex, as previously observed (Witt et al., 2007), thus increasing parasympathetic activity. While we cannot determine the chronological order of improvements in this study, we suggest that the improved parasympathetic activity led to the improvements in symptoms. Thus, interventions in PASC and ME/CFS that increase vagal tone could be an appropriate avenue of future investigation to increase quality of life for patients.

4.1. Limitations

As this study was limited in size, we did not include a time control group of PASC or ME/CFS patients who did not undergo the IMT. The inclusion of this control group and/or conducting a blinded randomized control trial could have helped to determine if the changes in symptomology and autonomic function observed over the 8 weeks could have been due to the normal passage of time rather than the IMT per se. However, objectively observing improvements in MIP, resting HR and HRV in all 3 groups due to time alone would be unlikely. Greater sample sizes in each group, thus improving statistical power, may have also led to statistically significant interaction effects between group and IMT in multiple variables (e.g. 6MWD).

The DSQ-1 and COMPASS 31 were completed approximately 3 days after the conclusion of the IMT and in-lab testing. The in-lab testing (in particular the 6MWD) had the potential to obscure our results due to any symptom exacerbation; however, if anything, this timing would have likely increased symptomatology rather than decreased it. Yet, we still observed a reduction in many symptoms. Further, we cannot discount the possibility that participants with ME/CFS or PASC could have been particularly symptomatic at baseline testing. Therefore, any improvements that we observed could have been due to a natural alleviation of symptoms over time. Lastly, the questionnaires were completed at home (with guidance by researchers) and therefore cognitive function and capacity along with education levels could be considered limitations. Participants were allowed to take their time and answer over longer periods of time; however, we do not have information pertaining to their education level.

The 6MWD test was used as a measure of cardiorespiratory fitness to minimize potential impact compared to other fitness testing protocols such as a full cardiopulmonary exercise test. However, this technique certainly has limitations and increases the risk of post-exertional malaise in those prone to developing it. A learning effect could also be present with the 6MWD for those unfamiliar with the protocol, thus improvements could have been due to familiarity. Recent guidelines have suggested that two 6MWD tests should be conducted to minimize this effect (Holland et al., 2014); however, we only conducted a single test to minimize the potential for post-exertional malaise with prolonged walking in these populations. Multiple clinical conditions that regularly use the 6MWD as a fitness indicator found that an improvement of approximately 30 m was the minimum clinically important difference (e.g. heart failure (Shoemaker et al., 2013), pulmonary arterial hypertension (Moutchia et al., 2023), and chronic obstructive pulmonary disease (Polkey et al., 2013)). On average, we only observed this level of improvement in the control group and the ME/CFS group. Lastly, an objective measure of physical activity such as a 3-day step count would have benefited this study as any observed changes could be due to varied activity levels.

We asked PASC patients to focus on the time since their COVID-19 infection when reporting their symptoms; however, we cannot exclude the possibility of pre-existing conditions unrelated to PASC. PASC patients were diagnosed by medical professionals, but we do not have information on the severity of their acute condition nor the clinical treatment that each patient underwent after their infection. Similarly, we do not have information on the respiratory and autonomic function of each patient prior to infection as this was a cross-sectional study. We deliberately chose to include only mild to moderate ME/CFS cases as a precaution. We urge caution for researchers, clinicians and patients embarking on an IMT protocol in case of an episode of post-exertional malaise. The protocol should be cautiously titrated, with prioritized pacing, and only when participants feel ready to increase their effort. All protocols involving physical activities have the potential to harm patients who experience post-exertional malaise and, therefore, must be implemented with a high degree of caution and with respectful, open, and understanding communication with patients. Lastly, we caution against extrapolating this data to larger populations given the small sample size within our study. Future studies on a larger scale are required.

5. Conclusions

We found that IMT improved symptoms in PASC and ME/CFS which could have been driven by concurrent improvements in parasympathetic activity. We recommend larger randomized clinical trials investigating IMT in these populations and potentially other post-viral conditions with similar symptomology. However, caution is urged for researchers to consider the potential for post-exertional malaise. We suggest that future studies directly measure sympathetic output via microneurography while specifically investigating any improvements in the respiratory metaboreflex via breathing against resistance. Further, we suggest that larger trials include a diverse group of participants with controls in place for medication use and co-morbidities.

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Lauren Sergio: Writing – review & editing, Funding acquisition, Conceptualization. **Smriti Badhwar:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Heather Edgell:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kathleen Kerr:** Writing – review & editing, Funding acquisition, Conceptualization. **Tania J. Pereira:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Farah Tabassum:** Writing – review & editing, Funding acquisition, Conceptualization. **Riina Bray:** Writing – review & editing, Funding acquisition, Conceptualization.

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Disclosures

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