Neurovascular Dysregulation and Acute Exercise Intolerance in ME/CFS

A Randomized, Placebo-Controlled Trial of Pyridostigmine

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BACKGROUND: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is characterized by intractable fatigue, postexertional malaise, and orthostatic intolerance, but its pathophysiology is poorly understood. Pharmacologic cholinergic stimulation was used to test the hypothesis that neurovascular dysregulation underlies exercise intolerance in ME/CFS.

RESEARCH QUESTION: Does neurovascular dysregulation contribute to exercise intolerance in ME/CFS, and can its treatment improve exercise capacity?

STUDY DESIGN AND METHODS: Forty-five subjects with ME/CFS were enrolled in a single-center, randomized, double-blind, placebo-controlled trial. Subjects were assigned in a 1:1 ratio to receive a 60-mg dose of oral pyridostigmine or placebo after an invasive cardiopulmonary exercise test (iCPET). A second iCPET was performed 50 min later. The primary end point was the difference in peak exercise oxygen uptake (V_{O_2}). Secondary end points included exercise pulmonary and systemic hemodynamics and gas exchange.

RESULTS: Twenty-three subjects were assigned to receive pyridostigmine and 22 to receive placebo. The peak V_{O_2} increased after pyridostigmine but decreased after placebo (13.3 ± 13.4 mL/min vs –40.2 ± 21.3 mL/min; $P < .05$). The treatment effect of pyridostigmine was 53.6 mL/min (95% CI, –105.2 to –2.0). Peak vs rest V_{O_2} (25.9 ± 15.3 mL/min vs –60.8 ± 25.6 mL/min; $P < .01$), cardiac output (–0.2 ± 0.6 L/min vs –1.9 ± 0.6 L/min; $P < .05$), and right atrial pressure (1.0 ± 0.5 mm Hg vs –0.6 ± 0.5 mm Hg; $P < .05$) were greater in the pyridostigmine group compared with placebo.

INTERPRETATION: Pyridostigmine improves peak V_{O_2} in ME/CFS by increasing cardiac output and right ventricular filling pressures. Worsening peak exercise V_{O_2}, cardiac output, and right atrial pressure following placebo may signal the onset of postexertional malaise. We suggest that treatable neurovascular dysregulation underlies acute exercise intolerance in ME/CFS.

CLINICAL TRIAL REGISTRATION: ClinicalTrials.gov; No.: NCT03674541; URL: www.clinicaltrials.gov.

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KEY WORDS: cardiopulmonary exercise test; dyspnea; myalgic encephalomyelitis/chronic fatigue syndrome; pyridostigmine

ABBREVIATIONS: Ca-v_{O_2} = arterial-venous oxygen content difference; iCPET = invasive cardiopulmonary exercise test; ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome; PASC = postacute sequelae of SARS-CoV-2 infection; PAWP = pulmonary arterial wedge pressure; POTS = postural orthostatic tachycardia syndrome; Qc = cardiac output; RAP = right atrial pressure; SFN = small fiber neuropathy; VE/V_{CO_2} = ventilatory efficiency; V_{O_2} = oxygen uptake

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Take-home Points

Study Question: Does neurovascular dysregulation contribute to exercise intolerance in ME/CFS, and can cholinergic stimulation with pyridostigmine improve exercise capacity?

Results: Comparing serial iCPET, peak V_{O_2} and associated changes in Qc and RAP were greater in the pyridostigmine group compared with placebo, driven by both improvement in the pyridostigmine group and worsening in the placebo group.

Interpretation: Pyridostigmine improves aerobic capacity by increasing Qc and right ventricular filling pressures, whereas worsening V_{O_2} and hemodynamics following placebo may signal the onset of postexertional malaise.

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a common, debilitating disorder that has a global impact on millions of people. Approximately 2.5 million Americans are diagnosed with ME/CFS, with a resulting loss in productivity amounting to $20,000 per patient, or $9.1 billion overall.[1] The National Academy of Medicine (formerly the Institute of Medicine) requires three major criteria for diagnosis (substantial impairment from fatigue for > 6 months, postexertional malaise, and unrefreshing sleep), plus either cognitive impairment or orthostatic intolerance.[2]

The pathophysiology underlying ME/CFS remains poorly understood. Proposed mechanisms include infectious,[3] inflammatory,[4] autoimmune,[5] neuroendocrine,[6] and genetic and environmental[7] causes. Due to considerable overlap among ME/CFS, postural orthostatic tachycardia syndrome (POTS), and fibromyalgia, small fiber neuropathy (SFN) has been implicated as a cause of these syndromes.[8-10] We recently showed that SFN was present in 31% of patients with ME/CFS undergoing invasive cardiopulmonary exercise tests (iCPETs),[11] similar to the reported 38% in POTS[12] and 50% observed in fibromyalgia.[13]

Immunohistochemical studies show that small fibers regulate microvascular tone through sympathetic and parasympathetic cholinergic synapses on perivascular myocytes.[14] It was therefore hypothesized that SFN contributed to hemodynamic phenotypes of low cardiac preload[15] from impaired venous return and impaired peripheral oxygen extraction, analogous to neurovascular dysregulation observed in POTS[16] and fibromyalgia.[17] We were unable to link neurite density to vascular dysregulation during incremental exercise.[11] The skin biopsy specimens were epidermal and not designed to detect small fibers innervating the sweat glands and, therefore, may not have captured the full spectrum of dysautonomia due to SFN.

An alternative interpretation is the presence of co-existing ganglionopathy, sometimes associated with antineuronal acetylcholine receptor antibodies and identical vascular dysregulation.[18] Pyridostigmine, an acetylcholinesterase inhibitor, is thought to enhance cholinergic stimulation of norepinephrine release at the post-ganglionic synapse and has shown significant improvement in both symptom burden and heart rate response in POTS.[19] The objective of the current trial was to use pyridostigmine to determine if neurovascular dysregulation underlies exertional intolerance in ME/CFS.

Study Design and Methods

Trial Design and Oversight

This was a single-center, randomized, double-blind, placebo-controlled trial. Subjects were assigned in a 1:1 ratio to receive a 60-mg dose of oral pyridostigmine or placebo after an iCPET. A second iCPET was performed 50 min later. Hemodynamics were recorded throughout the epoch of exercise during both tests.

A full list of trial personnel is provided in e-Appendix 1. The study was approved by the Partners Human Research Committee (IRB 2018P001871) and is registered at clinicaltrials.gov.[20]

Trial Population

The trial population consisted of patients aged ≥ 18 years with ME/CFS. All fulfilled the National Academy of Medicine requirement of three major criteria (ie, chronic fatigue for > 6 months, postexertional malaise, unrefreshing sleep) plus one minor criteria (ie, either cognitive impairment or orthostatic intolerance).[2] They were required to have a peak right atrial pressure (RAP) ≤ 6.5 mm Hg during their first, maximal iCPET,[15] along with the exclusion of resting or exercise pulmonary arterial or venous hypertension during their resting right heart catheterization and first iCPET.[21] Patients with conditions predictive of exercise limitation, such as anemia, BMI > 30 kg/m^2, and active cardiopulmonary disease, were excluded. A full list of inclusion and exclusion criteria is provided in e-Appendix 2.

Trial Procedures

Protocols for iCPET, hemodynamic measurements, and gas exchange measurements have been described previously.[22,23] Briefly, the pulmonary and radial arteries were catheterized with ultrasound and fluoroscopic guidance, and a standard right heart catheterization was then performed with oxygen saturation measurements to assess for intracardiac shunting.[24] Patients were transported to the cardiopulmonary exercise laboratory for maximum, incremental, upright exercise on a cycle ergometer as

ventilation and pulmonary gas exchange were continuously measured (MGC Diagnostics). Hemodynamics, including RAP, mean pulmonary artery pressure, and mean arterial pressure, were continuously recorded (Koninklijke Philips N.V.) and averaged throughout the respiratory cycle.[25] Pulmonary arterial wedge pressure (PAWP) and arterial and mixed-venous blood gases and pH were measured every minute and cardiac output (Qc) calculated by using the direct Fick principle. RAP and PAWP were measured as the mean of the "a" wave.

After confirmation of RAP ≤ 6.5 mm Hg, a maximal exercise effort (respiratory exchange ratio > 1.05 and/or heart rate > 85% predicted), and exclusion of exercise pulmonary or venous hypertension,[21] subjects were administered 60 mg of oral pyridostigmine or placebo in a 1:1 ratio. A second iCPET was performed after a combined dosing and rest period of 50 min. The full two-iCPET protocol is described in e-Appendix 3.

Modified Borg dyspnea and fatigue scales were administered immediately following both iCPETs. Subjects were asked to rate their dyspnea and fatigue from 0 ("nothing at all") to 10 ("maximal") during peak exercise.

Outcome Measures

The primary end point of the trial was the between-group difference in peak exercise oxygen uptake (V_{O_2}) following pyridostigmine or placebo administration. Secondary end points included between group differences in peak vs rest for V_{O_2}, Qc, RAP, PAWP, ventilatory efficiency (V_E/V_{CO_2}), peak arterial-venous oxygen content difference ($Ca\text{-}v_{O_2}$), and modified Borg dyspnea and fatigue scales.

Statistical Analysis

After demonstrating a normal distribution of the data by using the Kolmogorov-Smirnov test, a Welch's T test was used to compare exercise physiological variables between the groups. Two-sided *P* values, SEs, and 95% CIs are reported. For secondary end points, the *P* values and CIs were not adjusted for multiplicity and cannot be used to infer definitive treatment effects for these secondary end points. The Fisher exact test was used to compare the baseline characteristics between the two groups. With 80% power to detect a 10% difference in oxygen uptake at peak exercise, we estimated the need to enroll 50 patients.[26] This number also accounted for screening failures. The analysis was performed by using R version 4.1.0 (R Foundation for Statistical Computing).

Results

Patients

Of 362 subjects prescreened for eligibility, 50 were enrolled to undergo iCPET. Five subjects were excluded following the initial iCPET, with the remainder randomly assigned to receive pyridostigmine (23 patients) or placebo (22 patients). Male subjects were removed from analysis owing to a randomization error, yielding a 39-subject study sample (Fig 1). Baseline characteristics were similar in the two groups (Table 1). The mean age was 40 years. Few used diuretics or vasoactive drugs. Significant associated conditions included POTS, fibromyalgia, mast cell activation syndrome, and prior infection. Overall, 38% of the study population had objective evidence of SFN. Thirty patients had a paraneoplastic antibody evaluation performed at the Mayo Clinic Clinical Laboratory. Striational antibody was detected in one placebo patient's panel, and neuronal voltage-gated potassium channel antibody was detected in one treatment patient's panel. Acetylcholine receptor ganglionic neuronal antibodies were not detected in any patient panel.

Primary End Point

Peak V_{O_2} increased after pyridostigmine but decreased after placebo (13.3 ± 13.4 vs −40.3 ± 21.3; $P < .05$). The treatment effect of pyridostigmine was 53.6 mL/min (95% CI, −105.2 to −2.0) (Fig 2, Table 2).

Secondary End Points

Peak vs rest V_{O_2} (25.9 ± 15.3 mL/min vs −60.8 ± 25.6 mL/min; $P < .01$), Qc (−0.2 ± 0.6 L/min vs −1.9 ± 0.6 L/min; $P < .05$), and RAP (1.0 ± 0.5 mm Hg vs −0.6 ± 0.5 mm Hg; $P < .05$) were greater in the pyridostigmine group compared with the placebo group. There were no significant changes in PAWP, V_E/V_{CO_2}, or $Ca\text{-}v_{O_2}$ (Tables 2 and 3).

Discussion

ME/CFS is a common and often disabling disorder of unknown pathogenesis reportedly affecting 10% to 25% of patients in primary care practices,[27] 75 to 267 per 100,000 persons,[28] or 836,000 to 2.5 million people in the United States.[2] Nonspecific symptoms spanning multiple organ systems cause frequent evaluations by varied medical specialties, leading to combined direct and indirect US costs approaching $23 billion per year.[29] Emerging data suggest similar exercise pathophysiology[30] and an increased prevalence of ME/CFS[31] in patients with postacute sequelae of SARS-CoV-2 infection (PASC). Thus, insights into pathogenesis and treatment of ME/CFS are needed.

There are no approved treatments for ME/CFS,[32] with prior studies having looked at cognitive-behavioral therapy, graded exercise,[33] IV immunoglobulin,[34] and B-cell depletion.[35] Although pyridostigmine has shown improvement in symptom burden and heart rate response in POTS,[19] its effects on patients with ME/CFS are limited to case reports.[36] To our knowledge, the

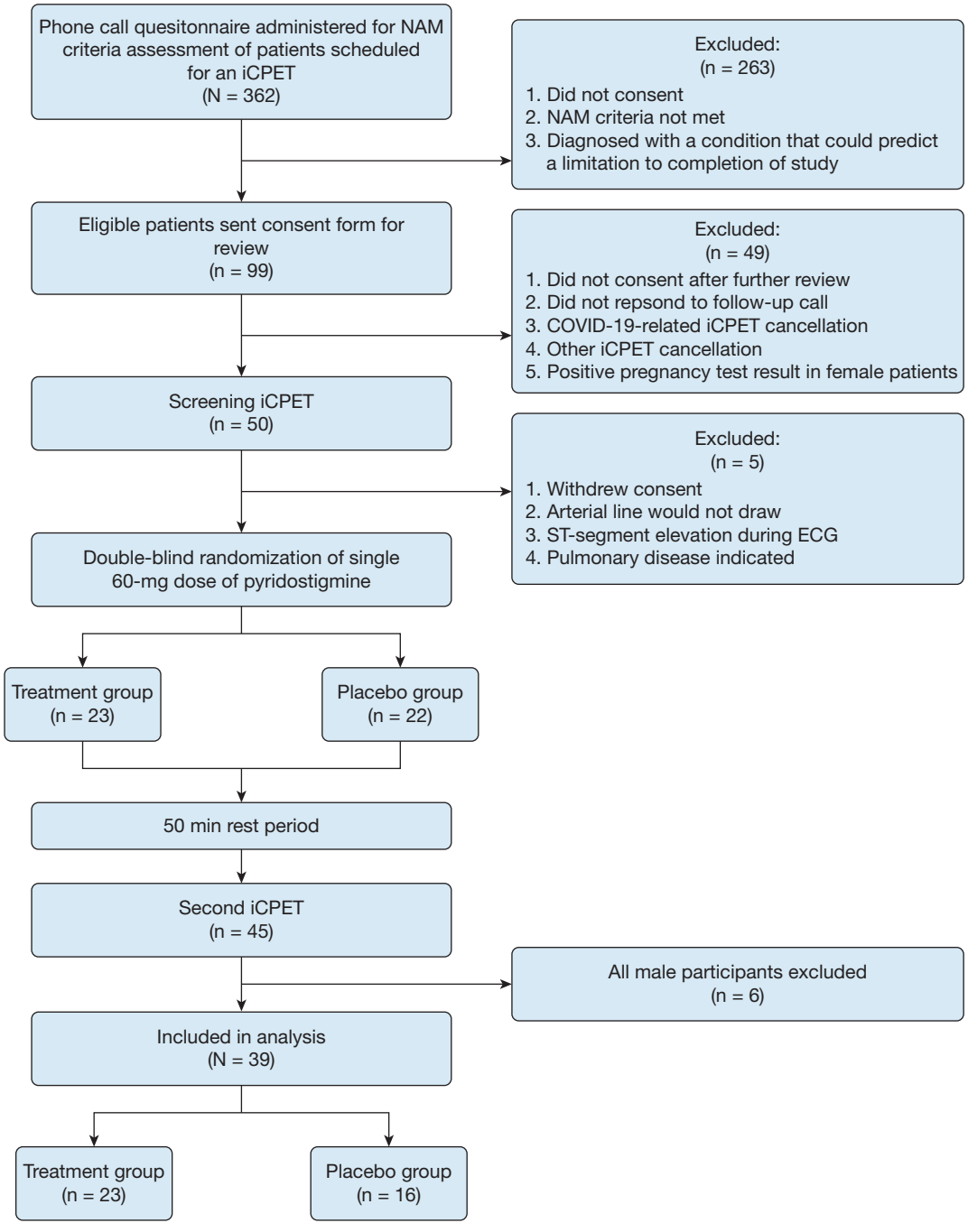

Figure 1 – *Screening, randomization, and assessment. iCPET = invasive cardiopulmonary exercise test; ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome; NAM = National Academy of Medicine; ST-segment = segment of ECG wave that represents the end of ventricular depolarization and the beginning of ventricular repolarization during the cardiac cycle. Elevation indicates potential myocardial ischemia or infarction.*

current study is the first blinded, randomized, placebo-controlled trial to evaluate the effects of pyridostigmine on acute exercise hemodynamics in ME/CFS.

Pyridostigmine Improves Exercise Hemodynamics

There was an increase in peak V_{O_2} following pyridostigmine administration due to an increase in Qc and, in turn, was related to improved RAP. Hence, our results suggest that acute treatment with pyridostigmine improves aerobic capacity by an increase in cardiac output from augmented preload. This is consistent with studies showing deficient preload as a cause of exertional intolerance in ME/CFS.[11,15]

TABLE 1] Baseline Characteristics

Characteristic	All (N = 39)	Pyridostigmine (n = 23)	Placebo (n = 16)
Age, y	40 ± 14	40 ± 16	40 ± 11
Female	39 (100%)	23 (100%)	16 (100%)
White race	33 (85%)	21 (91%)	12 (75%)
BMI, kg/m^2	23.5 ± 3.4	23.8 ± 2.7	23.0 ± 4.1
Hemoglobin, g/dL	14.0 ± 1.2	13.9 ± 1.3	14.0 ± 1.1
Comorbidities			
Hypertension	4 (10%)	3 (13%)	1 (6%)
Dyslipidemia	2 (5%)	1 (4%)	1 (6%)
Obesity	0	0	0
Cardiovascular family history	24 (62%)	13 (57%)	11 (69%)
Diabetes mellitus	0	0	0
Previous myocardial infarction	0	0	0
Coronary artery disease	0	0	0
Medications			
Statins	2 (5%)	2 (9%)	0
Beta-blockers	3 (8%)	2 (9%)	1 (6%)
ASA	2 (5%)	2 (9%)	0
Calcium-channel blockers	1 (2%)	1 (4%)	0
Diuretics	1 (2%)	1 (4%)	0
ACE inhibitors	0	0	0
Associated conditions			
Objective evidence of SFN by morphologic and/or functional testing	14/37 (38%)	11/22 (50%)	3/15 (20%)
Epidermal skin biopsy evidence of SFN (neurite density ≤ 5th percentile)	7/36 (19%)	5/21 (24%)	2/15 (13%)
Sweat gland skin biopsy evidence of SFN	5/12 (41%)	5/8 (63%)	0/4 (0%)
Functional testing (QSART and/or ESC) evidence of SFN	2/16 (13%)	1/10 (10%)	1/6 (17%)
POTS	18 (46%)	13 (57%)	5 (31%)
Fibromyalgia	11 (28%)	6 (26%)	5 (33%)
MCAS	7 (18%)	4 (17%)	3 (19%)
Preceding infection	20 (51%)	12 (52%)	8 (50%)
Positive ANA	10 (26%)	7 (30%)	3 (19%)

Data are expressed as mean ± SD unless otherwise indicated. ACE = angiotensin-converting enzyme; ANA = antinuclear antibody; ASA = acetylsalicylic acid; ESC = electrochemical skin conductance; MCAS = mast cell activation syndrome; POTS = postural orthostatic tachycardia syndrome; QSART = quantitative sudomotor axon reflex test; SFN = small fiber neuropathy.

These data suggest that neurovascular dysregulation underlies preload failure in ME/CFS. Decreased sympathetic outflow has been reported during orthostatic challenge and isometric exercise in ME/CFS.[37-39] In the similar syndrome of POTS, abnormal lower extremity venous pooling occurs upon standing.[40] Infused norepinephrine and phenylephrine resulting in excess peripheral venoconstriction is consistent with adrenergic receptor upregulation from denervation[41,42] and is further supported by low norepinephrine release following sympathetic nervous system stimulation in patients with POTS.[43]

Pyridostigmine is a reversible acetylcholinesterase inhibitor that acts by increasing levels of acetylcholine at the pre-ganglionic sympathetic synapse, resulting in a downstream increase in norepinephrine at post-ganglionic receptors. Norepinephrine release leads to venoconstriction and improved vascular tone, with subsequent augmentation of cardiac preload, Qc, and aerobic capacity (Fig 3).[44] In the related syndrome of POTS, a single 30-mg dose of pyridostigmine was previously shown to mitigate the heart rate increase during upright tilt.[19] Priming exercise has been shown to reduce intracardiac filling pressures in patients with

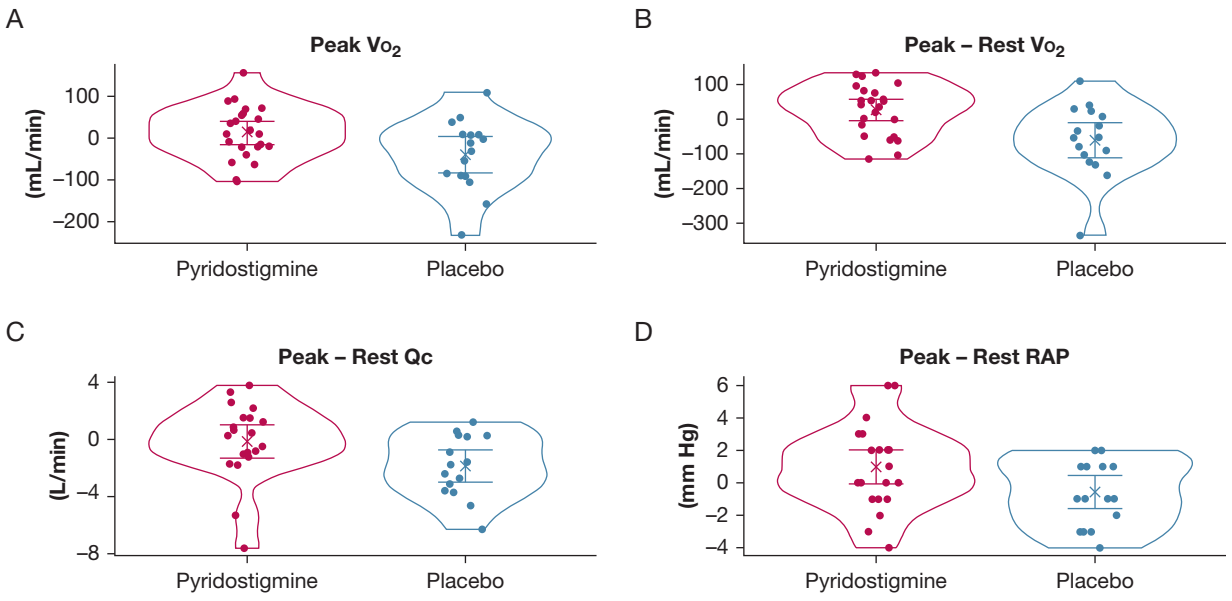

Figure 2 – *Primary and secondary outcomes, changes between iCPETs. Shown are mean ± 2 × SEM and the violin plot distributions of the changes between the two consecutive iCPETs. A, Peak* Vo_2. *B, Peak - Rest* Vo_2. *C, Peak - Rest Qc. D, Peak - Rest RAP. iCPET = invasive cardiopulmonary exercise test; Qc = cardiac output; RAP = right atrial pressure;* Vo_2= *oxygen uptake.*

heart failure with preserved ejection fraction, potentially explained by the shift of venous blood volume from stressed to unstressed compartments.[45] This may contribute to the decrease in peak Vo_2, Qc, and preload in the placebo group, reinforcing the steep portion of the Starling curve that these patients lie on.

Prior work suggests that impaired systemic oxygen extraction from microcirculatory or mitochondrial dysfunction may contribute to decreased aerobic capacity in ME/CFS.[11,46] Although there was a borderline reduction in systemic oxygen extraction in both treatment and placebo groups, this factor did not improve with acute pyridostigmine administration.

New Insights Into Postexertional Malaise

Postexertional malaise, a hallmark symptom of ME/CFS, is described as "flu-like" debilitating fatigue that typically

TABLE 2] Primary and Secondary Outcomes; Changes Between First and Second iCPET

End Point	Pyridostigmine (n = 23)	Placebo (n = 16)	Treatment Effect (95% CI)	*P* Value
Primary end point				
Peak Vo_2, mL/min	13.3 ± 13.4	−40.3 ± 21.3	53.6 (−105.2 to −2.0)	**.043**
Peak Vo_2, mL/kg/min	0.2 ± 0.2	−0.8 ± 0.4	1.0 (−1.9 to −0.7)	**.035**
Secondary end points				
Peak – rest Vo_2, mL/min	25.9 ± 15.3	−60.8 ± 25.6	86.7 (−148.1 to −25.2)	**.008**
Peak Qc, L/min	0.2 ± 0.2	−0.2 ± 0.3		.263
Peak – rest Qc, L/min	−0.2 ± 0.6	−1.9 ± 0.6	1.7 (−3.4 to −0.1)	**.039**
Peak RAP, mm Hg	1.2 ± 0.3	0.1 ± 0.5		.068
Peak – rest RAP, mm Hg	1.0 ± 0.5	−0.6 ± 0.5	1.5 (−3.0 to −0.04)	**.045**
Peak PAWP, mm Hg	1.0 ± 0.8	1.0 ± 0.5		1.0
Peak stroke volume, mL	3.0 ± 1.4	−1.1 ± 1.9		.093
Peak (Ca-vo_2)/[Hb]	0.0 ± 0.0	0.0 ± 0.0		.427
VE/Vco_2	−0.2 ± 0.8	1.0 ± 0.6		.262
Borg fatigue scale	0.1 ± 0.2	−0.6 ± 0.3	0.8 (−1.5 to −0.1)	**.038**
Borg dyspnea scale	−0.1 ± 0.2	−1.0 ± 0.5		.147

Data are represented as mean ± SD. Bold signifies statistical significance. Ca-vo_2 = arterial venous oxygen content difference; Hb = hemoglobin; PAWP = pulmonary artery wedge pressure; Qc = cardiac output; RAP= right atrial pressure; VE/Vco_2 = ventilatory efficiency; Vo_2 = oxygen consumption.

TABLE 3] Gas Exchange and Hemodynamics

End Point	Test	Pyridostigmine (n = 23)		Placebo (n = 16)	
		Rest	Peak	Rest	Peak
Primary end point					
Vo_2, mL/min	1	290.2 ± 62.8	1,221.5 ± 396.3	269.7 ± 54.0	1,304.3 ± 301.2
	2	277.6 ± 43.7	1,234.8 ± 404.1	290.2 ± 68.6	1,264.1 ± 309.4
Vo_2, mL/kg/min	1	4.7 ± 1.1	19.7 ± 6.7	4.3 ± 0.6	21.2 ± 5.7
	2	4.5 ± 0.9	19.9 ± 6.8	4.6 ± 0.8	20.5 ± 5.4
Secondary end points					
Qc, L/min	1	6.1 ± 1.6	11.0 ± 2.4	5.0 ± 1.1	11.5 ± 2.1
	2	6.4 ± 2.2	11.1 ± 2.5	6.6 ± 2.0	11.4 ± 1.8
RAP, mm Hg	1	−0.4 ± 1.6	−0.1 ± 2.1	−1.6 ± 2.3	0.4 ± 2.1
	2	−0.2 ± 1.4	1.1 ± 2.4	−0.9 ± 1.8	0.5 ± 2.9
PAWP, mm Hg	1	1.3 ± 2.0	5.1 ± 3.7	1.1 ± 1.3	4.4 ± 2.9
	2	2.0 ± 1.8	6.1 ± 4.5	1.6 ± 1.8	5.4 ± 2.5
SV, mL	1	73.2 ± 14.6	68.8 ± 10.5	63.1 ± 15.8	72.7 ± 11.4
	2	77.2 ± 27.7	72.0 ± 11.1	77.5 ± 26.4	73.8 ± 12.5
$(Ca\text{-}vo_2)/[Hb]$	1		0.8 ± 0.1		0.8 ± 0.1
	2		0.8 ± 0.1		0.8 ± 0.1
HR, beats/min	1	84.0 ± 15.0	159.2 ± 24.9	80.7 ± 9.9	156.9 ± 14.5
	2	85.3 ± 14.4	154.1 ± 25.6	86.6 ± 11.1	155.7 ± 14.1
VE/Vco_2	1	34.1 ± 7.7		28.3 ± 3.6	
	2	33.9 ± 8.2		29.3 ± 4.8	
Additional measures					
Vo_2 at AT, mL/min	1	647.6 ± 180.0		737.2 ± 184.3	
	2	699.0 ± 202.3		776.1 ± 165.4	
O_2 pulse % predicted	1	82.6 ± 20.1		91.7 ± 22.6	
	2	85.9 ± 20.0		88.2 ± 21.9	
$\Delta Qc/\Delta Vo_2$	1	5.3 ± 2.0		6.0 ± 2.2	
	2	4.2 ± 2.8		5.2 ± 2.5	
Peak Vo_2 % predicted	1	74.1 ± 24.9		79.8 ± 22.2	
	2	74.7 ± 25.2		77.2 ± 22.4	
Vo_2 at AT % predicted	1	39.5 ± 13.1		45.3 ± 14.5	
	2	42.8 ± 15.5		47.6 ± 13.5	
VD/VT	1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.1
	2	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1

Data are represented as mean ± SD. AT = anaerobic threshold; $Ca\text{-}vo_2$ = arterial venous oxygen content difference; Hb = hemoglobin; HR = heart rate; PAWP = pulmonary artery wedge pressure; Qc = cardiac output; RAP = right atrial pressure; SV = stroke volume; VE/Vco_2 = ventilatory efficiency; VD/VT = ratio of dead space to tidal volume; Vo_2 = oxygen consumption.

involves loss of physical stamina, cognitive impairment, impaired sleep, myalgias, arthralgias, and headaches.[47] The noninvasive, two-day CPET protocol of Keller et al[48] documented a significant decrease of peak Vo_2 on day 2, hypothesized to be related to postexertional malaise. The current study adds to the data of Keller et al, with invasive hemodynamics showing that the subsequent decrease in peak Vo_2 is driven by a decrease in Qc and cardiac preload. We hypothesize that postexertional malaise is related, in part, to neurovascular dysregulation precipitated by prior exercise.

In the placebo group, resting Vo_2 and Qc were increased prior to the second iCPET. Previous research suggests that immune-inflammatory mechanisms may play a role in the pathogenesis of ME/CFS by activating immune-inflammatory oxidative and nitrosative stress pathways.[49] Proinflammatory cytokines may be

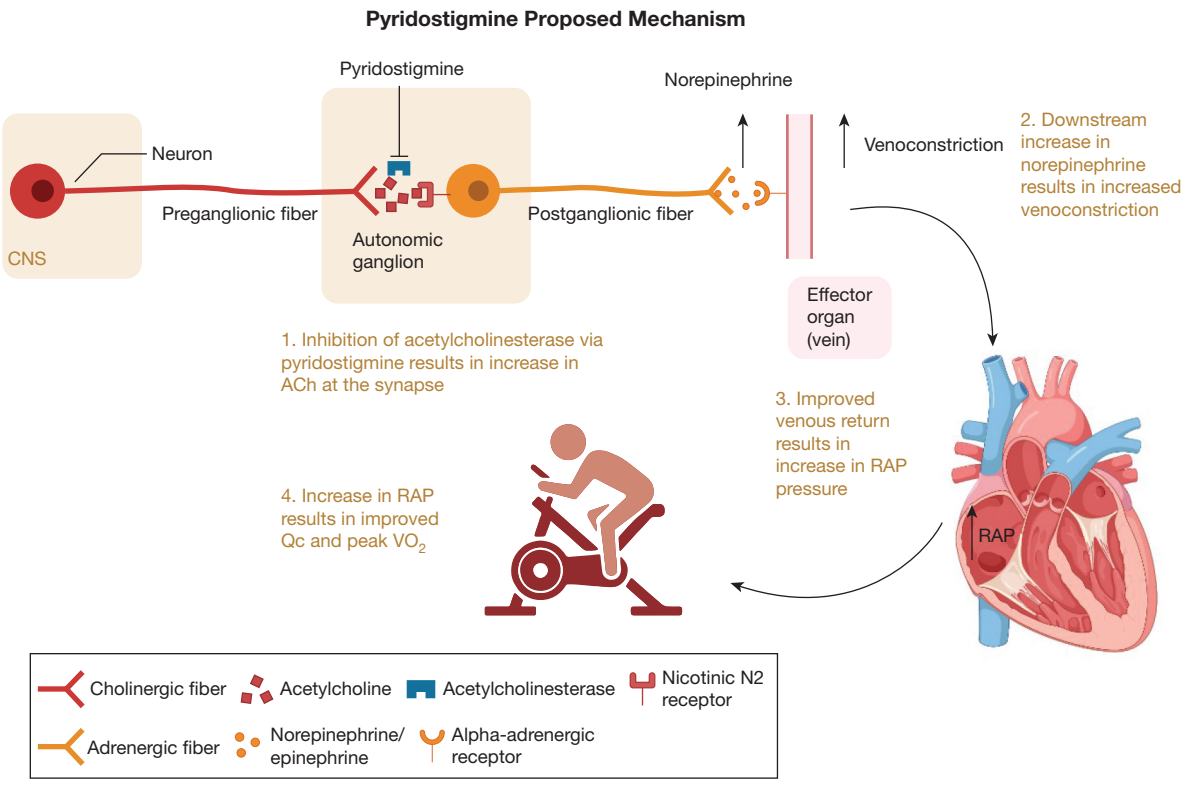

Figure 3 – *Pyridostigmine proposed mechanism. Ach = acetylcholine; NE = norepinephrine; Qc = cardiac output; RAP = right atrial pressure. (Adapted from BioRender.*[44]*)*

correlated with fatigue duration and disease severity, and cytokine profiling following submaximal exercise may help differentiate patients with ME/CFS from sedentary control subjects.[49,50] In addition, increased resting metabolism from raised sympathetic outflow in ME/CFS is supported by elevated plasma catecholamine levels and increased sympathetic nerve activity to the heart, skeletal muscle arterioles, and adrenals during rest. Moreover, adrenergic receptor upregulation may be contributing to sustained, resting sympathetic activity.[41,42] We speculate that an exaggerated immune-inflammatory and sympathetic response followed the first exercise test, resulting in elevated rest V_{O_2} and Qc. Further studies are required to investigate the metabolomic, proteomic, and inflammatory cytokine signatures for ME/CFS and provide insight into postexertional malaise.

Ventilatory Response

Dysfunctional breathing patterns may contribute to exercise limitations and impaired systemic oxygen extraction. Both groups showed ventilatory inefficiency according to elevated V_E/V_{CO_2} values. Hyperventilation or increased physiological dead space ventilation causes increases in V_E/V_{CO_2}, the latter not observed in this study. Thus, hyperventilation is the likely cause of dysfunctional breathing in this population, similarly observed in patients with PASC, some of whom have clinical overlap with ME/CFS.[30,31] Resulting alkalemia contributes to impaired systemic oxygen extraction by limiting the Bohr effect and causing a leftward shift of the oxygen-hemoglobin dissociation curve.[51] Ventilatory inefficiency may partly explain the lack of improvement in Borg fatigue and dyspnea scores, although underlying mechanisms remain unknown. Pyridostigmine did not influence ventilatory inefficiency. This may be due to a type II error. Another possibility is that increased cholinergic synaptic transmission in the sympathetic ganglion plays no role in the ventilatory response to acute exercise in ME/CFS.

Study Limitations

The physiological changes we describe are small and not clinically relevant, but within-group changes are concordant and between-group changes are statistically significant. The small changes in V_{O_2}, approximately 4% of peak values, may be a result of the use of a single dose of pyridostigmine studied acutely. It is possible that if we had used higher doses of pyridostigmine for a longer period, there may have been greater physiological changes. We justified this experimental protocol based

on a prior tilt table study of POTS using a very similar dosing regimen and a desire to use existing pulmonary and radial artery catheters.[19] Future studies should assess the chronic effects of pyridostigmine in subsets of patients with ME/CFS, varying in age, BMI, and pyridostigmine dose and duration needed to achieve clinically significant results.

Our previous work suggests two phenotypes of neurovascular dysregulation in ME/CFS: depressed Qc from impaired venous return and impaired peripheral oxygen extraction.[11] Although the latter was observed in the current study, acute administration of pyridostigmine did not influence peak ($Ca\text{-}vO_2$) values. We have found improved indices of aerobic capacity using long-term, increased dose of pyridostigmine in two published abstracts.[52,53] Future studies should investigate long-term use and higher doses of pyridostigmine and its effect on peripheral oxygen extraction through arteriolar regulation in the muscle bed.

The study population had a 38% prevalence of SFN, similar to the prevalence reported in POTS and fibromyalgia.[12,13] There was asymmetry in the distribution of length-dependent SFN between groups, but this finding did not reach significance. Lower leg biopsies designed to detect distal length-dependent SFN are less sensitive for patchy, proximal, or non-length-dependent SFN and are not expected to detect ganglionopathy,[54] the target of pyridostigmine.[19] The current study suggests that ganglionopathy and sympathetic outflow to systemic blood vessels are relevant to exertional intolerance in ME/CFS. It is also possible that enhancing sympathetic outflow from the ganglion with pyridostigmine overcomes the vasodilatory effects of SFN. Both pathways suggest that neurovascular dysregulation undermines exercise tolerance in ME/CFS.

Despite an improvement in peak VO_2 and exercise hemodynamics, scores on the Borg fatigue scale worsened slightly following pyridostigmine administration. We speculate that this may be due to more work achieved during the second iCPET.

This study consisted of 50 participants, five of whom were excluded (Fig 1). Of the 45 remaining subjects, 39 were female, and six were male. Although this gender distribution is reflective of the gender differences seen in ME/CFS,[55] our small sample size produced a randomization error in which all six male participants were placed in the placebo group. We elected not to include male patients in the primary analysis as their response to exercise was discordant compared with the female patients who received placebo. Peak VO_2, Qc, and RAP increased with serial iCPET. Based on Qc/VO_2 slopes and preserved systemic oxygen extraction, these subjects were characterized as the "low-flow" phenotype (e-Table 1). In addition, reducing the sample size increases the likelihood of type II errors. Studies with larger population sizes are required to ensure appropriate randomization and to assess the efficacy of pyridostigmine and differential exercise responses to both medication and placebo in male subjects.

Interpretation

Neurovascular dysregulation underlies acute exertional intolerance in ME/CFS. Pyridostigmine improves aerobic capacity in ME/CFS by increasing cardiac output through augmented right ventricular preload. A decrease in peak exercise VO_2, Qc, and RAP following placebo suggests a physiological mechanism underlying postexertional malaise. A similar approach using iCPET and pharmacologic intervention may prove useful in the study and treatment of PASC.[29,30]

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