The Exercise Response to Pharmacologic Cholinergic Stimulation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome – *Detailed Protocol*

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Protocol Title: The Exercise Response to Pharmacologic Cholinergic Stimulation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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I. Background and Significance

Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS), otherwise known as Chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME), is an under-recognized disorder whose cause is not yet understood. It is characterized by a constellation of symptoms that include debilitating fatigue for at least six months, post-exertional malaise, exertional dyspnea, cognitive impairment, orthostatic intolerance/autonomic dysfunction, widespread pain, gastrointestinal motility disorders, and unrefreshing sleep. Depending on varying definitions, ME/CFS is estimated to range from 10 to 25 percent of patients seen in primary care practices,[1] 75 to 267 cases per 100,000 persons,[2] or 836,000 to 2.5 million people in the United States.[3] In the United States, the lost productivity due to this disease results in an annual loss of $20,000 per person with CFS, or $9.1 billion.[4] Because of the often disconnected symptoms and resulting unnecessary medical tests and procedures, this number is thought to be an underestimation and may actually approach $23 billion in direct and indirect costs to society.[5]

The Institute of Medicine has published diagnostic criteria for ME/CFS.[3] Major criteria include substantial impairment from fatigue for six months, post-exertional malaise, and unrefreshing sleep. Minor criteria include cognitive impairment and orthostatic intolerance. As stated above, the cause of the syndrome is unknown with proposed mechanisms related to viral or infectious causes, immune system dysfunction, endocrine disorders, and neural dysregulation/autonomic dysfunction. Mounting evidence suggests that dysautonomia/autonomic dysfunction plays a prominent role in the pathophysiology of ME/CFS. Studies have shown an overlap in symptoms and diagnoses between postural orthostatic tachycardia syndrome (POTS), fibromyalgia, and ME/CFS.[6,7] Additionally, recent data have shown a high prevalence of a small fiber neuropathy (SFN) seen on skin biopsy in patients with chronic widespread pain syndromes,[8] fibromyalgia,[9] and POTS.[10]

Because of the significant exertional symptoms these patients suffer from, the Institute of Medicine and others have proposed changing the name of ME/CFS to Systemic Exertion Intolerance Syndrome.[11] Patients with SFN and unexplained dyspnea make up a prominent proportion of the patients seen in the Dyspnea Center at Brigham and Women's Hospital. These patients often go years without a diagnosis before an evaluation by a pulmonary physician at the Dyspnea Center. They are characterized as otherwise healthy individuals with diagnoses of ME/CFS, POTS, orthostatic hypotension, and/or fibromyalgia. Symptoms include exertional dyspnea, post-

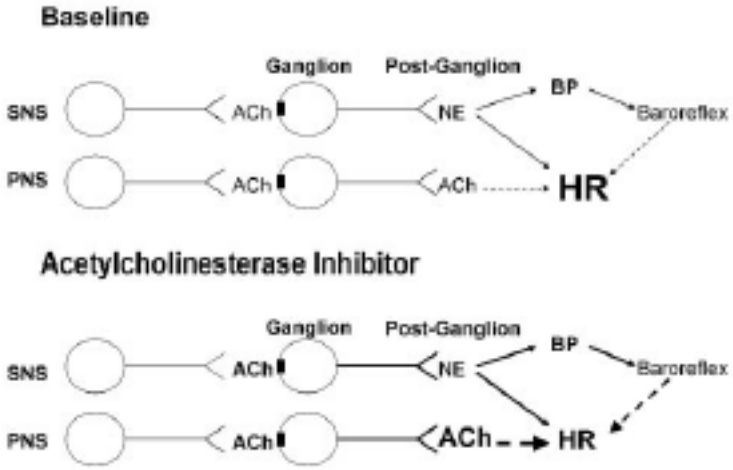
exertional malaise, pre-syncope, and perioral numbness and tingling due to hyperventilation.

A significant number of these patients have been evaluated clinically with a level 3 cardiopulmonary exercise tests (CPET). This involves the placement of a pulmonary artery catheter and radial artery catheter in the cardiac catheterization suite. After these are placed and secured, the patient performs upright exercise on a stationary cycle ergometer against increasing resistance using a ramp protocol.[12] Simultaneous measurements of pressures in three chambers of the heart (right atrium, right ventricle, pulmonary capillary wedge pressure/left atrial pressure/left ventricular end diastolic pressure), the pulmonary artery pressure, mean arterial pressure, and gas exchange variables (oxygen uptake and carbon dioxide production) are obtained from the resting state to the post-exercise state. Blood gas samples are drawn from both the arterial and venous compartment throughout exercise to analyze oxygen extraction, pH, lactate, partial pressure of oxygen (O2), and the partial pressure of carbon dioxide (CO2). Using the Fick principle, cardiac output is calculated, and various cardiopulmonary diseases are ruled out based on the combination of oxygen uptake, cardiac output, peripheral oxygen extraction, and cardiac chamber pressure measurements.

Our previous study has shown that low biventricular filling pressures ("preload failure", PLF) seen during level 3 CPET is the cause of unexplained dyspnea in patients with ME/CFS.[13] In this study, impaired patients, defined as oxygen uptake below 80% of predicted, were compared to normal patients after the exclusion of heart failure, pulmonary hypertension, and pulmonary mechanical limits to exercise. These data show that subjects in the impaired group have significantly lower and reversible biventricular filling pressures at peak exercise, suggesting this as a cause of lower oxygen uptake and decreased cardiac output. Of the impaired subjects who underwent evaluation for underlying causes, a significant number had positive findings for dysautonomia either by tilt table testing or nerve conduction studies.

As mentioned above, numerous studies have demonstrated up to an approximately fifty percent prevalence of SFN in chronic widespread pain, fibromyalgia, and POTS. Studies have shown that in POTS, there is abnormal venous pooling in the legs upon standing.[14] Furthermore, these patients demonstrate a hypersensitivity to norepinephrine or phenylephrine infusions in relation to peripheral venoconstriction, suggesting that they have an upregulation of adrenergic receptors in response to nerve atrophy and a lack of endogenous catecholamine production.[15,16] This is reinforced by the observation of decreased norepinephrine spillover after sympathetic nervous system evaluation in patients with POTS.[17]

Because of the evidence of impaired neural regulation of venous tone as a cause of symptoms in POTS, therapies aimed at increasing venoconstriction or intravascular volume have been attempted with varying degrees of success. These include midodrine, salt tabs, aggressive oral hydration, and fludrocortisone. Augmenting neurotransmitter release of norepinephrine with pyridostigmine has been studied with significant improvement in both symptom burden and heart rate response.[18] By inhibiting acetylcholinesterase, pyridostigmine is thought to enhance cholinergic stimulation of norepinephrine release at the post-ganglionic synapse.[18]



Recent work analyzing the Brigham and Women's Hospital CPET database has shown that among patients with low filling pressures (" preload failure"), approximately 50% has SFN seen on skin biopsy (data unpublished, manuscript in process). Off-label use of pyridostigmine for treatment of preload failure has shown a significant increase either in submaximum[19] or maximum aerobic capacity.[20] This is thought to be due to the same mechanisms described above; increased catecholamine release results in improved venoconstriction during exercise, thus increasing venous return to the heart.

While retrospective reviews have been published about SFN and dysautonomia, prospective treatment studies are scarce, and none has been published about the treatment of preload failure. In the past, dysautonomia and preload failure account for up to 20% of the diagnoses obtained from the CPET program[21], but more recently the prevalence is as high as 50%. The aim of this study is to assess the exercise response to pharmacologic cholinergic stimulation with pyridostigmine in preload failure. This will be evaluated in a randomized, double-blind, placebo-controlled trial. ME/CFS ~~is~~ remains a disease of unclear etiology with a significant worldwide burden. This study will enhance our knowledge into the pathophysiology behind ME/CFS and create new treatment strategies.

II. Specific Aims

The hypothesis of our study is that hemodynamic, ventilatory and oxygen exchange variables such biventricular filling pressures and systemic oxygen extraction can be improved by cholinergic stimulation in patients with ME/CFS.

The objective of this study is to examine the exercise response to pharmacologic cholinergic stimulation in ME/CFS patients already undergoing a clinically indicated level 3 cardiopulmonary exercise test (CPET). This will be achieved by inhibiting acetylcholinesterase with pyridostigmine, thus increasing acetylcholine levels, downstream levels of norepinephrine, and enhancing vascular regulation.

To test our hypothesis, we propose the following specific aims:

Primary Aim:

1. Define the response of peak oxygen uptake (VO2) to pyridostigmine

Secondary Aims:

1. Define the gas exchange responses, such as end-tidal CO2 and ventilatory efficiency to pyridostigmine.
2. Define the hemodynamic responses, such as right atrial pressures, pulmonary artery pressure, pulmonary capillary wedge pressures, cardiac output, heart rate, stroke volume, pulmonary vascular resistance and systemic vascular resistance to pyridostigmine.
3. Evaluate the response of skeletal muscle oxygen extraction and lactate to pyridostigmine.

These determinations will occur during a *clinically indicated* level 3 CPET, which includes exercising on a stationary cycle with a right heart catheter (RHC) and a radial arterial line in place. To stimulate the cholinergic response, a single dose of an oral acetylcholinesterase inhibitor, pyridostigmine, versus placebo will be given after the level 3 CPET. Recovery cycling will be performed after a rest period of 50 minutes. This will be administered in a randomized, double-blind, placebo-controlled trial.

The above variables have been well described as objective markers of exercise capacity and its use in diagnosing cardiopulmonary diseases.[22] Many of these variables are based on the Fick principle:

Oxygen uptake = Cardiac Output *(Arterial-venous oxygen content difference)

Oxygen uptake (VO2) and carbon dioxide output (VCO2) are continuously measured using a metabolic cart. Based on the equation above, oxygen uptake represents a composite of cardiac output and peripheral oxygen extraction, thus revealing the status of the cardiovascular system as a whole. This is further analyzed by obtaining both arterial and mixed venous oxygen saturations during exercise through the radial and pulmonary artery catheters, allowing a calculation of the arterial-venous oxygen content difference. Finally, oxygen uptake and the arterial-venous oxygen content difference is used to calculate cardiac output using the Fick principle outlined above.

The arterial-venous oxygen content difference is used to assess peripheral oxygen extraction. By adjusting this to the patient's hemoglobin, we can evaluate for macroscopic shunts, peripheral shunts, and mitochondrial dysfunction.

Ventilatory efficiency is defined as Minute Ventilation (VE)/Carbon Dioxide production (VCO2). Through the alveolar ventilation equation (see below), we are able to determine if there is increased dead space ventilation or hyperventilation. Additionally, end-tidal CO2 measurements will be used to determine if hyperventilation is present.

$V_A = \frac{VCO2xK}{PACO2}$; $VA = R(VT - VD)$

Where
V_A: alveolar ventilation (mL/min)
V_{CO2}: Rare of CO2 production (mL/min)
PA_{CO2}: Alveolar PCO2 (mmHg)

R: Respiratory rate (breaths/min)
K: Constant (863 mmHg)
VT: Tidal volume (mL)
VD: Physiologic dead space (mL)

Right atrial pressures and pulmonary capillary wedge pressure measurements are obtained through the pulmonary artery catheter and measured with the Philips Xper Information Management System. Normal values and tracings have been well described in the past and the physician investigators are well versed in interpreting these values and waveforms.[23] Based on prior studies, a right atrial pressure of 6.5 mmHg will be considered the lower limit of normal at peak exercise.[13]

III. Subject Selection

All adults between 18 and 80 years old who are referred for a clinically indicated level 3 CPET (stationary cycle exercise testing with a RHC and radial arterial line place) for the evaluation of unexplained exercise intolerance, most often from BWH outpatient pulmonary and cardiology clinics will be pre-screened for this study. Prospective subjects's medical records will be initially pre-screened for the following initial eligibility criteria:

Initial (Pre-screening) Inclusion criteria:

1) Meets the Institute of Medicine (IOM) criteria for ME/CFS, or
2) Has medical comorbidities associated with ME/CFS such as dysautonomia, low ventricular filling pressures, postural orthostatic tachycardia syndrome (POTS), orthostatic hypotension, or fibromyalgia. Further confirmation that a subject meets the IOM criteria for ME/CFS by the telephone pre-screening questionnaire will be needed for subjects that meet this Initial Pre-screening inclusion criteria.

Initial (Pre-screening) Exclusion criteria:

1) Obesity (BMI > 30 kg/m2)
2) Non-controlled asthma
3) Anemia (Hb < 10 g/dl)
4) Active or treated cancer
5) History of interstitial lung disease (ILD)
6) Chronic obstructive pulmonary disease (COPD)
7) Pulmonary hypertension (PH)
8) Congestive heart failure (CHF)
9) Active arrhythmias
10) Valvular heart disease
11) Coronary artery disease (CAD)
12) Other conditions that could predict a limitation or not completion of the study (as determined by the PI).

Based on the medical history obtained from the electronic medical record, potential subjects will receive a recruitment letter at least one week prior to their CPET, and if a potential subject is interested in the study, a consent form will be given no less than two days before their test.

On the date of their test, potential subjects, who agree to participate and sign the consent form, will be evaluated for the following final eligibility criteria.

Final (Screening) Inclusion criteria:

1) Completing the clinically indicated level 3 CPET

Final (Screening) Exclusion criteria:

1) Pregnancy test positive in female patients.
2) Submaximal testing in clinically indicated level 3 CPET: peak HR < 80% predicted OR peak RER < 1.05. [24]
3) Pulmonary mechanical limitation to exercise in clinically indicated level 3 CPET: VE /MVV < 0.7 at AT.[25]
4) Pulmonary arterial hypertension in clinically indicated level 3 CPET rest mPAP > 25 mmHg and rest PCWP < 15 mmHg and PVR ≥ 240 dynes. [26]
5) Pulmonary venous hypertension in clinically indicated level 3 CPET: rest mPAP >25 mmHg and rest PCWP >15 mmHg.[27]
6) Exercise induced pulmonary arterial hypertension in clinically indicated level 3 CPET: In patients > 50 years of age: peak mPAP > 33 mmHg and PVR > 168 dynes; and in patients > 50 years of age: peak mPAP > 33 mmHg and PVR > 168 dynes. [28]
7) Exercise induced pulmonary venous hypertension in clinically indicated level 3 CPET: rest mPAP ≥ 25 mmHg and PCWP ≤ 15 mmHg and, in patients ≤ 50 years of age: PCWP > 19 mmHg and PVR < 108 WU; or in patients > 50 years of age peak PCWP > 17 mmHg and PVR ≤ 168 WU. [27]
8) Persistent hypotension during or after the clinically indicated level 3 CPET: SBP < 90 mmHg for more than 5 minutes.
9) Refractory arrhythmia during or after the clinically indicated level 3 CPET.

IV. Subject Enrollment

Subjects will be recruited from the population of adults between 18 and 80 years old who are scheduled to undergo a clinically indicated level 3 CPET at Brigham and Women's Hospital (BWH). These subjects are scheduled for this test for the evaluation of unexplained exertional intolerance and, for the most part, are seen in the Dyspnea Clinic at Brigham and Women's Hospital prior to testing. This clinic is staffed by multiple pulmonary physicians besides the principal investigator. Dr. Systrom, the principal investigator, who is also the director of the Dyspnea Clinic and the Advanced CPET Program at BWH, will be the physician who will conduct the cardiopulmonary exercise tests for every patient.

Subjects will be pre-screened as described above. Based on the medical history obtained from the electronic medical record, potential subjects will be recruited. A recruitment letter signed by Dr. Systrom, as the physician who will perform the cardiopulmonary test for all eligible subjects, will preferably be mailed, or, if not possible, emailed through a send secure e-mail to potential eligible subjects.

Prospective subjects will be given a one-week period to contact the research staff at either their emails or their phone numbers to notify if they are or not interested in knowing more about the study. If after that period, the research staff has not heard from the patient, the prospective subject will be called by a research staff to see if they are interested. If a prospective subject is interested in knowing more about the study, a detailed explanation of the study protocol will be given and pre-screening questions will be asked to determine eligibility, if data was not clearly stated in their electronical medical records, (please see attached telephone script). If a subject meets the initial eligibility criteria, ample time will be provided to ask questions, and the study staff will reinforce the subject that participation is voluntary, that they do not have to participate, and that the decision not to participate will not affect their care, now or in the future. If the subject agrees, a consent form will be sent through a send secure e-mail or mailed to read. Study staff will encourage the subject to share the consent form with health care providers and/ or relatives. A follow up phone call or email will be held with the patient to assess if they would like to participate in the study at least 48 hours after they receive the consent form and before his/her scheduled iCPET.

Subjects may be enrolled directly from Dr. Systrom's Dyspnea Clinic visit at BWH. In this case, the research study will be mentioned in a clinic visit and, if the prospective subject expresses interest in the study, the consent form will be given to the prospective subject to read at home and research staff will call the prospective subject to go over the study, assess eligibility, answer questions, and see if the prospective subject would like to participate in the study. Prospective subjects will be encouraged to discuss this study with other healthcare providers to obtain additional guidance.

Prospective subjects, who are not Dr. Systrom's patients, but who communicate with any of the research team to express their interest to participate in the study, regardless of how they find out about the study, will be pre-screened and provided with the consent form if potentially eligible.

Prospective subjects who are taking pyridostigmine would be asked to discuss holding their medication at least one week before the study with their prescribing physician.

The day of their level 3 CPET, prospective subjects will be given another copy of the consent form and, once all questions are answered and if the prospective subjects still want to participate, they will sign the consent form. Study staff will then take a physical copy and an electronic copy of the consent form signed by either Dr. Systrom or a licensed co-investigator and the subject, give the physical copy of the signed consent form to the subject and add the original signed consent form to the subjects' source documents and upload the electronic copy to their electronic medical records.

All subjects will also be participating in the Pulmonary Vascular Disease Research & Clinical Care Tissue Bank (Protocol # 2011P000272).

V. Study Procedures

Patients will undergo a clinically indicated level 3 CPET. The standard of care for this involves placing a pulmonary artery catheter/right heart catheter and a radial artery catheter in the cardiac catheterization lab. Patients are then transported to the exercise lab for a cardiopulmonary exercise test with both catheters in place. Usual practice involves obtaining hemodynamic measurements at rest, then during free-wheeling (no resistance), then with increasing resistance until they reach peak exercise determined by heart rate response and gas exchange measurements. Hemodynamics are recorded every minute during exercise. Arterial and mixed venous blood samples are collected every minute during exercise. The CPET concludes when the patient asks to stop the exercise test or after peak exercise has been achieved. Hemodynamic measurements and blood draws also occur during the recovery period.

The details of the study after the clinically indicated CPET are as follows:

1. After a clinically indicated level 3 CPET, a subject's hemodynamic waveforms and ventilatory variables will be reviewed for the final exclusion criteria. The clinically indicated level 3 CPET will be supervised by the physician investigators.
2. The subjects who meet the final eligibility criteria and who attest that they can do the recovery cycling will be given a single dose of pyridostigmine 60 mg PO versus placebo. This will be administered by either the physician investigators, or a physician's assistant. Both the patient and physician will be blinded to the study drug. Drug selection will be randomized.
3. The subject will remain in the exercise lab to rest for 50 minutes. They will be under the supervision of a physician or physician's assistant for this time-period and continuously monitored using a portable cardiac monitor. The pulmonary artery and radial artery catheters will be flushed with heparinized-saline by the exercise physiologists, physician investigator or physician's assistant to ensure that the lines will remain patent, as it would be usually done in order to draw the 1-hour post lactate samples.
4. After 50 minutes, the patient will perform the recovery cycling. Usual practice of the cardiopulmonary exercise test involves obtaining arterial and mixed venous blood samples every minute during exercise. During recovery cycling, arterial and mixed venous blood samples will only be obtained two times: during rest and at peak exercise. This adds 6 mL of blood drawn in addition to the blood drawn during the clinically indicated CPET. Recovery cycling will be performed under supervision of the physician investigators.
5. Key differences between recovery cycling and the clinically indicated CPET include:
    - Pulmonary capillary wedge pressure and other hemodynamic parameter's measurements taken only two times (rest and peak exercise), minimizing the already small risk of pulmonary artery rupture.
    - Elimination of a lactate level drawn one hour after exercise completion, expediting catheter removal.
    - Minimization of blood drawn to 6 mL, compared to 45 mL drawn during the clinically indicated CPET.

6. Additionally, a demographics questionnaire and the ME/CFS ICC 1-10 Symptom Severity Questionnaire[29] will be administered to all subjects. These will either be administered through REDCap or on paper and results stored in REDCap and in the subjects study chart.

7. The modified Borg Scale[30] for assessing dyspnea and fatigue will be administered after the clinically indicated CPET and after recovery cycling with the study drug. This will be used to assess subjective responses to the study drug.

8. Subjects will receive a follow up phone call or email within the following week, ideally within two days after the study to assess if they experience any adverse events from the study. This will be documented for every patient and if severe adverse events happen, action will take place to improve subject's health and the IRB will be notified.

VI. Biostatistical Analysis

Statistical analysis will be performed with either STATA, SAS, or Graphpad Prism. With 80% power to detect a 10% difference in oxygen uptake at peak exercise, we estimate the need to enroll 60 patients. This number also accounts for screening failures.

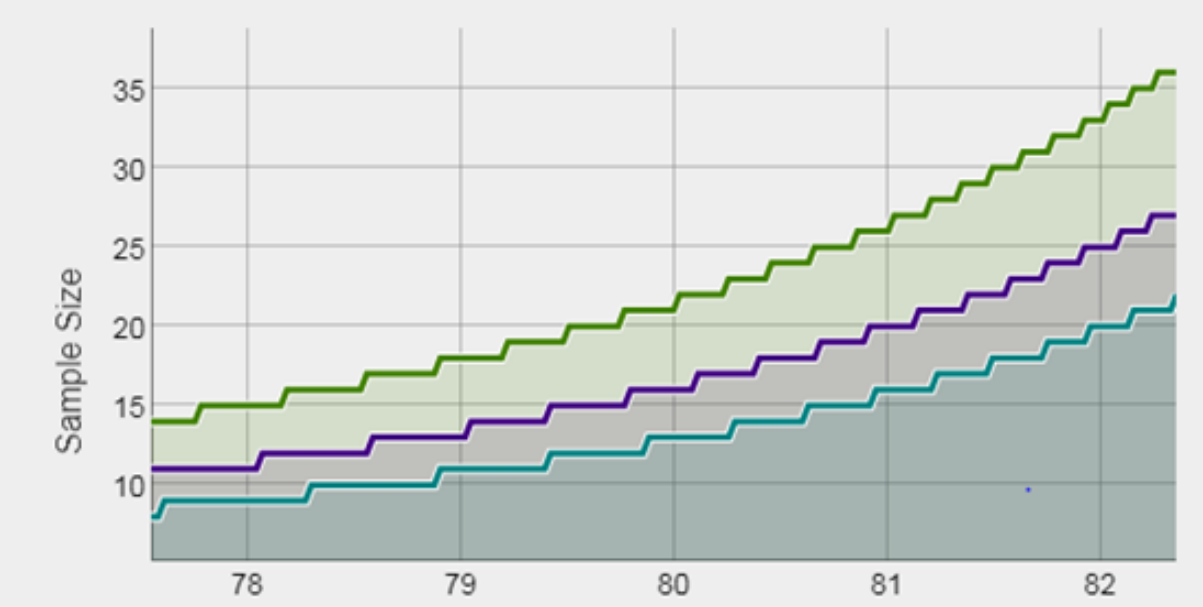
$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(10^2 + 10^2/1)(1.96 + 0.84)^2}{10^2}$$

$$n_1 = 16$$

$$n_2 = K * n_1 = 16$$



Y-axis: Sample size
X-axis: Single-arm sample size
Upper green line: 90% power
Middle purple line: 80% power
Lower teal line: 70% power

VII. Risks and Discomforts

There are risks of side effects from pyridostigmine administration, such as nausea, diarrhea, muscle cramping and twitching, excessive drooling, bradycardia, and excessive ocular tearing.

Risks of blood draws include a small risk of infection, lightheadedness, and/or fainting because of the additional blood that is being drawn.

Risks of the arterial catheter remaining in place for recovery cycling include pain, bleeding, swelling, or redness where the catheter is placed, brief loss of pulse due to blood flow problems in the artery, damage to the artery wall or nearby nerves, inadvertent removal, and infection (infection is rare and happens in less than 1 in 100 cases). Placement of a radial arterial line in the wrist can cause decreased blood flow to the hand that may require surgery to correct. This is very rare and has not happened when the catheter is left in place for only a few hours for research purposes.

There are risks related to the pulmonary arterial (PA) catheter remaining in place for the rest period and recovery cycling. Risks may include arrhythmias, tachycardia, bradycardia, and infection. This is minimized by careful observation for any of these events during the clinically indicated CPET.

There is a risk of pulmonary artery injury during the PCWP measurements (balloon inflation). This is minimized by limiting this to three inflations during recovery cycling and knowing exactly how much air is needed to inflate the balloon based on the prior CPET. Among approximately 7,000 invasive CPETs performed between

Massachusetts General Hospital and Brigham and Women's Hospital, there has only been one case of pulmonary artery rupture.

While exercise testing is generally safe, risks related to recovery cycling include heart attack, stroke, and dangerous arrhythmias. The risks are minimized by requiring the successful and uneventful preceding, clinically indicated CPET.

The risks listed above are identical to those during the clinically-indicated CPET. Both the administration of pyridostigmine or placebo and cardiopulmonary exercise testing will be performed under the direct supervision of the physician investigators.

There is a risk that the subject's private health information will be released to the public. All subjects participating will be assigned a specific study number. All information will be de-identified.

For those patients who are taking pyridostigmine and are asked to withhold it before the study, mild discomfort might arise. Although no significant withdrawal symptoms have been linked to pyridostigmine withholding, in some subjects some mild ME/CFS symptoms might return transiently. Subjects who are asked to withhold pyridostigmine before the study will be instructed to resume taking it as soon as the test is completed.

VIII. Potential Benefits

ME/CFS remains a prominent medical issue with very little known about its pathophysiology and treatment. At present, little is also known about the relationship between the autonomic nervous system and cardiopulmonary hemodynamics during exercise. Information obtained from this research will contribute to our understanding of normal human physiology and the pathophysiology behind ME/CFS.

Subjects enrolled in this study may not benefit from this study. Research obtained from their participation may benefit other patients in the future who suffer from exertional intolerance.

IX. Monitoring and Quality Assurance

The principal investigators will perform this function. This study is an investigator-initiated study and will be conducted only at Brigham and Women's Hospital. The range of possible study events that could have an important impact on the risks and benefits of research participants is narrow. Continuous monitoring of events by the investigator will prompt reporting of serious adverse events to the IRB and, when applicable, the FDA, or others.

All reports of adverse events, breaches of confidentiality, complaints, and unanticipated problems will be reviewed by the principal investigator and will be submitted to the IRB per the Partners HRC policy.

The principal investigators will be required to guarantee that research staff prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All study data recorded will be documented in the database maintained by the exercise lab and

source documents. The research team will meet weekly to review the accuracy and completeness of the consent forms and data collected per protocol.

To ensure privacy and confidentiality, codes will be substituted for identifiers and access to the data will be restricted to the principal investigator and the research team. The research staff will be reminded at regular intervals of the importance of maintaining research subject confidentiality. Research records are kept electronically in either a Partners encrypted laptop or desktop computer kept at the Thorn building and, whenever possible, physically in a locked cabinet at BWH, PBB-Clinics 3. No information on subjects and biological samples will be sent outside of the Partners network. Materials will not be maintained for use outside the scope of this protocol.

X. References

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