The attached includes:

Protocol: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

- Statistical Plan included within the protocol

NCT02669212

Document Date: 11/10/2020

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome
ME/CFS at NIH
Protocol Number: 16-N-0058
Date of This Submission/Version: 11/10/2020, Version 18.0

Principal Investigator

Name, Degree	Branch/Institute	Bldg/Rm	Phone	E-mail
Avindra Nath, MD	SINS/ NINDS	10/ 7C-103	301-496-1561	natha@ninds.nih.gov

Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff: None

Total requested accrual: 346
(50) Post-infectious Myalgic Encephalomyelitis/Chronic Fatigue Syndrome participants
(50) Healthy Volunteers
(50) Recovered COVID-19 infection—asymptomatic participants
(176) Focus Group participants
(20) Technical Development sub-study

Project Uses Ionizing Radiation: ☐ No ☒Yes
☐Medically-indicated only
☒ Research-related only
☐Both

IND/IDE ☒ No ☐Yes

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes
Covered Protocol Requiring DEC Clearance (per SOP 21) ☐ No ☒ Yes

Approved for Short Form Consent Process for Non-English Speakers ☒ No ☐Yes

Flesch-Kincaid reading level of ME/CFS, HV consent form: 8.9
Flesch-Kincaid reading level of COVID-19 HV consent form: 9.2
Flesch-Kincaid reading level of Focus Group A consent form: 8.6

Flesch-Kincaid reading level of Focus Group B consent form: 9.0
Flesch-Kincaid reading level of Technical Development Substudy consent form: 9.7
Flesch-Kincaid reading level of Genetic consent form: 10.5

Précis:

Objective The primary objective is to explore the clinical and biological phenotypes of post-infectious myalgic encephalomyelitis/chronic fatigue syndrome (PI-ME/CFS). The secondary objective is to explore the pathophysiology of fatigue and post-exertional malaise (PEM).

Study population Up to 346 persons will be enrolled as part of this protocol. Up to 150 persons aged 18-60 will be part of 3 study groups: 50 ME/CFS patients whose fatigue began after an infection, 50 non-fatigued participants with a documented history of a full recovery from a COVID-19 infection, and 50 healthy volunteers. The study has a target of completing all study procedures on 20 enrolled participants in each group. Up to an additional 176 persons reporting a community diagnosis of ME/CFS will be enrolled into focus groups to discuss the experience of post-exertional malaise. Up to an additional 10 healthy volunteers and 10 ME/CFS patients may be enrolled to refine the protocol's electrophysiological and neuroimaging techniques.

Design This is a single-center, exploratory, cross-sectional study of PI-ME/CFS. Participants will have a *phenotyping visit*, which will encompass a 2-5 day long inpatient admission at the NIH Clinical Center. Case status for ME/CFS participants will be determined after the phenotyping visit by a *case adjudication* process utilizing an expert physician committee and published guidelines. Adjudicated participants meeting inclusion criteria will be invited back to participate in an *exercise stress visit,* which will encompass a 5-10 day long inpatient admission. Detailed subjective and objective measurements and biological specimens will be serially collected before and up to 96 hours after a peak exercise test capable of inducing post-exertional malaise during this visit. All procedures will be completed on all three study groups to allow for optimal inter-group comparisons.

Outcome measures
The primary purpose of this protocol is to perform exploratory analysis of collected samples for the generation of new hypotheses regarding ME/CFS. The types of analyses to be performed will be wide ranging. Planned areas of focus include:

1. Characterization of the immune system and inflammatory signaling in collected samples at baseline and following maximal exercise exertion.
2. Characterization of the pattern of microbiome in collected samples at baseline and following maximal exercise exertion.
3. Characterization of bioenergetics, autonomic, and metabolic function in collected samples at baseline and following maximal exercise exertion.
4. Characterization of physical and cognitive fatigue using functional magnetic resonance imaging and transcranial magnetic stimulation at baseline and following maximal exercise exertion.
5. Characterization of neurocognition at baseline and following maximal exercise exertion.

6. Characterization of brain function and connectivity at baseline and following maximal exercise exertion.
7. Characterization of autonomic function at baseline and following maximal exercise exertion.
8. Characterization of gene expression profiles in collected samples at baseline and following maximal exercise exertion.

Contents

Précis: 2
List of Abbreviations 8
1 Introduction 10
1.1 HISTORY....10
1.2 DEFINITION....11
1.3 ETIOLOGY....11
1.4 EPIDEMIOLOGY 13
1.5 DIAGNOSTIC CHALLENGES 13
1.6 SOCIO-ECONOMIC BURDEN 14
1.7 DISABILITY 14
1.8 MORTALITY....14
2 Study Objectives....15
2.1 Primary objective....15
2.2 Secondary objective 15
3 Subjects 15
3.1 Description of study populations 15
3.2 Inclusion criteria 16
3.2.1 Inclusion criteria for all participants 16
3.2.2 Additional inclusion criteria for participants with PI-ME/CFS for the phenotyping visit 16
3.2.3 Additional inclusion criteria for participants with PI-ME/CFS for the exercise stress visit 17
3.2.4 Additional inclusion criteria for healthy volunteer group....17
3.2.5 Additional inclusion criteria for Documented Lyme Infection – Asymptomatic group 17
3.3 Exclusion criteria 18
3.3.1 Exclusion criteria for all participants 18
3.3.2 Additional exclusion criteria for participants with PI-ME/CFS forphenotyping visit 19
3.3.3 Additional exclusion criteria for participants with PI-ME/CFS for exercise stress visit 19
3.3.4 Additional exclusion criteria for healthy volunteer group....19
3.3.5 Additional exclusion criteria for Documented Lyme Infection – Asymptomatic

group 19
4 Study Design and Methods....19
4.1 Study overview....19
4.2 Recruitment....21
4.3 Focus Groups....22
4.31 Focus Group Study A....22
4.31.1 Inclusion criteria for focus group A participants....22
4.31.2 Exclusion criteria for focus group participants....23
4.32 Focus Group Study B....24
4.4 Technical Development Sub-Study....27
4.5 Phenotyping Visit (Visit 1: Phenotyping)....29
4.6 Study procedures: Exercise Stress visit (Visit 2)....43
4.6.1 : Updated History and Physical Examination....44
4.6.2 : Intravenous line placement....44
4.6.3 : Blood collection....44
4.6.4 : Urine collection....44
4.6.5 : Saliva sample collection....44
4.6.6 : Patient-reported outcome....45
4.6.7 : Physical activity monitoring....47
4.6.8 : Metabolic Chamber....47
4.6.9 : Body Composition Measurement by Dual Energy X-ray Absorptiometry....48
4.6.10 : Metabolic Diet and Nutritional Assessment....48
4.6.11 Resting Energy Expenditure Measurements by Ventilated Hood....48
4.6.12 Peak Exercise Testing....49
4.6.13 : Qualitative measurement of Post-Exertional Malaise....49
4.6.14 : Blood measurements before and after CPET....50
4.6.15 : Neurocognitive testing....50
4.6.16 : Holter monitoring....50
4.6.17 : fMRI....50
4.6.18 : Effort-Expenditure for Rewards Task (EEfRT)....52
4.6.19 : Lumbar puncture....53
4.6.20 : Stool Sample Collection....53
4.6.21 : Sleep Electrocephalogram....53

5 Descriptions of Optional Produres 53
5.1.1 Sedation during LP 53
5.1.2 Fluoroscopic-guided LP 54
5.1.3 Medical, Psychiatric, and Rehabilitation Consultation 54
5.1.4 Whole genome sequencing 54
5.1.5 Skin Biopsies 54
5.1.6 Muscle Biopsy 54
5.1.7 Functional Magnetic Resonance Imaging (fMRI) 55
5.1.8 Transcranial Magnetic Stimulation (TMS) 55
5.1.9 Metabolic Chamber wearing EEG cap and accelerometers 56
6 End of participation 56
7 Management of Data and Samples 57
7.1 Storage 57
7.2 Data (including genomic data) and sample sharing plan 57
8 Additional Considerations 58
8.1 Research with investigational drugs or devices 58
8.2 Gene therapy 59
9 Risks and Discomforts 59
9.1 Risks of history and physical examination 59
9.2 Risks of IV insertion 59
9.3 Risks of blood collection 59
9.4 Risks of HIV testing 59
9.5 Risks of urine collection 59
9.6 Risks of symptom assessment/patient reported outcome measures 60
9.7 Risks of psychological assessments and psychological inventories 60
9.8 Risks of neurocognitive testing 60
9.9 Risks of Occupational therapy evaluation 61
9.10 Risks of MRI 61
9.11 Risks of muscle strength testing 62
9.12 Risks of activity monitoring and fatigue diary 63
9.13 Risks of nutritional assessment and metabolic diet 63
9.14 Risks of saliva collection 63
9.15 Risks of buccal swab collection 63

9.16 Risks of stool sample collection 63
9.17 Risks of Holter monitoring 63
9.18 Risks of LP 63
9.19 Risks of sedation for LP 63
9.20 Risks of fluoroscopic-guided LP 63
9.21 Risks of autonomic testing 64
9.22 Risks of cytapheresis 64
9.23 Risks of immune cell blood draw 64
9.24 Risks of medication washout 64
9.25 Risks of metabolic chamber 64
9.26 Risks of DEXA 64
9.27 Risks of resting energy expenditure measurements by ventilated hood 65
9.28 Risks of peak exercise testing 65
9.29 Risks of Qualitative Interviews 65
9.30 Blood measurements before and after CPET 65
9.31 Risks of Effort-Expenditure for Rewards Task 65
9.32 Risks of TMS 65
9.33 Risks of sleep electroencephalogram 66
9.34 Risks of Medical, Psychiatric, Sleep Service and Rehabilitation Consultation 66
9.35 Risks of genetic testing 66
9.36 Risks of Skin Biopsies 67
9.37 Risks of Muscle Biopsy 67
10 Subject Safety Monitoring 67
11 Outcome Measures 67
12 Statistical Analysis 67
12.1 Analysis of data/ study outcomes 67
12.2 Power analysis 69
13 Human Subjects Protection 69
13.1 Subject selection 69
13.2 Justification for exclusion of children 70
13.3 Justification for exclusion of pregnant women 70
13.4 Justification for exclusion of women who have given birth in the past year 70
13.5 Justification for exclusion of those who lack consent capacity 70

13.6 Justification of exclusion of non-English speakers 70
13.7 Justification for inclusion of employees 70
13.8 Justification for sensitive procedures 71
13.9 Safeguards for vulnerable populations and sensitive procedures 71
14 Anticipated Benefit: Indirect 72
15 Consent Documents and Process 72
15.1 Consent procedures 72
15.2 Consent documents 73
16 Data and Safety Monitoring 73
16.1 Data and safety monitor 73
16.2 Data and safety monitoring plan 73
16.3 Criteria for stopping the study or suspending enrollment or procedures 73
17 Quality Assurance 73
17.1 Quality assurance monitor 73
17.2 Quality assurance plan 73
18 Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations 74
19 Alternatives to Participation 74
20 Privacy 74
21 Confidentiality 74
21.1 For research data and investigator medical records 74
21.2 For stored samples 74
21.3 Special precautions 74
22 Conflict of Interest 74
22.1 Distribution of NIH guidelines 74
22.2 Conflict of interest 75
23 Technology Transfer 75
24 Research and Travel Compensation 75
25 Attachments/ Appendices 78
25.1 Schedule of Events 79
25.2 Questionnaires 82
25.3 Case Report Forms (CRFs) 82
25.4 Focus Group Forms 82
25.5 Advertisements 82

25.6 Website Language 82
25.7 Screening Questionnaires 82
25.8 Thank you letters 82
25.9 Letter for Retroactive Increase in Compensation for Technical Development Substudy 82
25.10 Adjudication Board Members 82
26 Consent Forms 82
27 References 83

List of Abbreviations

AE	adverse event
AI	associate investigator
ANA	anti-nuclear antibody
AT	anaerobic threshold
BPI	brief pain inventory
CBC	complete blood count
CC	cubic centimeter
CDC	Centers for Disease Control and Prevention
CNS	central nervous system
CFS	chronic fatigue syndrome
CO	carbon monoxide
CO2	carbon dioxide
CO2HB	carbaminohemoglobin
COPD	chronic obstructive pulmonary disease
CPET	cardiopulmonary exercise test
CSF	cerebrospinal fluid
CRP	c-reactive protein
CTCAE	common terminology criteria for adverse events
DEXA	dual energy X-ray absorptiometry
DSM	diagnostic and statistical manual of mental disorders
DoH	Declaration of Helsinki
EBV	Epstein-Barr Virus
EEfRT	Effort-Expenditure for Rewards Task
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
EKG	electrocardiogram
FACIT	functional assessment of chronic illness therapy
FDA	Food and Drug Administration
fMRI	functional magnetic resonance imaging
FSQ	fibromyalgia survey questionnaire
FWA	federal-wide assurance

HV	healthy volunteer
HHV-6	human herpesvirus-6
HIV	human immunodeficiency virus
HSPU	human subjects protection unit
ID	identification
INR	international normalized ratio
IRB	Institutional Review Board
ISI	inter-stimulus interval
LAI	lead associate investigator
LIP	Licensed Independent Practitioner
LP	lumbar puncture
MASQ	multiple ability self-report questionnaire
MD	medical doctor
ME	myalgic encephalomyelitis
MFI	multidimensional fatigue inventory
ML	milliliter
MPQ	McGill pain questionnaire
mREM	milliRoentgen Equivalent in Man
MRI	magnetic resonance imaging
MSEC	millisecond
NIH	National Institutes of Health
NIH-BFI	National Institutes of Health_ brief fatigue inventory
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NMR	nuclear magnetic resonance
NPS	neuropathic pain scale
O2	oxygen
O2HB	oxyhemoglobin
OHSRP	Office of Human Subjects Research Protection
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEM	post exertional malaise
PI	principal investigator
PI-ME/CFS	Post-Infectious Myalgia Encephalomyelitis/Chronic Fatigue Syndrome
PPI	pixels per inch
PROMIS	patient reported outcomes measurement information system
PROMIS-F	patient reported outcomes measurement information system-fatigue
PRPL	patient recruitment and public liaison
PSQI	Pittsburgh sleep quality index
PT	prothrombin time
PTT	partial thromboplastin time
QA	quality assurance
RF	rheumatoid factor
RPR	rapid plasma reagin
SAE	serious adverse event

SCID	structural clinical interview and diagnosis
SEID	systemic exertion intolerance disease
SF-36	short-form 36
SOP	standard operating procedure
SSS	symptom severity score
T	tesla
TMS	transcranial magnetic stimulation
TSH	thyroid stimulating hormone
T3	triiodothyronine
T4	free thyroxine
V02	oxygen consumption
WPI	widespread pain index

1 Introduction

1.1 HISTORY

Chronic fatigue syndrome (CFS), myalgic encephalomyelitis (ME), and systemic exertion intolerance disease (SEID) are names used to describe a disorder characterized by persistent and disabling fatigue, exercise intolerance, dyscognition, and physical discomfort often following an acute infection. These disorders were described as part of epidemics, with benign ME being defined after an epidemic at the Royal Free hospital in London, England (Royal Free Syndrome)[1,2] and CFS being defined after an epidemic in Incline Village, Nevada.[3] These diagnoses are historically controversial, with scientists and physicians disagreeing over whether these disorders are better considered to be a distinct disease entity with a clear organic basis[4] or related to psychological factors and social contagion.[5,6] Although various infectious agents have been implicated, including brucella[7], poliovirus[8], Epstein-Barr Virus (EBV)[9], Human T-cell Lymphoma Virus II (HTLV-II)[10], and the Xenotropic Murine Leukemia Virus-Related Virus (XMRV)[11], no infection has yet been found to be necessary to cause ME/CFS. This is in contrast to other types of known infectious encephalitis, as has been demonstrated with strains of herpesvirus, enterovirus, alphavirus, flavivirus, buynavirus and even evidenced by the discovery of a submicroscopic, filter-passing agent during the encephalitis lethargica epidemic of 1916-1927.[12,13] However, epidemiological evidence that viral infections are sufficient to cause ME/CFS is fairly strong, with prospective studies estimating the incidence of ME/CFS after several different documented infections to be between 10-12%. [14,15] No physiological mechanism responsible for the persistence of fatigue and related symptoms has yet been determined. Despite the ambiguities and controversies of ME/CFS, the illness experience is very much “real”. ME/CFS is an illness of physical and emotional disablement, with dramatic ramifications on the lives of those afflicted, those that care about the afflicted, and the societies they live in.

1.2 DEFINITION

ME/CFS lacks a specific biomarker or a precise phenotype. Hence, it is not surprising that there are currently over 20 different case definitions or diagnostic criteria.[16] All these diagnostic criteria were recently reviewed in great detail by the Institute of Medicine (now called the National Academy of Medicine) and published in 2015. Importantly, they point out that there is significant overlap between symptoms of major depression,

fibromyalgia, and other syndromic illnesses. Hence, the report proposed that post-exertional malaise and neurocognitive symptoms should be considered the core symptoms of ME/CFS. Since fatigue may be a normal response to a stressful event, the diagnosis of ME/CFS typically requires that the fatigue be present for longer than six months, with the exception of the 2011 International Consensus Criteria which does not require a six month waiting period. All the various criteria require that other explanations for a patient's symptoms to be ruled out before a diagnosis of ME/CFS can be made, although the list of exclusionary conditions differs across the criteria. There are many other challenges in establishing the diagnosis. The more recent criteria have developed a constellation of symptoms that need to be present for the diagnosis to be considered. However, the mere presence of the symptoms can lead to classification of healthy controls as having ME/CFS, hence close attention need to be given to the severity and frequency of the symptoms.

1.3 ETIOLOGY

The cause of ME/CFS remains unknown, although in many cases symptoms are thought to be triggered by an infection or other prodromal events such as "immunization, anesthetics, physical trauma, exposure to environmental pollutants, chemicals and heavy metals, and rarely blood transfusions".[17]

The IOM in its recent report researched the possible association of infections with ME/CFS. They concluded that while numerous infectious agents have been associated with ME/CFS, only Epstein Barr Virus (EBV) has been consistently associated with the syndrome. Their major findings related to the association with EBV are described below. The possibility that EBV infection can be a trigger for ME/CFS is suggested by results of some prospective studies.[14,18-20] Several studies also found high titers of certain antibodies to EBV in CFS patients, including viral capsid antigen (VCA) IgG, persistent titers of VCA Immunoglobulin M (IgM), or the persistence of early antigen IgG, whereas healthy individuals who were previously infected with EBV had only VCA IgG and nuclear antigen IgG antibodies.[21-25] Some studies, however, including a study of twins discordant for disease[26], were unable to find this difference.[27-30] The severity of the acute illness may be a predictor of ME/CFS. A population-based study of ME/CFS patients after mononucleosis infection showed severity of illness, as measured by baseline autonomic symptoms and days spent in bed, to be a significant predictor of ME/CFS 6 months after the infection.[31] One double-blind, placebo controlled trial showed symptom improvement after 6 months in patients with elevated IgG antibody titers against EBV and HHV-6 following treatment with valganciclovir. There were also statistically significant changes in monocyte and cytokine levels, suggesting that immunomodulation may have been a factor in their improvement. However, the number of patients studied was small (N = 30), there were no differences in viral antibody titers between the two arms, and the patients were followed for only 9 months.[32] A prospective review of 106 ME/CFS patients with elevated serum antibody titers to EBV, CMV, or HHV-6 showed that 75 percent responded to long-term treatment with valacyclovir and/or valganciclovir (mean duration = 2.4 years). A patient was categorized as a responder if the Energy Index Point Score effect was greater than or equal to 1.[33] A single-blind, placebo-controlled trial found a significant increase in NK cell activity in ME/CFS patients following

treatment with isoprinosine.[34] However, another double-blind, placebo-controlled trial of ME/CFS patients with elevated antibodies to EBV failed to show a difference in clinical improvement between acyclovir-treated participants and placebo controls at 37 days follow-up.[35] This study included a small number of patients (N = 27) and did not assess immune parameters. In this model, ME/CFS represents the consequence of a persistent infection and successful intervention would require identifying the infective agent and designing therapies to thwart it.

Another possibility is that there is no ongoing infection but rather the infection acts as a trigger for the syndrome which is mediated by the persistent immune[19,36] or mitochondrial[37] abnormalities. A wide variety of immune abnormalities have been described in this population, including alterations in cytokines, expansion of specific immunoglobulin classes, alterations in T-cell metabolism, and NK cell function [1-3] There is also a greater association with autoimmune diseases and the most consistent finding seems to be polyclonal B cell activation, as first suggested from a placebo controlled clinical trial using rituximab.[38] In this study 30 ME/CFS patients were randomized to either Rituximab 500 mg/m2 or saline, given twice two weeks apart, with follow-up for 12 months. The primary end-point, defined as effect on self-reported fatigue score 3 months after intervention, was negative. However, patients reported improvement at secondary end- points. Major or moderate overall response, defined as lasting improvements in self- reported fatigue score during follow-up, was seen in 10 out of 15 patients (67%) in the Rituximab group and in two out of 15 patients (13%) in the Placebo group ($p = 0.003$). Mean response duration within the follow-up period for the 10 responders to Rituximab was 25 weeks (range 8–44). Four Rituximab patients had clinical response durations past the study period. General linear models for repeated measures of Fatigue scores during follow-up showed a significant interaction between time and intervention group ($p = 0.018$ for self-reported, and $p = 0.024$ for physician-assessed), with differences between the Rituximab and Placebo groups between 6–10 months after intervention. There were no serious adverse events. Two patients in the Rituximab group with pre-existing psoriasis experienced moderate psoriasis worsening. The authors concluded that the delayed responses starting from 2–7 months after Rituximab treatment, in spite of rapid B-cell depletion, suggests that ME/CFS is an autoimmune disease and may be consistent with the gradual elimination of autoantibodies preceding clinical responses.[38] However, a more recent double-blind placebo controlled by the same authors found that rituximab treatment did not lead to clinical improvement over the course of twelve months.[39] In this model, a successful intervention could target a specific immune or metabolic deficit whose restoration would ameliorate symptoms.

A third possibility is that ME/CFS a syndrome with a primarily neuropsychological biology. ME/CFS shares many features with other syndromic diagnoses, including demographics, correlations with psychiatric co-morbidity, the types of symptoms experienced, discordance between clinical observation and subjective experience, and limited impact on mortality. Viewed this way, ME/CFS may reflect the somatoform consequence of infection that is analogous to the post-injury pain of whiplash and post-traumatic fibromyalgia, the headaches and dyscognition of post-concussive syndrome and mild traumatic brain injury, the fatigue and dyscognition following cancer therapies, the post-Lyme disease syndrome following infections with Lyme disease, the event-

specific triggers of functional movement disorders, and perhaps the emerging problem of "long haul" COVID-19.[4] Alterations in immune function, neuroendocrine performance, and other biological parameters would represent epiphenomenon in this model, objective evidence of system-wide performance changes that occur with perceived distress.[39,40] Interventions targeting a single physiological system could temporarily improve symptoms related to bidirectional communication with, and influence on, neural systems. However, such interventions would be unlikely to lead to self-evident and long-lasting benefit due to our essential tendencies to acclimate to chronic physiological changes and to develop collateral systems to circumvent dysfunction occurring in any single physiological mechanism. Successful interventions in the model would require dissecting the "master" regulatory signals and develop interventions that would downregulate all of the altered physiological systems in synchrony.

However, it is probable that the cause of symptoms throughout the whole population of persons diagnosed with ME/CFS is not homogeneous. It is possible that all three of the aforementioned possibilities are correct, but only for subpopulations of ME/CFS sufferers. These possibilities may not be relevant to persons who credit the onset of ME/CFS with non-infectious exposures and life stressors nor those with substantial premorbid psychiatric illness. A host of unusual or poorly described medical phenomena, such as mast cell activation disorder, Chiari malformation, myotonic muscular dystrophy, paraneoplastic phenomenon that can accompany occult neoplasms, and alterations in the microbiome, may also contribute to the development of ME/CFS symptoms in certain individuals. *For the purpose of reducing heterogeneity in the current study, we will only enroll patients who have a clinical history of infection followed by chronic fatigue and post-exertional malaise. We have introduced the term, post-infectious ME/CFS (PI-ME/CFS) to refer to these patients.*

1.4 EPIDEMIOLOGY

An estimated 84 to 91 percent of patients affected by the condition are not yet diagnosed,[41] and people with ME/CFS often struggle with their illness for years before receiving a diagnosis. In multiple surveys, 67 to 77 percent of patients reported that it took longer than 1 year to receive a diagnosis, and about 29 percent reported that it took longer than 5 years.

1.5 DIAGNOSTIC CHALLENGES

Seeking and receiving a diagnosis can be a frustrating process for patients with ME/CFS for several reasons, including a lack of understanding of diagnosis and treatment of the condition among health care providers and skepticism about whether it is in fact a true medical condition. Less than one-third of medical schools include ME/CFS-specific information in their curriculum [42], and only 40 percent of medical textbooks include information on the condition [43]. Some studies on awareness of ME/CFS have found high awareness among health care providers, but many providers believe it is a psychiatric/psychological illness or at least has a psychiatric/psychological component.[44,45] ME/CFS often is seen as a diagnosis of exclusion, which also can lead to delays in diagnosis or misdiagnosis of a psychological problem. [46,47] Patients often

struggle with their illness for years before receiving a diagnosis. Hence there is a need for a faster and more accurate diagnosis of this syndrome.

1.6 SOCIO-ECONOMIC BURDEN

Once diagnosed, moreover, many people with ME/CFS report being subject to hostile attitudes from their health care providers [48], as well as to treatment strategies that exacerbate their symptoms. [49]

ME/CFS can cause significant impairment and disability that have negative economic consequences at the individual and societal levels. At least one-quarter of ME/CFS patients are house- or bedbound at some point in their lives.[50] The direct and indirect economic costs of ME/CFS to society are estimated to be between $17 and $24 billion annually,[51] $9.1 billion of which can be attributed to lost household and labor force productivity.[52] Together, high medical costs and reduced earning capacity often have devastating effects on patients' financial situations.[52]

1.7 DISABILITY

Several ME/CFS symptoms—including fatigue, cognitive dysfunction, pain, sleep disturbance, post-exertional malaise, and secondary depression or anxiety—may contribute to impairment or disability [53,54]. Patients with ME/CFS have been found to be more functionally impaired than those with other disabling illnesses, including type 2 diabetes mellitus, congestive heart failure, hypertension, depression, multiple sclerosis, and end-stage renal disease [44,55]. Symptoms can be severe enough to preclude patients from completing everyday tasks, and 25-29% of patients report being house- or bedbound by their symptoms. Many patients feel unable to meet their family responsibilities and report having to reduce their social activities. However, these data include only patients who were counted in clinics or research studies and may underrepresent the extent of the problem by excluding those who are undiagnosed or unable to access health care.[56,57]

1.8 MORTALITY

Literature on mortality associated with ME/CFS is sparse. One study found that cancer, heart disease, and suicide are the most common causes of death among those diagnosed with ME/CFS, and people with ME/CFS die from these causes at younger ages than others in the general population. However, the authors note that these results cannot be generalized to the overall population of ME/CFS patients because of the methodological limitations of the study [58].

2 Study Objectives

2.1 Primary objective

To explore the clinical and biological phenotypes of post-infectious chronic fatigue syndrome (PI-ME/CFS)

2.2 Secondary objective

To explore the pathophysiologies of fatigue and post-exertional malaise (PEM). Fatigue will be explored using tasks designed to create muscular and cognitive fatigue. PEM will be explored using an exercise stress and measuring the symptomatic and biological alterations that occur before and afterwards.

3 Subjects

3.1 Description of study populations

Up to 346 persons will be enrolled as part of this protocol. Up to 150 persons aged 18-60 will be part of 3 study groups: 50 PI-ME/CFS patients, 50 healthy volunteers (HV), and 50 asymptomatic and healthy persons with a documented history of COVID-19 infection (COVID-19 HV). The study has a target of completing all study procedures on 20 enrolled participants in each group. Any participant who withdraws prior to the completion of the study procedures may be replaced. Up to an additional 176 persons reporting a community diagnosis of ME/CFS will be enrolled into focus groups to discuss the experience of post-exertional malaise. Up to an additional 10 healthy volunteers and 10 ME/CFS participants may be enrolled to refine the protocol's electrophysiological and neuroimaging techniques.

The PI-ME/CFS group will consist of persons reporting persistent and severe fatigue and post-exertional malaise as the consequence of an acute infection without a prior history of medical or psychiatric illness. PI-ME/CFS pre-screening will review medical records and will seek to identify persons with a community diagnosis of ME/CFS that started within 6 months after an infectious illness that do not have other potential medical causes for their symptoms.

Potential PI-ME/CFS participants that meet inclusion and exclusion criteria for a *phenotyping visit* on pre-screening will be invited to participate and undergo a full week of medical interviews and research measurements. This collected information will be used in a *case adjudication process* to determine if a participant meets criterion to return for a separate *exercise stress* visit. *Exercise stress* visit participants are required to meet more stringent inclusion and exclusion criteria to best ensure that participants have ME/CFS that started after an infection, meet published diagnostic criteria, have an appropriate level of symptom severity and physical function to undergo the protocol's exercise stress procedure, and do not have exclusionary co-morbid health issues. This process has necessitated two sets of inclusion criteria for PI-ME/CFS participants: phenotyping criteria (Sections 3.2.2 and 3.3.2) and exercise stress visit criteria (Sections 3.2.3 and 3.3.3).

All PI-ME/CFS phenotyping participants will have a ME/CFS diagnosis from their health care providers and, therefore, represent the illness as it is considered in the community. For this reason, PI-ME/CFS participants will undergo all of the phenotyping procedures even if information that would exclude the participant from the study is discovered during the visit. Data collected from PI-ME/CFS participants that fail to meet *exercise stress* criterion will be used in comparative analyses and serve as a control group for comparisons to PI-ME/CFS participants that qualify for the exercise stress visit.

Two other control groups have also been chosen for comparisons.

The healthy volunteer (HV) group will consist of demographically matched persons without clinical fatigue, free from medical disease, and presumed to have a properly functioning immune system. The HV group represents a standard control population for comparison to the PI-ME/CFS group. HV participants will take part in both the phenotyping and exercise stress visits.

Asymptomatic persons with a history of COVID-19 infections and a full recovery is a salient comparator group to PI-ME/CFS. Similar to PI-ME/CFS, COVID-19 has been implicated in causing a syndrome of fatigue, pain, and neurocognitive difficulties, currently referred to as "long haul COVID".[4-6] The COVID-19 HV group will consist of healthy persons who had a laboratory documented COVID-19 infection within the last 5 years and currently do not have clinically significant fatigue and PEM. The COVID-19 HV control group will allow for biological amongst persons with an infectious process that did not progress to chronic debility. COVID-19 HV participants will take part in the phenotyping visit but not the exercise stress visit of the protocol.

3.2 Inclusion criteria

3.2.1 Inclusion criteria for all participants

1. Adult participants aged 18-60 years at the time of enrollment.
2. Self-reported completion of at least the 7th grade of school.
3. Ability to speak, read, and understand English.
4. Willing and able to complete all study procedures
5. Participant has a primary care physician at the time of enrollment.
6. Able to provide informed consent.

3.2.2 Additional inclusion criteria for participants with PI-ME/CFS for the phenotyping visit

1. A self-reported illness narrative of the development of persistent fatigue and post-exertional malaise as the consequence of an acute infection. The persistent fatigue may have an acute onset or become progressively worse over 6 months.
2. Licensed Independent Practitioner documentation of ME/CFS onset:
    - Medical documentation of absence of symptoms prior to ME/CFS onset. This may include medical records, letters, or information gathered from telephone calls with study personnel.
    - Documentation of a medical evaluation for symptoms of an acute infection or documentation of a medical evaluation of persistent symptoms within 2 months following an assumed infection.
3. Persistent fatigue and PEM onset less than 5 years prior to enrollment.

3.2.3 Additional inclusion criteria for participants with PI-ME/CFS for the exercise stress visit

1. Be unanimously considered to be a case of PI-ME/CFS by the protocol's adjudication committee.
2. Meet the 1994 Fukuda Criteria OR the 2003 Canadian Consensus Criteria for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome OR the Institute of Medicine Diagnostic Criteria.[61-63]
3. Have moderate to severe clinical symptom severity:
    - Severe fatigue as determined using the Multidimensional Fatigue Inventory (MFI): score of ≥ 13 on the general fatigue subscale **or** ≥10 on the reduced activity subscale.
    - Functional impairment as determined using the Short-Form 36 (SF-36): score of ≤70 physical function subscale, **or** ≤50 on role physical subscale, **or** ≤75 on social function subscale

3.2.4 Additional inclusion criteria for healthy volunteer group

None

3.2.5 Additional inclusion criteria for COVID-19 Healthy Volunteers

Documented prior COVID-19 infection as evidenced by:

1. A history of clinical manifestations compatible with COVID-19
2. Laboratory evidence of COVID-19 infection:
    - Detection of SARS-CoV-2 RNA or antigen in nasopharyngeal swab, sputum, other sample source with Emergency Use Authorization/approval from the FDA; OR
    - A positive antibody test using an assay that has received Emergency Use Authorization/approval from the FDA
3. Has been recovered from the COVID-19 infection for at least six months and no more than five years.

3.3 Exclusion criteria

3.3.1 *Exclusion criteria for all participants*

1. Current or past psychotic disorder including depression with psychosis, bipolar disorder, and schizophrenia
2. Current DSM-5-defined major depression disorder, generalized anxiety disorder, post-traumatic stress disorder, panic disorder, or obsessive-compulsive disorder unless managed for more than six months with a stable treatment regimen
3. Current or substance use disorder within last 5 years as diagnosed on the Structured Clinical Interview for DSM-5 (SCID-5).
4. Current suicidal ideation
5. History of head injury with loss of consciousness or amnesia lasting greater than a few seconds within last five years or lasting greater than 5 minutes at any point during their lifetime. Persons with medical record evidence of post-concussive symptoms lasting more than six months are also excluded.
6. Women who are pregnant, actively seeking to become pregnant, or have been pregnant in the year prior to study enrollment.
7. Current or previous malignancy. Certain dermatologic malignancies (e.g. basal cell carcinoma) will be allowed. A history of malignancy that have fully resolved with surgical resection only (i.e. no chemotherapy, radiation therapy, or immunotherapy) will be allowed.
8. Current systemic immunologic disorders (e.g. Type 1 diabetes, rheumatoid arthritis) will be excluded. Allergies requiring anti-histamines may not be an exclusion, but allergies requiring immunosuppressants may be an exclusion.
9. Current or previous long term immune suppressive or immunomodulatory therapy. Systemic steroid use, even short-term, must not have been used within the month prior to enrollment
10. Any medical condition (eg, congestive heart failure, coronary artery disease, chronic obstructive pulmonary disease, severe osteoarthritis, poorly controlled asthma) that would make the study procedures risky for the participant (e.g. exercise-induced angina and asthma) or that may confound the study results (e.g. untreated obstructive sleep apnea, severe osteoarthritis).
11. Participation in a clinical protocol (e.g. anti-inflammatory drug intervention study) which includes an intervention that may affect the results of the current study.
12. Inability to perform the bicycling exercise task.
13. Clinically significant claustrophobia
14. Not willing to allow for research samples to be shared with other researchers.
15. Employees or staff at NIH that are directly supervised by the primary investigator or associate investigators.

3.3.2 *Additional exclusion criteria for participants with PI-ME/CFS for phenotyping visit*

1. Significant neurological disorder (e.g. neurodegenerative disorder, stroke, epilepsy).

2. PI-ME/CFS disease severity that makes it impossible for the volunteer to leave the home or requires inpatient treatment.
3. Suspected, probable, or confirmed Lyme disease per 2011 CDC Lyme Disease National Surveillance Case Definitions.
4. Underlying illness that may cause fatigue such as thyroid dysfunction, hepatitis, or other systemic diseases.

3.3.3 Additional exclusion criteria for participants with PI-ME/CFS for exercise stress visit

1. Current (within 1 week) use of prescription or over-the-counter medications, herbal supplements, or nutraceuticals that may influence brain excitability that the potential participant is either unwilling or clinically unable to safely wean off for the duration of the period of the exercise stress visit. The possibility for a potential participant to be weaned off medication will be cooperatively determined by both the clinical investigative team and personal physicians. Examples of medications that influence brain excitability include tricyclic antidepressants, hypnotic, antiepileptic, antipsychotic medication, stimulants, antihistamines, muscle relaxants, dopaminergic medications, and sleep medications.

3.3.4 Additional exclusion criteria for healthy volunteer group

1. Substantial daily fatigue as determined using PROMIS-SF Fatigue: score of > 17.
2. Significant neurological disorder (e.g. neurodegenerative disorder, stroke, epilepsy).
3. Current (within 1 week) use of prescription or over-the-counter medications, herbal supplements, or nutraceuticals that may influence brain excitability

3.3.5 Additional exclusion criteria for COVID-19 healthy volunteer group

1. Substantial daily fatigue as determined using PROMIS-SF Fatigue: score of > 17
2. Significant neurological disorder (e.g. neurodegenerative disorder, stroke, epilepsy).
3. Current (within 1 week) use of prescription or over-the-counter medications, herbal supplements, or nutraceuticals that may influence brain excitability

4 Study Design and Methods

4.1 Study overview

This is a single center, cross-sectional, phase I pilot study to explore the clinical and biological phenotype of PI-ME/CFS in order to deeply characterize the syndrome and compare it to relevant control conditions. Biological specimens will also be collected for the development of neuronal and animal models of PI-ME/CFS. Study procedures will include an initial **phenotyping visit**, where clinical and biological information will be collected. The collected information from the phenotyping visit for PI-ME/CFS participants will be used in a **case adjudication** process utilizing an expert diagnostic committee to determine case validity. PI-ME/CFS participants adjudicated to be valid cases, meet recognized diagnostic and symptom severity criteria, and are able to taper off

medications that alter brain excitability will return to the NIH Clinical Center for an **exercise stress visit**. The results from participants with PI-ME/CFS will be compared against results from two distinct control groups: healthy volunteers and COVID-19 HVs. Prior to conducting exercise stress visits, the investigative team will establish and conduct up to four telephone **focus groups** to gather information for use in developing the qualitative PEM interview guide used during the exercise stress visit. Up to an additional 20 focus groups will be conducted to expand on the information collected in these initial focus groups. The purpose of this data collection would be to provide more extensive information about the similarities and differences of PEM in daily life compared with that occurring after CPET. Additionally, a small number of healthy volunteers and ME/CFS patients will be enrolled in **technical development sub-study** that will be used to refine the protocol's electrophysiological and neuroimaging techniques used during the exercise stress visit.

It is anticipated that most participants in both the PI-ME/CFS group and the healthy volunteer group will not be currently enrolled on other NIH protocols at the time of study participation. Participants from the COVID-19 HV group may be co-enrolled in NIH protocols or may be self-referred. Focus group participants are not anticipated to be currently enrolled in other NIH protocols. Participation in the technical development sub-study will be made available to all healthy volunteers inquiring about participation in the protocol.
Healthy volunteers in the technical development sub-study may also participate in the phenotyping and exercise stress visits. Participation in the technical development sub-study will be made available to documented ME/CFS patients that inquire about participation in the protocol and would otherwise be eligible to participate but do not meet study criteria for post-infectious causation or for having symptoms for less than 5 years. ME/CFS patients that participate in the technical development sub-study are excluded from participation in the phenotyping and exercise stress visits but may participate in focus groups.

The phenotyping visit (Visit 1) is designed to clearly define and document the characteristics of the study populations as well as collect biological samples. Eligible participants will undergo a set of research measurements and procedures. The results from the phenotyping visit will enable cross-sectional comparisons between the three study groups.

The exercise stress visit (Visit 2) will occur up to 12 months after the phenotyping visit. The exercise stress visit is designed around an exercise intervention designed to evoke the characteristic post-exercise malaise of PI-ME/CFS. All participants will undergo careful clinical description and a variety of biological measurements before, during, and after peak exercise testing. Qualitative interviews, subjective reporting on questionnaires, physiological measurements, neuroimaging, and biological samples will be serially collected. Only HV and PI-ME/CFS participants will be invited to participate in the exercise stress visit. These measurements will enable cross-sectional comparisons between these two study groups as well as comparisons between pre- and post-exercise state within and between study groups.

Participants in this study will undergo a great number of procedures in a relatively short period of time. All of the procedures that are to be performed in this study are standard research measurements, with well-defined risks and discomforts. No particular measurement or procedure is exceedingly burdensome, however the cumulative burden of undergoing all of these procedures is significant. This burden is a reasonable one, as it is the intent of this study to induce fatigue and PEM in its participants. However, PI-ME/CFS patients are defined by their vulnerability to exhaustion. It is probable that some participants will be unable complete the entire study. Some participants may also be so exhausted by the study that they may require some time to recover prior to travelling home. Thus, the study team will be solicitous of the participants in regard to their comfort and flexible to provide for their needs.

This 'Phase I' study will analyze the collected data in an exploratory manner. The goal of these analyses is to identify physiological alterations for the purpose of hypothesis generation. A broad array of laboratory methods that query different aspects of human physiology will be performed on collected samples. The protocol is looking for positive results that would generate testable hypotheses and for negative results that will provide a sense of the biological measurements and physiological mechanisms where large differences are unlikely to be seen with future study. De-identified data and biological samples will be shared with a wide range of collaborators, both within NIH and across the world. A larger 'Phase II' study is planned that will use these results to determine potential mechanisms for intervention and estimate effect sizes for larger clinical and interventional studies.

4.2 Recruitment

Recruitment for the study will vary by group.

- PI-ME/CFS patients may be referred to this study from the CDC CFS Multi-site Clinical Study. This ongoing natural history study is funded to assess ME/CFS patients until September 2017.
- COVID-19 HV participants may be referred from NIH clinics who study these conditions, in particular the investigators involved in Protocol 000084: *Natural History of Post-Coronavirus Disease 19 Convalescence at the National Institutes of Health.* Participants will not be directly recruited by physicians with whom they have a therapeutic relationship with.
- Patients from all three groups may be recruited from the Patient Recruitment and Public Liaison (PRPL) office and by self-referral. A study investigator will contact potential participants via the contact information provided by the PRPL inquiry form to evaluate eligibility.
- Participants may also be recruited from other NIH protocols. Non-Associate Investigators working on other NIH protocols will be provided with IRB-approved recruitment flyers. If appropriate, these non-AI investigators may send flyers to their study participants (both healthy volunteers and patients) by mail with a cover letter to inform them of the study opportunity or provide flyers to study participants in person.

All potential participants will undergo a pre-screening process to assist in the determination of eligibility. Persons that have inquired about potential participation will

be contacted by phone. A check-list based on the study inclusion and exclusion criteria will be used by the investigative team to collect information relevant to determining eligibility. Persons obviously not meeting eligibility criteria will be informed at this time. Potential PI-ME/CFS and COVID-19 HV participants will be informed that medical record review is one of the eligibility criteria. Study staff will work with potential participants in obtaining relevant medical records. Costs associated with obtaining medical record will be paid for by the protocol. Medical records obtained will be kept confidential and locked in a filing cabinet when not in use. If medical record review determines that a person is ineligible to participate, they will be notified. Medical records on ineligible persons may be kept until the completion of the study. Participants may request their records to be returned or destroyed at any time.

Permission to contact relevant medical care providers may also be requested. Some potential participants will be taking medications that would require being tapered off during the study, while others may need to have facilitated access to medical care after study visits.

Employees of the NIH may participate in this protocol. There will be no direct solicitation of employees or staff members by supervisors. All recruitment materials, including those specific for NIH, will be approved by the IRB prior to use. Advertisements, study-specific website language, and Telephone Screening Forms are described in detail in Appendix 26.5, 26.6, and 26.7 respectively.

Once the potential participant is determined to be eligible for the study, a date will be set for the phenotyping visit. It is anticipated that 1-2 potential participants will be identified and pre-screened per week.

4.3 Focus Groups

4.31 Focus Group Study A

In order to gather information for use in creating a meaningful interview guide for the PEM qualitative interview planned during the Exercise Stress Visit, the investigative team will conduct up to 10 focus groups. Each group will contain between three to nine ME/CFS patients, with the maximum number of focus group participants being 36. Focus group participants will not be eligible to participant in other study procedures and represent their own recruiting pool. NIH employees will not be eligible to participate in the focus groups. The focus groups will take place over the telephone to accommodate a non-mobile population.

Focus group participants will be persons with ME/CFS in the community willing to participate in telephone discussions with the investigative team to assist in the development of a qualitative interview tool for eliciting the experience of PEM.

4.31.1 *Inclusion criteria for focus group A participants*

1. Adult 18 years or older
2. Self-report of being diagnosed with ME/CFS in the community.

3. Self-report of having experienced post-exertional malaise.
4. Ability to speak, read, and understand English.
5. Able and willing to participate in focus group telephone call.

4.31.2 Exclusion criteria for focus group participants

1. Participation in the phenotyping and exercise stress studies.
2. Decline to be audio-taped during the focus group.

A letter of invitation (Appendix 26.4) will be sent to community advocacy groups to be shared with their members and may posted on-line at NIH ME/CFS websites. Persons interested in participating in the focus group will contact the investigative team. Potential focus group participants will be screened for inclusion/exclusion using a focus group participation checklist (Appendix 26.4). Potential focus group participants will be informed that focus group participation precludes participation in the phenotyping and exercise stress studies. The investigative team will contact interested individuals to answer any questions and address concerns, and schedule times for obtaining telephone informed consent.

Persons found to be eligible will be mailed a copy of the focus group consent form (Appendix 26.4). The individual will be instructed to read the consent form and be ready at the appointed date and time to review the consent document with an investigator authorized to obtain consent. Decision making capacity will be assessed and documented. If the subject is determined not to have decision making capacity, the telephone consent process will be halted, and the participant will be determined ineligible for participation in the focus group.

Potential participants with decision making capacity will be asked: "Do you agree to voluntarily participate in this study?" The consenting investigator will document the date and time oral consent was obtained on a consent procedure note. The participant will sign and date the consent form in the presence of a witness. The witness will also sign and date the consent form. The subject will be instructed to mail the consent form back to the site, where the investigator will sign and date the form. The investigator will then send a copy of the form with all signatures to the subject. The original consent document will be placed in the research chart.

After the investigative team receives the informed consent form, the focus group participant will be contacted to schedule a focus group teleconference time.

The purpose of the focus groups is to gather information to create the interview protocol to be used for the semi-structured interviews. Each focus group will be a joint discussion in which all of the scheduled participants will participate together. An overview guide to the focus group discussion has been developed (Appendix 26.4). Barbara Stussman will moderate the focus groups. Persons who decline to be audio-taped will not be included in the focus groups. Focus groups will be rescheduled if less than three participants make themselves available for the telephone call.

Recruitment for the focus groups will seek to categorize participants by level of disability: persons with symptoms that have maintained their activity level, persons whose activity has been limited by symptoms, and persons fully disabled and/or housebound from their symptoms.

Data collected during focus groups will consist of audio recordings which will be sent to a professional service for transcription via a secure website. Data, recordings, and transcriptions will be stored in locked cabinets. Transcriptions will NOT contain any identifying information and will be labeled with a number only. Basic demographics will be collected on all focus group participants but will not be associated with the corresponding transcriptions and will be kept secure and confidential. Demographics to be collected from focus group participants include: (sex, age (18-29; 30-39; 40-49; 50-59; 60+), race (Asian, Black, White, Other), ethnicity (Hispanic; non-Hispanic), years of diagnosis of CFS (0-4; 5-9; 10-14; 15-19; 20+). Contact information that contains personally identifying information for focus group participants will be kept on file for five years after the conclusion of the study.

The risks of focus group participation include potential breach of confidentiality and emotional feelings that may arise with discussing personal feelings in a group setting. There is no direct benefit from focus group participation. Focus group participants will not be compensated for their participation.

4.32 Focus Group Study B

As of October 2018, the results of the initial focus groups (Focus Group Study A) have been used to create the interview guide being used during the exercise stress visit. The initial qualitative analysis of the collected data provides a description of post-exertional malaise in which there may be four key symptom concepts (cognitive difficulties, extreme fatigue, flu-like symptoms, and muscle pain/weakness) and a time course in which to expect the onset of PEM. The medical literature has a paucity of detailed qualitative studies about PEM in ME/CFS; there is currently only a single study of 19 persons.[64] An expansion of the work performed in Focus Group Study A work has the potential to contribute to broader knowledge of PEM.

Focus group Study B will expand the scope of our focus group study activities. This expansion will include collecting more extensive demographic information, information about symptom severity, information about CPET experiences, and collection of medical records for diagnostic confirmation purposes. The purpose of this data collection would be to provide more extensive information about the similarities and differences of PEM in daily life compared with that occurring after CPET.

For Focus Group Study B, the investigative team will conduct up to 20 focus groups. Each group will contain between three to seven ME/CFS patients, with the maximum number of focus group study B participants being 140. Focus group participants will not be eligible to participant in the main study (Visit 1 phenotyping and Visit 2 exercise stress) but may participate in the Technical Development Substudy (TDS) and represent their own recruiting pool. NIH employees will not be eligible to participate in the focus

groups. The focus groups will take place over the telephone to accommodate a non-mobile and geographically dispersed population.

Focus group participants will be persons with ME/CFS in the community willing to participate in telephone discussions with the investigative team to describe their experiences of PEM and provide medical records for diagnostic confirmation.

Inclusion criteria for focus group B participants

1. Adult 18 years or older
2. Self-report of being diagnosed with ME/CFS in the community.
3. Self-report of having experienced post-exertional malaise.
4. Ability to speak, read, and understand English.
5. Able and willing to participate in focus group telephone call.
6. Willing to share their medical records with the investigative team for diagnostic confirmation.

Exclusion criteria for focus group B participants

1. Participation in the phenotyping and exercise stress studies.
2. Decline to be audio-taped during the focus group.

Initially, participants will be recruited from persons who have contacted the investigative team about participating in any aspect of this protocol. These potential focus group participants will be contacted by phone and screened for inclusion/exclusion using Focus Group B Participation Checklist (Appendix 26.4). Potential focus group participants will be informed that focus group participation precludes participation in the phenotyping and exercise stress studies. The investigative team will contact interested individuals to answer any questions and address concerns, work with participants in completing paperwork to obtain pertinent medical records, and schedule times for obtaining telephone informed consent. The NIH will cover the costs of obtaining medical records. If recruitment goals are not met using the protocol's recruitment pool, a letter of invitation (Appendix 26.4) will be sent to community advocacy groups to be shared with their members and may posted on-line at NIH ME/CFS websites. Persons interested in participating in the focus group will contact the investigative team and undergo screening as detailed above. The investigative team may target specific under-represented ME/CFS sub-groups, such as minorities with ME/CFS, males with ME/CFS, persons aged 18-29 with ME/CFS, and bedbound ME/CFS participants.

Persons found to be eligible will be mailed a copy of the focus group consent form (Appendix 26.4). The individual will be instructed to read the consent form and be ready at the appointed date and time to review the consent document with an investigator authorized to obtain consent. Decision making capacity will be assessed and documented. If the subject is determined not to have decision making capacity, the telephone consent process will be halted, and the participant will be determined ineligible for participation in the focus group.

Potential participants with decision making capacity will be asked: "Do you agree to voluntarily participate in this study?" The consenting investigator will document the date and time oral consent was obtained on a consent procedure note. The participant will sign and date the consent form in the presence of a witness. The witness will also sign and date the consent form. The subject will be instructed to mail the consent form back to the site, where the investigator will sign and date the form. The investigator will then send a copy of the form with all signatures to the subject. The original consent document will be placed in the research chart.

After the investigative team receives the informed consent form, the focus group participant will be contacted to schedule a focus group teleconference time and to coordinate the collection of medical records.

The purpose of the focus groups is to gather information to collect information about the experience of PEM in daily life and the experience of PEM after CPET. Each focus group will be a joint discussion in which all of the scheduled participants will participate together. Overview guides for the Focus Group Study B discussions have been developed (Appendix 26.4). Barbara Stussman will moderate the focus groups. Persons who decline to be audio-taped will not be included in the focus groups. Focus groups will be rescheduled if less than three participants make themselves available for the telephone call.

Recruitment for the focus groups will seek to categorize participants by level of disability: persons with symptoms that have maintained their activity level, persons whose activity has been limited by symptoms, and persons fully disabled and/or housebound from their symptoms.

Data collected during focus groups will consist of audio recordings which will be sent to a professional service for transcription via a secure website. Data, recordings, and transcriptions will be stored in locked cabinets. Transcriptions will NOT contain any identifying information and will be labeled with a number only. Basic demographics will be collected on all focus group participants but will not be associated with the corresponding transcriptions and will be kept secure and confidential. The demographic and descriptive information that will be collected can be reviewed in Focus Group B Participation Checklist (Appendix 26.4). Contact information that contains personally identifying information for focus group participants will be kept on file for five years after the conclusion of the study.

The risks of focus group participation include potential breach of confidentiality and emotional feelings that may arise with discussing personal feelings in a group setting. There is no direct benefit from focus group participation. Focus group participants will not be compensated for their participation.

An in-depth qualitative analysis process, thematic analysis, will be used to systematically code and categorize the textual data. Two investigators will read over the transcripts several times to allow immersion into the data, and then agreed upon a preliminary

categorical structure or "coding scheme." They then independently "coded" all of the transcripts (i.e. the textual data), which involved identifying salient categories, collating those categories into overarching themes, and assigning names to the themes.[65,66] After the two researchers independently coded all the textual data, codes will be compared to identify disagreements and meetings will be conducted to discuss coding differences at-length and reach consensus. A qualitative software package (such as MAXQDA) will be used to highlight text within the transcripts and create categories and sub-categories electronically.

4.4 Technical Development Sub-Study

During the Exercise Stress Visit (Visit 2), participants will undergo a sequence of transcranial magnetic stimulation (TMS), functional magnetic resonance imaging (fMRI), and electroencephalography (EEG). An overview of those neurological measurements are described in full in Sections 4.6.15 and 4.6.16 below. To refine these techniques, up to 10 healthy volunteers and up to 10 ME/CFS participants will be enrolled on an outpatient basis for the purpose of having the TMS, fMRI, and EEG measures streamlined. This sub-study will be used to refine the timing and sequencing of different measurements and tasks, to ensure that the various equipment involved works harmoniously with each other, provide the investigative team experience in synchronizing these different measurements and using them in ME/CFS volunteers.

4.4.1 Inclusion criteria for all participants in the technical development sub-study

1. Adult participants aged 18-60 years at the time of enrollment.
2. Ability to speak, read, and understand English.
3. Willing and able to complete all study procedures
4. Right-handed
5. Able to provide informed consent.

4.4.2 Exclusion criteria for the technical development sub-study

1. Active infection (e.g. influenza, urinary tract infection) by history, physical examination. These participants may be re-evaluated after the acute infection resolves.
2. Current or past psychotic disorder including depression with psychosis, bipolar disorder, and schizophrenia
3. Current DSM-5-defined major depression disorder, generalized anxiety disorder, post-traumatic stress disorder, panic disorder, or obsessive-compulsive disorder unless managed for more than six months with a stable treatment regimen
4. Current substance use as determined during medical history and by urine toxicology. Marijuana use will not be considered an exclusion.
5. Current suicidal ideation as determined during medical history
6. Women who are pregnant,
7. Current or previous malignancy. Certain dermatologic malignancies (e.g. basal cell carcinoma) will be allowed. A history of malignancy that have

fully resolved with resection only (i.e. no chemotherapy, radiation therapy, or immunotherapy) will be allowed.

8. Current immunologic disorder (e.g. Type 1 diabetes, rheumatoid arthritis)
9. Current or previous long term immune suppressive or immunomodulatory therapy.
10. Any medical condition that would exclude from participation in the phenotyping and exercise stress visits (eg, congestive heart failure, coronary artery disease, chronic obstructive pulmonary disease, severe osteoarthritis, poorly controlled asthma) or make the study procedures risky for the participant (e.g. seizure disorder).
11. Participation in a clinical protocol (e.g. centrally-acting drug intervention study) which includes an intervention that may affect the results of the technical development sub-study.
12. Clinically significant claustrophobia
13. Not willing to allow for research samples to be shared with other researchers.
14. Employees or staff at NIH that are directly supervised by the primary investigator or associate investigators.

4.4.3 Additional inclusion criteria for ME/CFS participants in the technical development study

1. Licensed Independent Practitioner documentation of ME/CFS

4.4.4 Additional exclusion criteria for ME/CFS participants in the technical development study

1. Significant neurological disorder (e.g. neurodegenerative disorder, stroke, epilepsy).
2. ME/CFS disease severity that makes it impossible for the volunteer to leave the home or requires inpatient treatment.
3. Current treatment for underlying illness that may cause fatigue such as thyroid dysfunction, hepatitis, or other systemic diseases.

Right-handed healthy volunteers interested in participating in the protocol may also be invited to participate in the technical development sub-study. Qualified healthy volunteers may participate in both the sub-study and the phenotyping/exercise stress visits. For participants interested in both parts of the study, participation in the technical development sub-study must occur prior to the phenotyping visit (Visit 1). Healthy volunteers participating in the technical development sub-study will complete a specific informed consent form; participants in both parts of the study will need to complete two informed consent forms.

Participation in the technical development sub-study will be made available to right-handed ME/CFS patients with that inquire about participation in the protocol, are generally healthy, have no medical issues that would increase risk of having TMS and MRI, but are found not meet the protocol's strict criteria for post-infectious causation or

symptom duration. PI-ME/CFS participants that enroll in the phenotyping and exercise stress visits will be ineligible for participating in the technical development sub-study.

All participants in the technical development sub-study will have a detailed history and physical examination and urine toxicology. ME/CFS diagnostic status will be determined using the Fukuda, Canadian Consensus Criteria, and the Institute of Medicine Diagnostic Criteria. Participants will need to meet at least one of these criteria. Women of childbearing age will have urine pregnancy testing performed within 24 hours of fMRI and/or TMS. For participants undergoing fMRI that have not had a clinical MRI scan at the NIH in the past year, the investigative team will collect the clinical MRI sequences prior to fMRI experiments. It is anticipated that all measurements in the technical development sub-study, including TMS, MRI, and EEG, can be completed during 1-3 outpatient visits.

The timing of each test in the electrophysiology and fMRI sequences is crucial for obtaining precise images and determining how the different measured parameters change following the physical and mental fatiguing tasks. Once a task begins, it will trigger a chain of operations. For the TMS procedure, this includes applying single and double TMS pulses at precise intervals during the electrophysiology sessions. For the fMRI procedure, this includes synchronizing the markers of the fMRI sequence with both the EEG and the behavioral performance during the fatiguing tasks. Normal differences between individuals' task adherence can introduce a jitter in synchronization, ranging from differential ability to perform a task at different time points to missed trials. This sub-study will train the team to troubleshoot missed trials in real-time, identify potential weak points in the chain of events that need special attention, and adapt the procedure accordingly. The sub-study will also identify the average and minimum number of block/task that healthy volunteers and ME/CFS patients yield, which will help the investigators determine the minimum set of recordings that are required to extract the complete amount of information desired.

Participants will also complete questionnaires as part of the technical development sub-study. These include: NIH-BFI. MFI-20, MASQ, SF-36, PROMIS, BDI-II, BAI, and PDS. These questionnaires are described in detail in Sections 4.5.5 and 4.6.6.

4.5 Phenotyping Visit (Visit 1: Phenotyping)

The phenotyping visit will take place at the NIH Clinical Center up to 12 months before the exercise stress visit and is expected to last 5 days for HV and COVID-19 HV participants and 10 days for PI-ME/CFS participants. Phenotyping may occur on either an outpatient or an inpatient admission basis, depending on convenience. Information collected during the phenotyping visit will be used to determine PI-ME/CFS case status as well as to determine eligibility for the exercise stress visit. All procedures are for research purposes.

Consent will be obtained before any study procedures, including screening procedures, are done. Eligibility criteria will be reviewed during the consent process. An eligibility criteria checklist will be reviewed on Visit 1 completion and documented in the protocol's research records.

During the phenotyping visit, clinical information that is collected may impact participant eligibility. All participants in the PI-ME/CFS group will have documentation of being diagnosed with the illness in the community. In this way, PI-ME/CFS participants found not to meet inclusion/exclusion criteria during the phenotyping visit still represents what ME/CFS is in the community. For this reason, PI-ME/CFS participants that are found to have alterative or concomitant diagnoses that would otherwise exclude from study participation may still be invited to complete all of the phenotype visit procedures.

Participants in the HV and COVID-19 HV groups may be found to meet exclusion criteria during Visit 1. Such participants are also permitted to complete Visit 1 procedures, as long as they do not have substantial daily fatigue nor concerns for safety. Samples and data from these participants will be used in the primary analysis. HV and COVID-19 HV participants found to have substantial daily fatigue will have their study participation ended immediately and will not be used in primary analyses. Study participation for HV individuals meeting exclusion criteria will be complete at the end of Visit 1. Study participation for all COVID-19 HV individuals will be complete at the end of Visit 1.

Some participants may be found to have clinical and/or laboratory evidence of an acute and reversible inflammatory reaction. Such reactions may be related to infections (common cold, urinary tract infections), toxic exposures (food poisoning), or allergies. If acute reversible inflammation is noted, participants will be provided appropriate therapy to speed resolution. Certain measurements will be influenced by acute reversible inflammation (i.e. immunological measurements); these measurements will be drawn after all signs of inflammation have resolved. All questionnaires and case report form information are detailed in Appendix 26.2 and Appendix 26.3.

During the active recruiting period of this protocol, a series of Amendments have expanded the optional research procedures offered during Visit 1. Also, some participants did not complete certain measurements because of equipment failures or staffing issues during their Visit 1. Participants who have completed Visit 1 prior to Amendment 11 may be offered the opportunity to return to NIH to undergo these additional measurements and any missed Visit 1 measurements. Such participants will be reconsented prior to participation. Travel, accommodations, and payment will be provided as detailed in Section 25: Travel and Compensation. There is no time limit in regard to re-contacting participants for returning for additional Visit 1 measurements.

The following procedures will be completed during the phenotyping visit:

4.5.1 : History and Physical Examination: A comprehensive clinical assessment will take approximately 2-3 hours and will include measurement of vital signs, a detailed history, and physical examination. Detailed information about symptoms that comprise major PI-ME/CFS diagnostic criteria will be collected.[61,62,67] Additional time is anticipated to be necessary for the PI-ME/CFS group. Participants will be seen by a LIP each day they are in the hospital. Progress notes will be documented in CRIS.

4.5.2 : Intravenous line placement: An intravenous line will be placed. This will allow for obtaining serial blood samples, administration of gadolinium, and cytapheresis. IV placement typically takes between 5-30 minutes.

4.5.3 : Blood collection: The following tests will be done at the NIH Clinical Center laboratory to determine study eligibility:

a. Acute care panel[1]
b. Mineral panel
c. Hepatic panel
d. Complete blood count with differential
e. Prothrombin time (PT), international normalized ratio (INR), and partial thromboplastin time (PTT)[2]
f. Thyroid stimulating hormone (TSH), free thyroxine (T4), triiodothyronine (T3)
g. Iron, ferritin, transferrin saturation
h. Lipid panel[1]
i. Hemoglobin A1c
j. Anti-nuclear antibody (ANA), Rheumatoid factor (RH), anti-cyclic citrullinated antibody (anti-CCP), anti-Smith antibody, anti-RNP, ssA, and ssB[2]
k. Vitamin B12, 25(OH) Vitamin D, $1,25(OH)_2$ Vitamin D, folate[2]
l. Creatine Kinase
m. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and d-dimer
n. Quantitative immunoglobulins
o. Flow cytometry for lymphocyte subsets
p. Human immunodeficiency virus (HIV) by enzyme-linked immunosorbent assay (ELISA)[2]
q. PCR for EBV and CMV
r. Antibodies to C6 peptide
s. Hepatitis panel[2]
t. Rapid plasma reagin (RPR)[2]
u. Serum tryptase level
v. Heavy metal screening
w. Serum pregnancy testing (may be performed for women of childbearing potential if problems arise with obtaining urine pregnancy testing).
x. Additional blood testing may be needed if clinically indicated to investigate for underlying systemic illnesses or other causes of fatigue.

[1]Fasting labs
[2]Results within 12 weeks of screening may be used in lieu of these tests

Additional blood may be drawn for use in live cell research measurements and/or stored for future use. A blood draw will take about 30 minutes. No more than 150 ml of blood will be drawn in a single day during Visit 1. No more than 550mL of blood will be drawn within an 8-week period.

4.5.4 : Urine collection: Urine will be collected for urine toxicology screening and may be used to test for pregnancy in of women of childbearing potential. A sample of urine will also be collected and stored for future use. Urine collection takes up to 5 minutes.

4.5.5 : Symptoms Assessment: Symptom and health questionnaires will be administered. Some of these instruments will be scored and used to determine symptom severity as detailed in the exercise stress visit inclusion/exclusion criteria.

a. Short-Form 36 (SF-36): The SF-36 is easily administered and reliably reflects health-related quality of life outcomes. This questionnaire has 36-items that assess eight health issues: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, general mental health, social functioning, energy/fatigue, and general health perceptions. The SF-36 has been tested and validated extensively in a number of clinical populations and was developed for self-administration by patients. This questionnaire is part of the PI-ME/CFS research definition (7-10 minutes).[68]

b. CDC Symptom Inventory (CDC-SI): The CDC-SI will be used to collect information on occurrence, frequency, and intensity of symptoms common in ME/CFS and other fatiguing illnesses. (5-10 minutes).[69]

c. Multidimensional Fatigue Inventory (MFI): The MFI is a validated 20-item self-report instrument designed to measure fatigue severity.[70] The items are designed to assess general, physical, emotional, and mental manifestations of fatigue as well as vigor, an estimate of the patient's energy level. The MFI-20 has been validated in chronic fatigue syndrome patients.[71] This questionnaire is part of the PI-ME/CFS research definition (10 minutes).

d. Patient Reported Outcomes Measurement Information System – Short Forms (PROMIS-SF) PROMIS is a system of highly reliable, precise measures of patient–reported health status for physical, mental, and social wellbeing. These computer adaptive tests are generally less than 6 questions and developed from more than 1000 datasets from multiple disease populations including cancer, heart disease, rheumatoid and osteoarthritis, psychiatric conditions, spinal cord injury, and chronic obstructive pulmonary disease. Initial psychometric properties showed internal consistency reliability coefficient of 0.81. PROMIS-SF include Fatigue, Pain Behavior, Pain Interference, Pain Intensity, Global Health, Emotional Distress – Anxiety, Emotional Distress- Depression, Sleep-Related Impairment and Sleep Disturbances (10 minutes).[72]

e. The National Institutes of Health – Brief Fatigue Inventory (NIH-BFI) is a 7-item clinician-administered questionnaire. To assess reliability, a principle component analysis was applied and yielded a single component. Further, the NIH-BFI produced Cronbach alphas ranging from .81-.88. The NIH-BFI is

strongly correlated with fatigue items from validated clinician-administered depression and mania scales. (2 minutes).[73]

f. The McGill Pain Questionnaire (MPQ): a list of 20 groups of adjectives to describe sensory, affective and evaluative aspects of pain. Using the MPQ, participants will be asked to describe their pain experience (2 minutes).[74]

g. The Neuropathic Pain Scale (NPS): a questionnaire designed to assess distinct qualities associated with neuropathic pain. It measures both the quality and the intensity of the neuropathic sensations. (5 minutes).[75]

h. Polysymptomatic Distress Scale: Polysymptomatic Distress Scale (PSD): The polysymptomatic distress scale is a self-administered instrument that determines both the distribution of painful areas across the body and an estimate of related symptom burden. Evidence supports the reliability and validity of the PSD in the general population (5 minutes).[76]

i. Patient Health Questionnaire-15 (PHQ-15): A validated questionnaire used to assess somatic symptom severity and the potential presence of somatization and somatoform disorders (3-5 minutes).[77]

j. Pittsburgh Sleep Quality Index (PSQI): The PSQI measures sleep quality over a 1-month period with 19 questions in 7 clinically-derived component scores. This instrument has a sensitivity of 89.6% and a specificity of 86.5% for identifying a sleep disorder (PSQI<5) in a clinical sample (15 minutes).[78]

k. Fatigue Catastrophizing Scale is a 10-item paper/pencil questionnaire to measure catastrophizing related to the fatigue experience. This questionnaire takes less than two minutes to complete. It uses a 5-point rating scale (1= never true) to (5 = all the time true) with proven high internal consistency reliability (coefficient alpha = 0.85 – 0.92) (2 minutes).[79]

l. The Multiple Ability Self-Report Questionnaire (MASQ): A 38-item questionnaire that assesses the subjective appraisal of cognitive difficulties in five cognitive domains: language, visual-perceptual ability, verbal memory, visual-spatial memory, and attention/concentration. The MASQ sub-scales are scored on an 8–40 point scale with high scores indicating greater perceived difficulties (10 minutes).[80]

m. Belief about Emotions scale: A validated questionnaire designed to measure the beliefs of expressing negative thoughts and feelings (3-5 minutes).[81]

4.5.6 : Psychological Assessment: A licensed mental health professional will perform psychological evaluation and the Structured Clinical Interview for DSM-5 (SCID-5) (approximately 2 hours). Psychological assessment will be optional for the DLI-A group.

4.5.7 : Psychological inventories

a. Psychological inventories will be administered. These may include: Composite International Diagnostic Interview Trauma Section (CIDI-Trauma): A validated survey that characterizes a participant's previous traumatic experiences. The CIDI-Trauma quantifies and evaluates the number of adverse events experienced by subject, and relates these to a host of possible disorders under the umbrella of the DSM-IV diagnostic manual (10-15 minutes).[82]
b. Post-traumatic Stress Diagnostic Scale (PDS): A validated instrument for the epidemiologic diagnosis of Post-traumatic Stress Disorder (10 minutes).[83]
c. Childhood Trauma Questionnaire Short Form (CTQ-SF): A validated instrument that characterizes potential traumatic life experiences in early childhood (10 minutes).[84]
d. Sexual and Physical Abuse Questionnaire (SPAQ): A validated questionnaire that characterizes the type and age of occurrence of traumatic life experiences (5 minutes).[85]
e. Beck Depression Inventory –II (BDI-II): A 21-question validated self-report inventory for measuring the severity of depression (3-5 minutes).[86]
f. Beck Anxiety Inventory (BAI): a 21-question validated self-report inventory for measuring the severity of anxiety (3-5 minutes).[87]
g. Center for Epidemiologic Studies Depression Scale – Revised (CESD-R): A 20-item validated self-report inventory for screening for depression (3-5 minutes).
h. Gratitude Questionnaire –Six Item Form: A six-item self-report questionnaire designed to assess individual differences in the proneness to experience gratitude in daily life. The gratitude questionnaire will only be administered to the PI-ME/CFS participants.[88]

4.5.8 : Neurocognitive testing

a. Wechsler Test of Adult Reading (WTAR) (The Psychological Corporation, 2001) requires the examinee to read words aloud.[89]
b. Wechsler Adult Intelligence Scale – Fourth Edition (WAIS-IV) subtests including Coding, Symbol Search and Digit Span will be administered. For these subtests, examinees memorize strings of numbers or complete speeded tasks involving unfamiliar symbols.[90]
c. Test of Variables of Attention requires examinees to rapidly respond using a button press to certain target stimuli and not distractor stimuli.[91]
d. Hopkins Verbal Learning Test-Revised (HVLT-R) requires examinees to remember words that were read to them earlier.[92]
e. Brief Visual Memory Test-Revised (BVMT-R) requires examinees to remember designs that were shown to them earlier.[93]
f. Wisconsin Card Sort Test (WCST-64) requires examinees to utilize corrective feedback to learn how to sort cards.[94]
g. Controlled Oral Word Association Test (COWAT; FAS and Animals) requires examinees to generate words to various cues.[95]

h. Paced Auditory Serial Addition Test (PASAT) requires examinees to rapidly perform serial addition.[96]
i. Grooved Pegboard Test requires examinees to rapidly insert pegs in holes.[97]
j. Word Memory Test requires the examinee to view words and later remember them.[98]
k. B Test asks the examinee to rapidly discriminate between letter stimuli.[99]
l. Dot Counting Test asks the examinee to count dots as rapidly as possible.[100]
m. Minnesota Multiphasic Personality Inventory – 2 Restructured Form (MPI2-RF) requires examinees to complete true-false items that best describes themselves.[101]
n. Effort-Expenditure for Rewards Task (EEfRT) is a multi-trial game in which participants are offered a choice between two task difficulty levels for a reward. It is fully described in Section 4.6.19.

The complete battery will take about 2 to 3 hours.

4.5.9 : Occupational Therapy Evaluation (only ME/CFS): Only ME/CFS participants may have an evaluation from occupational therapy. A licensed occupational therapist will perform an open-ended, focused interview intended to elicit perceptions of fatigue and its effect on function, in an unstructured format. The interviewer is free to ask additional questions to more fully understand the participant. Interviews to be digitally recorded and transcribed.

The Activity Card Sort – 2nd edition will also be administered.[102] This is an instrument used for assessing participation in occupational performance of instrumental, social-cultural, and leisure activities. The tool uses a Q sort method in which respondents sort 89 cards depicting people engaged in various activities into 5 mutually exclusive categories, such as “never done,” “continue to do,” “recently added,” “given up,” and “do less.” The Activity Card Sort yields a total score and four domain scores which can be used to determine a "retained activity level" score that is the percentage of activities in which a person is currently engaged in divided by those with whom he or she was involved in the past. The occupational therapy evaluation will take 60-90 minutes.

4.5.10 : Magnetic Resonance Imaging (MRI): A MRI of bilateral thighs and a structural brain MRI will be performed. Scanning will be performed either at Clinical Center Radiology or on a dedicated MRI scanner in the NMR Center. Scans may include clinical anatomic images, MPRAGE, DWI (for DTI) and T2w sequences. For the structural brain MRI, intravenous gadolinium contrast to determine cortical enhancement or meningeal inflammation will be given if there are no contraindications. MRI scans may include intravenous administration of an FDA-approved, macrocyclic, gadolinium-based MRI contrast agent (if applicable: including in healthy volunteers). Unless otherwise specified in the protocol, contrast agents will be used at FDA-approved doses. NIH Clinical Center Radiology and Imaging Sciences guidelines for gadolinium administration will be followed. Under certain circumstances, participants in this study may receive a linear chelate. Examples of situations in which this may occur include the following: (1) prior sensitivity to all macrocyclic agents; (2) participation in a longitudinal study in which a linear chelate was previously given, there is a clear advantage to maintaining the same

gadolinium protocol, and T1 signal change in the basal ganglia or dentate nuclei was not observed; or (3) after consultation with, and approval by, a radiologist credentialed at the NIH Clinical Center. A list of the instances in which a linear chelate was administered, along with the justification for its administration, will be provided to the IRB at the time of continuing review. NIH Clinical Center Radiology and Imaging Sciences guidelines for gadolinium administration will be followed. Under certain circumstances, participants in this study may receive a linear chelate. Examples of situations in which this may occur include the following: (1) prior sensitivity to all macrocyclic agents; (2) participation in a longitudinal study in which a linear chelate was previously given, there is a clear advantage to maintaining the same gadolinium protocol, and T1 signal change in the basal ganglia or dentate nuclei was not observed; (3) after consultation with, and approval by, a radiologist credentialed at the NIH Clinical Center. A list of the instances in which a linear chelate was administered, along with the justification for its administration, will be provided to the IRB at the time of continuing review.

All NMR Center scans will be performed with dedicated radiofrequency coils that have received approval from the NIH Safety Committee. All women of childbearing potential will have a urine pregnancy test performed no more than 24 hours before each MRI scan. The blood and/or urine collections will be completed prior to MRI to screen for renal dysfunction or pregnancy that would be contraindicated for MRI (45-60 minutes).

4.5.11 : Muscle strength testing: Hand-held dynamometers are simple, portable, and relatively inexpensive method to measure isometric muscle strength. It has been shown to be a reliable tool as it correlates well with muscle strengths (hip abductors, knee extensors, pectorals, shoulder flexors) of individuals with COPD.[103] Grip strength will be measured on both hands in a neutral position of the arm, forearm, and wrist. Two consecutive attempts at 1-minute intervals will be measured in kilograms with the hand-held dynamometer. Each attempt will require a participant to exert a maximum possible grip force for about 5 seconds. After a minute of rest, the third attempt will measure fatigue resistance by asking the participant to hold the maximum hand grip as long as able until the 50% maximum grip force (based on the highest maximum hand grip force obtained from the first two attempts) is reached, which is often reached in less than a minute. The test will be repeated on the other hand. The same dynamometer will be used for all participants (30 minutes).

4.5.12 : Activity Monitor and Fatigue Diary Instruction (ME/CFS, HV): Eligible participants will receive instruction from a study investigator regarding the use of an activity monitor and a fatigue diary at home prior to the participant's return to the Clinical Center for the inpatient admission. The study investigator will explain how and when to use the activity monitor and fatigue diary and how it will be mailed to the participant at home approximately two weeks prior to the scheduled admission. The fatigue diary is designed to occur in conjunction with activity monitoring. Measurements will be made at intervals throughout the day, starting at the time of awakening and concluding at bedtime. The participant will use the activity monitor for at least five days prior to the scheduled admission and throughout the inpatient stay. It will be worn continuously (i.e. while awake and while asleep) during this time period. It can be

removed if the patient showers, bathes, or swims. Medication withdrawal will be complete prior to activity monitoring. COVID-19 HV participants will not receive this instruction.

4.5.13 : Nutritional Assessment:
Nutrition staff will either meet in person with the participants during their Visit 1 admission or contact consented participants by telephone prior to the Visit 2 admission. During this meeting, participants will be instructed on food record keeping and educated about the controlled metabolic menu that they will have for Visit 2. Selections will be made for the menus that they prefer to have during Visit 2 and food allergies and intolerances will be assessed. Changes to controlled menus will only be made if possible to accommodate food allergies. COVID-19 HV participants will not receive this assessment.

All participants will fill out an internet-based food frequency questionnaire (Diet History Questionnaire (DHQII), National Cancer Institute, Rockville, MD) which asks them 134 questions regarding their dietary intake over the past year and 8 questions about dietary supplement intake. This questionnaire is completed online and typically takes approximately 45-60 minutes to complete. This may be completed during Visit 1 or Visit 2.

Participants will be asked to provide detailed food records for 7 days prior to their admission during the same week that they are wearing the activity monitor and recording the fatigue diary. These food records will be reviewed by NIH nutrition staff for accuracy using 3-dimensional models and then entered into Nutrition Data Systems for Research (NDSR, University of Minnesota, Minneapolis, MN) to obtain nutrient intake. COVID-19 HV participants will not receive this assessment.

4.5.14 : Saliva Sample Collection: Saliva will be collected using 1-2 salivary swabs taken from the anterior floor of the mouth and near the Stenson's ducts. Samples will be collected from a clean mouth at least two hours after the participant's last meal. It takes about one minute to collect a sample.

4.5.15 : Buccal Swab Sample Collection: Buccal swabs will be collected using a brush rotated against the buccal surface. Buccal swabs will be used for microbiome and micronutrient profiling. (<1 minute)

4.5.16 : Stool sample collection: A fresh stool sample will be collected in sterile cup and frozen immediately. Stool samples will be used for microbiome and micronutrient profiling.

4.5.17 : Holter monitoring: Heart rate variability will be assessed using standardized Clinical Center EKG/Holter Monitor data, which will be collected per NIH Cardiology standard routines. The EKG monitoring will last less than 24 hours.

4.5.18 : Lumbar puncture: All participants will undergo an LP to collect cerebrospinal fluid (CSF) for research. The LP will be done at the Clinical Center at the bedside or in

Radiology if done under fluoroscopy. Inpatients will recover on the unit and outpatients will recover in the outpatient day hospital. For outpatients, study clinical team member will contact the subject the next day to assess for potential adverse events and provide contact information if problems arise after the procedure. The NIH Radiation Safety Committee guidelines will be followed for participants undergoing LP under fluoroscopy. Sedation with low-dose benzodiazepines may be used for subjects with anxiety during the LP. Analysis of CSF may include (but will not be limited to): opening pressure, cell count, total protein, glucose, PCR for pathogens, cytokine assays, lipid profile, and flow cytometry for phenotyping of immune cells (CD4 and CD8 T cells including central memory, central effector, regulatory and naïve T cell subsets, B cells and monocytes), cytokine/chemokine profile, growth factors, proteome and metabolome, autoantibodies to brain antigens and neurotransmitters. CSF will also be aliquoted and stored for future use. Additionally, specialized laboratory testing such as detection of autoantibodies to novel antigens will be performed as needed. The upper limit of CSF withdrawn will be 24 ml. (30-60 minutes)

4.5.19 : Autonomic testing (optional): All participants may undergo provocative autonomic testing, which includes:

- Breathing maneuvers:
  - *Deep breathing:* The participant will lie supine with head on pillow and breathes deeply at a rate of 5 to 6 breaths per minute for 3 minutes.
  - *The Valsalva maneuver*: Assessment of beat-to-beat blood pressure and heart rate during and after performance of the Valsalva maneuver is a well-accepted autonomic function test.1 The participant lies supine on a tilt table with head on pillow. The participant then blows or strains against a resistance for 12 seconds at 30 mmHg and then relaxes. If a "square wave" phenomenon is observed, the subject may be tilted at 20 degrees head up and the procedure repeated. At least 3 Valsalva maneuvers will be performed. The maneuvers will be repeated (at least 1 minute between repetitions) until a technically adequate recording is obtained. If the participant cannot perform a technically adequate Valsalva maneuver by blowing against a resistance, then a Valsalva maneuver may be done alternatively by having the subject strain against a closed glottis.

- Head-up tilt table testing: The participant will lie supine for at least 15 minutes after placement of monitoring leads. Baseline hemodynamic measures are taken and blood is drawn. Then the participant is tilted head-up according to the following procedures:
  - The participant is tilted head up at a 70-degree angle.
  - At approximately 4-minute intervals, hemodynamic measures are taken and blood is drawn.
  - Hemodynamic measures may also be taken and blood drawn if the investigative team feels that neurally mediated hypotension or syncope may occur soon (e.g., if the participant talks or skin electrical conductance increases, or the blood pressure becomes notably more variable).

- Upright tilt is continued for up to 40 minutes. Tilting will be ceased in the event of sudden bradycardia, hypotension, or syncope.
- After the tilting the participant is brought to the supine position and observed for at least 10 minutes.
- At 10 minutes of recovery, hemodynamic measures are taken and a blood sample drawn.

- Hemodynamic and non-invasive monitoring: For monitoring, electrocardiographic and impedance cardiographic leads may be applied to the chest, abdomen, or neck; a laser-Doppler flowmeter placed on a finger; a skin thermistor placed on a finger; skin conductance electrodes placed on two fingers; a finger cuff applied for non-invasive measurement of beat-to-beat blood pressure; an automated blood pressure cuff applied for standard measurement of brachial blood pressure; a forearm plethysmograph transducer and rapid-inflation brachial cuff placed around the same arm for forearm blood flow measurements; a device applied for monitoring respiration; or a pulse oximeter applied to a finger. One or more of these devices may be omitted if there are technical or practical problems, without the omission being considered a protocol deviation. Of these non-invasive monitors, the following are not standard, though they are validated, for autonomic testing: forearm plethysmography, impedance cardiography, laser-Doppler flowmetry.

- Blood sampling: Blood may be sampled through direct venipuncture or 3-way stopcocks attached to indwelling i.v. catheters, for assays of catecholamines and other neurochemicals. Autonomic disturbance from decreased ganglionic neurotransmission, identified by abnormal hemodynamic responses to the Valsalva maneuver, low plasma norepinephrine levels, and neuroimaging evidence for intact postganglionic sympathetic nerves, can result from a circulating antibody to the ganglionic nicotinic acetylcholine receptor. Plasma from patients with this constellation of clinical laboratory findings may be assayed for such an antibody.

- Urine collection: Urine may be collected from participants and will be analyzed to understand catecholamine and other neurochemical metabolism. Urine may be collected at multiple time points, for example, before and after tilt table testing. Participants unable or unwilling to give urine samples will still be able to participate in other aspects of the protocol.

Participants will not undergo autonomic testing if they are taking medications that would interfere with interpretation of the results, have inner ear injury or disease, are known to be unable to tolerate autonomic testing, or are unwilling to participate related to cumulative procedural burden.

4.5.20 : Immune Cell collection: All participants will be given a choice to undergo cytapheresis or an immune cell blood collection.

- Cytapheresis (Option A): Participants choosing cytapheresis will undergo apheresis to collect of immune cells for immunology testing. Cytapheresis takes about 2.5 hours
- Immune cell blood collection (Option B): For participants unwilling or unable to undergo cytapheresis, a blood draw will be performed instead. Up to 100mL of blood will be drawn for the storage of serum, plasma, and PBMCs. Blood draws take about 30 minutes. Typically, no more than 150ml of blood will be drawn in a single day. No more than 550ml of blood will be drawn over an 8-week period, per NIH guidelines.

Participants may be asked to undergo immune cell collection procedures an additional time during the period of their study participation.

4.5.21 : Medication Review and Wash-out Planning (ME/CFS only): Medications that may interfere with measures of brain excitability have the potential to confound several of the proposed study results. Examples of such medications include tricyclic antidepressants, hypnotic, antiepileptic, antipsychotic medication, stimulants, antihistamines, muscle relaxants, dopaminergic medications, opiates, and sleep medications. In clinical practice, participants with PI-ME/CFS often take large numbers of medications for their disorders, including those that might impact brain excitability. However, there are currently no medications that have been demonstrated to effectively treat PI-ME/CFS, and many of the medications these patients take have a negligible impact on the symptom of their illness. Therefore, to increase study homogeneity and reduce potential confounding from medications, a medication review will be conducted and a medication wash-out plan determined for its participants prior to participation in the exercise stress visit or the optional fMRI and TMS procedures.

Medication wash-out will be considered for any medication that is not medically necessary in the opinion of study investigators and the participant's physician care providers. Examples include medications used only on an 'as needed' basis (PRN), medicines used solely to address symptoms (i.e. fatigue, pain, sleep quality, mood), syndromic diagnoses (i.e. fibromyalgia, ME/CFS), medications prescribed for questionable indications, and medications given at sub-therapeutic doses. Participants taking stable doses of medications for medical diseases or psychiatric diagnoses will not be withdrawn from their medications. Participants taking medications that cannot be withdrawn as an outpatient or would place participants at high-risk for withdrawal-related complications (i.e. long-acting opiates or benzodiazepines), will not undergo withdrawal and will be excluded from study participation.

A study LIP will develop the medication withdrawal plan in conjunction with the participant and relevant physicians. All withdrawal plans will require the agreement and full cooperation with the participant's primary care physician. If psychiatric medications prescribed by the participant's psychiatrist are being withdrawn, agreement and full cooperation from the psychiatrist will also be obtained. Participant will be informed that

if they develop urgent or life-threatening problems during the medication withdrawal that they should immediately go to an Emergency Room for immediate evaluation and request that the evaluating physicians contact the study investigators and physician care providers. For non-urgent problems, participants will be instructed to call either the study investigators or local physician care providers.

The nature of the medication taper will depend on the particularities of the medication. Some medications will be able to be stopped immediately. Other medications may require a tapering regimen over days to weeks. The longest taper anticipated will be with the SSRI/SNRI class of medications which can require up to 6 weeks to taper. The timing of the medication withdrawal will depend on the length of time required to successfully withdraw a medication, the number of medications that require withdrawal, and the anticipated time for the inpatient visit. In the case of participants taking multiple medications, withdrawal will likely be sequential. The goal is for participants to be off of potentially interfering medications for at least 5 plasma half-lives prior to their inpatient visit.

Participants will be monitored at a minimum of weekly per telephone during outpatient withdrawal period by a clinically-credentialed member of the study staff. An AE baseline assessment will be performed per phone at the time of beginning of tapering, at least once during the tapering period, and at least once during the period off study drug prior to admission. Closer monitoring may occur at the initiation of the withdrawal or if the participant develops withdrawal issues. Participants with severe withdrawal-related AEs will receive appropriate care from their involved physicians, which may include hospitalization and/or restarting the medication. Participants unable to withdraw off medications will be excluded from the study. After completion of the exercise stress visit, participants may be titrated back onto their baseline type and level of medication in conjunction with the care of their physician care providers.

4.5.22 : Sedation during LP (optional): Sedation with low-dose benzodiazepines may be used for participants with anxiety during the LP. All participants receiving sedation during the study will be monitored for side effects.

4.5.23 : Fluoroscopic-guided LP (optional): If the study investigators determine that the LP should be done under fluoroscopy because of a participant's body habitus, the procedure will be done in Radiology at the Clinical Center. The fluoroscopic-guided LP involves exposure to radiation. The radiation exposure is not required for medical care and is for research purposes only.

4.5.24 : Medical, Psychiatric, Sleep Service, and Rehabilitation Consultation (optional): Medical, psychiatric, sleep and/or rehabilitation consultation may be requested if it is clinically required or desirable in order to provide for the inpatient needs of study participants.

4.5.25 : Case adjudication: The determination of PI-ME/CFS status will occur after the completion of all clinical and research measurements of the phenotyping visit. Relevant

descriptive information, medical documentation, and clinical laboratory measurements will be de-identified and compiled into an adjudication packet. These adjudication packets will then be submitted to the protocol's adjudication panel for review and case status determination.

The adjudication panel will be chaired by the primary investigator (Dr. Avindra Nath). Five other members will serve on the panel. Panel members will be current or previously licensed independent practitioners with community-recognized experience with the diagnosis and care of persons with ME/CFS that have also published on the subject in peer-reviewed journals. The names of the Adjudication Board members are listed in Appendix 26.10. The primary investigator reserves the right to invite and dismiss panel members as best serves the needs of the protocol.

The case adjudication packet for each participant will be sent to the chairman and each adjudicator for review. All adjudicators will be asked to review each PI-ME/CFS participant's case packet within 6 weeks of the completion of the phenotyping visit. Adjudicators will review the packets and vote as to whether the reviewed case is or is not consistent with the study criteria for PI-ME/CFS. Adjudicators will be given two weeks in which to conduct their review and vote. Votes will be cast using a password-protected online voting system. A unanimous decision is one in which all adjudicators responding within the 2-week response window all agree that a case meets the protocol's PI-ME/CFS criteria. The chairman and at least two members of the adjudication panel must vote to be considered a voting quorum.

In the case of diagnostic disagreement, a teleconference will be arranged to discuss the case and determine if consensus can be reached. At least three members of the adjudication panel, including the chairman and all dissenting panel members, must be part of the teleconference to be considered a voting quorum. In cases where adjudicators do not agree, the individual cases will not be considered a case of PI-ME/CFS and individual case opinions will be recorded.

To be invited back for the exercise stress visit, a PI-ME/CFS participant must be unanimously adjudicated to meet the inclusion/exclusion criteria detailed in Sections 3.2 and 3.3. In particular, the adjudication committee will review the inclusion and exclusion criteria where clinical judgment is required. Examples of such include the determination of whether a case is post-infectious, if symptoms are related to an alternative diagnosis or related to concomitant medical and/or psychological illness, and if a case meets published diagnostic criteria.
Participants that do not meet these criteria will be notified that participation in the study has ended. Participants that do not meet criteria for participation in the exercise stress visit will be provided one or more of the following general explanations:

- Members of the adjudication panel thought that an alternative diagnosis that was discovered during phenotyping could be related to ME/CFS symptoms
- Members of the adjudication panel thought that other medical issues are contributing, at least in part, to ME/CFS symptoms

- Members of the adjudication panel did not reach consensus agreement that ME/CFS symptoms started after an infection
- Members of the adjudication panel found that the participant did not meet the published ME/CFS diagnostic criteria used in this study
- Members of the adjudication panel found that the participant did not meet the protocol's symptom severity criteria
- The level of functional impairment observed during the phenotyping visit would interfere with being able to perform the exercise stress tasks

The case status of healthy volunteers and COVID-19 HV participants will be adjudicated by the study team, not the adjudication panel.

4.6 Study procedures: Exercise Stress visit (Visit 2)

PI-ME/CFS and HV participants deemed eligible for further study participation after the phenotyping visit and case adjudication process will be scheduled for an exercise stress visit. The exercise stress visit will be an inpatient admission lasting approximately 5-10 days, with the possibility of extending admissions for clinically-related reasons. The home ward for participants during Visit 1 will be the metabolic unit and the hospital bed for the admission will be within a metabolic chamber.

Peak exercise testing is the major experimental stimulus for this protocol. It is anticipated that performance of this task will induce post-exertional malaise in PI-ME/CFS patients but not in either of the control groups.

In this protocol, the period of days prior to the peak exercise test will be referred to as the **Pre-Exercise** period. The day the exercise challenge is administered is referred to as **Exercise Day 0**. Each day following the exercise challenge will be additively referred to as a **Post-Exercise Day** (i.e. 24 hours after peak exercise testing would be Exercise Day 1; 72 hours after exercise would be Exercise Day 3).

PI-ME/CFS participants are expected to develop post-exertional malaise within 24-48 hours. All participants will be observed and measured for 72 hours after exercising (Post-exercise day 3).

The NIH team will not provide primary treatment for ME/CFS or other medical problems that may arise during this study except in the event of medical necessity due to acuity of illness or lack of access to timely and appropriate care.

The procedures performed during the exercise stress visit will be scheduled based on availability of testing and investigators and will be scheduled to minimize patient inconvenience. Optional procedures for research purposes may also be performed during the exercise stress visit. The optional procedures will be scheduled based on availability of testing and investigators and will be scheduled to minimize patient inconvenience. A study schematic is attached as Appendix 24.1.

Urine pregnancy testing will always be done prior to MRI, fMRI, TMS, and the LP (if done under fluoroscopy) for women of childbearing potential.

4.6.1 : Updated History and Physical Examination

A comprehensive clinical assessment usually lasts 1-2 hours and will include measurement of vital signs, and a history and physical examination. Inpatients will be seen by a LIP daily. Progress notes will be documented in CRIS.

4.6.2 : Intravenous line placement

An intravenous line will be placed and may be kept in place during the hospital admission. This will allow for obtaining serial blood samples. Intravenous lines will be changed per clinical center protocol. IV placement takes between 5-30 minutes.

4.6.3 : Blood collection

Blood will be collected at various times during the inpatient admission. Blood draws take about 30 minutes. A schedule of blood draws is depicted in the study schematic (Appendix 24.1). No more than 250 ml of blood will be drawn in a single day. No more than 550 ml of blood will be drawn over an 8-week period, per NIH guidelines.

Blood will be serially drawn before and after CPET. This is fully described in Section 4.6.13.

Blood may optionally be drawn for tests that may include, but will not be limited to:

- Acute care panel
- PT/PTT (needed prior to LP)
- Hepatic panel
- Complete blood count with differential
- Serum pregnancy testing (for women of childbearing potential if problems arise with obtaining urine pregnancy testing).

4.6.4 : Urine collection

A urinalysis for urine toxicology will be completed during the inpatient admission and may be used to test for pregnancy in women of childbearing age. A sample of urine will also be collected and stored for future use. Collecting a urine sample takes up to 5 minutes.

4.6.5 : Saliva sample collection

Samples will be collected by participants chewing briefly on collection swabs (or simply spitting into a collection vial) at three times points (At 9pm, at 7:30 am, and 12pm). Samples will be immediately placed on dry ice until they are able to be stored in a -80°C freezer. These measures will be made on the same days as MRI and TMS testing during the Pre-Exercise period. A third set of salivary cortisol measures will be made on the same day as exercise testing. A fourth and fifth set of salivary cortisol measures will be made on the same days as MRI and TMS testing during the Post-Exercise period. Salivary cortisol testing will be completed on these samples according to standardized

testing by a commercial laboratory. Additionally, aliquots of saliva may be stored for future use (1 minute).

4.6.6 : Patient-reported outcome

Patient reported outcome measurements will be made during the exercise stress admission. Select questionnaires may be performed at multiple time points in order to determine subjective change before and after the peak exercise intervention.

CFS Symptom Inventory: Visual Analogue Scale for CFS Symptoms (VAS-CFS Symptoms) consists of 10 items related to physical fatigue, mental fatigue or mental fog, muscle aches, join aches, lightheadedness, "flu-like" symptoms, sore throat, gastrointestinal discomfort, shortness of breath, and environmental sensitivity. This questionnaire will be administered at multiple time points during the inpatient stay (2 minutes).[69]

The Brief Pain Inventory (BPI): a validated measurement of pain severity and pain interference. Both are scored on 0 -10 point scales with higher scores indicating more severe pain. This questionnaire will be administered at multiple time points during the inpatient stay (10 minutes).[104]

The approximate time points at which these tests will be administered include: up to 4 hours before CPET, up to one hour after CPET, 4-6 hours after CPET, 20-28 hours after CPET, 44-52 hours after CPET, and 68-76 hours after CPET.

Other tests to be administered only once include:

1. Multidimensional Fatigue Inventory (MFI): The MFI is a validated 20-item self-report instrument designed to measure fatigue severity.[70] The items are designed to assess general, physical, emotional, and mental manifestations of fatigue as well as vigor, an estimate of the patient's energy level. The MFI-20 has been validated in ME/CFS patients (10 minutes).[71]

2. Patient Reported Outcomes Measurement Information System – Short Forms (PROMIS-SF) PROMIS is a system of highly reliable, precise measures of patient–reported health status for physical, mental, and social wellbeing. These computer adaptive tests are generally less than 6 questions and developed from more than 1000 datasets from multiple disease populations including cancer, heart disease, rheumatoid and osteoarthritis, psychiatric conditions, spinal cord injury, and chronic obstructive pulmonary disease. Initial psychometric properties showed internal consistency reliability coefficient of 0.81. PROMIS-SF measures that are part of the Core Screening Evaluation include Fatigue, Pain Behavior, Pain Interference, Pain Intensity, Global Health, Emotional Distress – Anxiety, Emotional Distress- Depression, Sleep-Related Impairment and Sleep Disturbances (10 minutes).[72]

3. The National Institutes of Health – Brief Fatigue Inventory (NIH-BFI) is a 7-item clinician-administered questionnaire. To assess reliability, a principle component

analysis was applied and yielded a single component. Further, the NIH-BFI produced Cronbach alphas ranging from .81-.88. The NIH-BFI is strongly correlated with fatigue items from validated clinician-administered depression and mania scales (2 minutes).[73]

4. The McGill Pain Questionnaire (MPQ): a list of 20 groups of adjectives to describe sensory, affective and evaluative aspects of pain. Using the MPQ, participants will be asked to describe their pain experience (2 minutes).[74]

5. The Neuropathic Pain Scale (NPS): a questionnaire designed to assess distinct qualities associated with neuropathic pain. It measures both the quality and the intensity of the neuropathic sensations (5 minutes).[75]

6. Polysymptomatic Distress Scale: Polysymptomatic Distress Scale (PSD): The polysymptomatic distress scale is a self-administered instrument that determines both the distribution of painful areas across the body and an estimate of related symptom burden. Evidence supports the reliability and validity of the PSD in the general population (5 minutes).[76]

7. Patient Health Questionnaire-15 (PHQ-15): A validated questionnaire used to assess somatic symptom severity and the potential presence of somatization and somatoform disorders (3-5 minutes).[77]

8. PROMIS sleep and wake disturbances. These short forms are 8-item questionnaires developed from more than 1000 datasets from multiple disease populations including cancer, heart disease, rheumatoid and osteoarthritis, psychiatric conditions, spinal cord injury, and chronic obstructive pulmonary disease. Initial psychometric properties showed internal consistency reliability coefficient of 0.81 (4 minutes).[72]

9. Pittsburgh Sleep Quality Index (PSQI): The PSQI measures sleep quality over a 1-month period with 19 questions in 7 clinically-derived component scores. This instrument has a sensitivity of 89.6% and a specificity of 86.5% for identifying a sleep disorder (PSQI<5) in a clinical sample (15 minutes).[78]

10. Fatigue Catastrophizing Scale is a 10-item paper/pencil questionnaire to measure catastrophizing related to the fatigue experience. This questionnaire takes less than two minutes to complete. It uses a 5-point rating scale (1= never true) to (5 = all the time true) with proven high internal consistency reliability (coefficient alpha = 0.85 – 0.92) (2 minutes).[79]

11. The Multiple Ability Self-Report Questionnaire (MASQ): A 38-item questionnaire that assesses the subjective appraisal of cognitive difficulties in five cognitive domains: language, visual-perceptual ability, verbal memory, visual-spatial memory, and attention/concentration. The MASQ sub-scales are scored on

an 8–40 point scale with high scores indicating greater perceived difficulties (10 minutes). [80]

12. Short-Form 36 (SF-36): The SF-36 is easily administered and reliably reflects health-related quality of life outcomes. This questionnaire has 36-items that assess eight health issues: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, general mental health, social functioning, energy/fatigue, and general health perceptions. The SF-36 has been tested and validated extensively in a number of clinical populations and was developed for self-administration by patients (7-10 minutes).[68]

13. Belief about Emotions scale: A validated questionnaire designed to measure the beliefs of expressing negative thoughts and feelings (3-5 minutes).[81]

14. Beck Depression Inventory –II (BDI-II): Beck Depression Inventory –II (BDI-II): A 21-question validated self-report inventory for measuring the severity of depression (3-5 minutes).[86]

15. Beck Anxiety Inventory (BAI): a 21-question validated self-report inventory for measuring the severity of anxiety (3-5 minutes).[87]

16. Center for Epidemiologic Studies Depression – Revised (CESD-R): A 20-item validated self-report inventory for screening for depression (3-5 minutes).[105]

4.6.7 : Physical activity monitoring

Participant will arrive for the exercise stress visit having monitored physical activity at home with an activity monitor for at least five days. They will continue to be monitored throughout the exercise stress visit. An activity log form will be provided to the participants so they can enter the incidents (date, time, type of activity) when the monitor is removed during the exercise stress visit. This activity log will assist in interpreting the data captured by the physical activity monitor. Participants will return the monitor at the end of the exercise stress visit.

4.6.8 : Metabolic Chamber

The metabolic chamber is a whole-room indirect calorimeter that allows detailed assessment of energy and nutrient balance. Measurements are conducted at stable interior (room) temperature, humidity and barometric pressure, which are continuously measured. Airtight sampling ports and a four-way air-locking food and specimen passage are designed to allow blood draws and specimen retrievals with minimal disturbance to the chamber environment. Blood pressure and heart rate values are recorded in the morning of the study upon entry into the chamber, and immediately after the completion of the respiration chamber stay. Outside air is continuously drawn into the chamber, and the flow rate of air at the outlet is measured at using a pneumotachograph with a differential manometer. A fraction of the extracted air is analyzed at 1-minute intervals for O2 and CO2 concentrations with a thermomagnetic O2 analyzer. This allows for a continuous assessment of oxygen consumption ($V\dot{}O2$), carbon dioxide elimination ($V\dot{}CO2$), and

calculation of overall energy expenditure (EE). The ratio between V˙CO2 and V˙O2 (the respiratory quotient [RQ]) reflects preference for carbohydrate or fat oxidation. Continuous measurement of RQ at 1-minute resolution as well as a measurement of 24-hr urinary nitrogen excretion enables accurate determination of macronutrient oxidation rates.

The metabolic chamber will be the participant's "hospital bed" during the exercise stress visit. Participants will generally spend their downtime between study procedures and sleep in the metabolic chamber. In this way, metabolic measurements will be made throughout the entire exercise stress visit.

4.6.9 : Body Composition Measurement by Dual Energy X-ray Absorptiometry

Upon completion of their stays in the metabolic chamber, subjects will undergo a DEXA scan (iDXA, GE Healthcare, Madison WI). With this technique, one can determine total and regional body fat and lean soft tissue masses, bone mineral content and density. DEXA produces photons at two different energy levels, 40 and 70 KeV. The photons pass through tissues and attenuate at rates related to elemental composition. Bone mineral, with highly attenuating calcium and phosphorous, is readily distinguished from soft tissues. The different elemental profiles of fat and bone-mineral free lean components allows for the analysis of soft tissue fat content, so that bone mineral, fat, and bone mineral fat-free lean components may be resolved. The effective radiation dose is less than 1 mrem, which is below the guideline of 5000 mrem per year allowed for research subjects by the NIH Radiation Safety Committee. All women of childbearing potential will have a urine pregnancy test performed no more than 24 hours before the DEXA scan. DEXA scan will not be repeated during Visit 2 if it was performed as part of an optional procedure during Visit 1 (Section 5.1.9).

4.6.10 : Metabolic Diet and Nutritional Assessment

All participants will consume a controlled metabolic diet for the entire Exercise Stress Visit. Energy needs for weight maintenance will be estimated using the Mifflin St. Jeor equation with an activity factor of 1.5.[106] The diets will be standardized to have 55% of energy from carbohydrate, 30% of energy from fat and 15% of energy from protein. All food and food ingredients will be weighed to the nearest gram weight in the NIH CC Metabolic Kitchen prior to being served to the participant. Though participants should consume all foods provided to them, any food not eaten will be weighed back and then, if possible, nutrients will be replaced the same day.

Participants whom did not previously complete the Diet History Questionnaire (DHQII) will do so. This questionnaire is completed online and typically takes approximately 45-60 minutes to complete. Also, a structured dietary interview to assess dietary avoidances will be conducted by NIH nutritional staff (see appendix 26.3).

4.6.11 Resting Energy Expenditure Measurements by Ventilated Hood

While the metabolic hood is the 'gold standard' for metabolic measurements, the technique requires participants to spend long periods of time enclosed in a small room.

As a contingency if a participant is found to be unable to tolerate prolonged time in the metabolic chamber, we will perform indirect calorimetry using the ventilated hood technique. This entails having the participants breathe into a transparent plastic hood placed over the head for up to 40 minutes. Ventilated hood measurements will be timed to occur in the morning after a 12 hour fast.

Hood measurements will be made in all participants prior to performing CPET to establish a baseline measurement. No further ventilated hood measurements will be made if the participant is able to tolerate staying in the metabolic chamber. If a participant is found to be unable to tolerate the metabolic chamber, ventilated hood measurements will be made each morning that the participant would have otherwise been in the metabolic chamber.

4.6.12 Peak Exercise Testing

The testing will be completed at the NIH Clinical Center Rehabilitation Department. All participants need to refrain from smoking for at least four hours before the cardiopulmonary testing.

Cardiopulmonary Exercise Tests (CPET). CPET will be performed using a cycle ergometer. A ramp protocol will be used where the work rate would be gradually increased until volitional fatigue is reached by the patient. The target endpoint is exertional intolerance defined as the subjects expressed desire to stop cycling despite strong verbal encouragement from the testing staff. When the test is stopped, the ergometer work rate will be decreased to cool down levels and may be stopped immediately at the subject's request. Thus, the subject determines the stopping point and is never pushed beyond their expressed ability to continue. Endpoints for stopping the tests will be those recommended by the American Heart Association.[107]

Supplemental, noninvasive cardiac output (Qt) and muscle oxygenation measurements will be made during the CPET. Qt will be measured by bioimpedance plethysmography (ZCG) and muscle oxygenation measurements will be measured by near infrared spectrometry (NIRs). A fingertip blood pressure cuff may also be used to estimate blood pressure and Qt. For further determination of maximal oxygenation values of NIRs measurements, a thigh occlusion test will be performed prior to the CPX. Following seated rest, an occlusion cuff will be rapidly inflated to and held at ~ 250 mmHg, until a plateau is observed in the oxygenation signal (not to exceed 8 minutes). Blood may also be sampled through an IV during CPET. Subjects will be allowed to recover sufficiently after performing the CPX. The total time required for each CPET is approximately 90 minutes.

CPET has been shown to reliably induce post-exertional malaise in one small study.[108] To ensure that the exercise task is properly reaching maximal work rates and inducing post-exercise malaise, the relevant data from the first five PI-ME/CFS and HV participants will be reviewed.

4.6.13 : Qualitative measurement of Post-Exertional Malaise

Qualitative interviews will help illuminate how PI-ME/CFS patients experience PEM and how these experiences differ from those of HV and DLI-A subjects. To date, PEM has not been well studied using qualitative methods. Qualitative interviewing offers the ability to understand the unique experience of ME/CFS patients and provide a deep, more nuanced understanding PEM within their social worlds.

Up to 10 brief 10-30 minute semi-structured qualitative interviews will be conducted during the exercise stress visit to capture their first-hand experiences with PEM (if any). The actual questions used in the qualitative interview will be developed using a focus group methodology previously described (Section 4.3). As the peak of PEM has been estimated to occur 24-72 hours after exercise in ME/CFS, qualitative interviews will be performed up to 4 hours before CPET, up to one hour after CPET, 4-6 hours after CPET,, 20-28 hours after CPET, 44-52 hours after CPET, and 68-76 hours after CPET in all participants. This serial collection will allow participants to describe their experience while it is fresh in their minds and to see if the PEM changes over time.

Interviews may be conducted over the telephone as necessary due to professional conflicts and weekend interviewing. An interviewer guide (see Appendix 26.4) will structure and direct the interviews so that patients are prompted to explain in detail their experiences with PEM, but interviewers will have discretion to modify as appropriate. Data collected during qualitative interviews will consist of audio recordings, which will be sent to a professional service for transcription via a secure website. Data, recordings, and transcriptions will be stored in locked cabinets. Transcriptions will NOT contain any identifying information and will be labeled with a number only. Persons who decline to be audio-taped will not be included in the qualitative portion of the study.

4.6.14 : Blood measurements before and after CPET

Between 25- 80mL of peripheral blood will be serially drawn during the exercise stress visit. The timing of these blood draws will be parallel to the times when qualitative data about PEM and questionnaire-based measurements of subjective fatigue are collected. The approximate time points at which blood measurements will be collected include: 4 hours before CPET, up to one hour after CPET, 4-6 hours after CPET, 20-28 hours after CPET, and 44-52 hours after CPET and 68-76 hours after CPET in all participants.
No more than 250 ml of blood will be drawn in a single day. No more than 550 ml of blood will be drawn over an 8-week period, per NIH guidelines.

4.6.15 : Neurocognitive testing

Neurocognitive testing will be conducted before and after CPET. The TOVA, HVLT-R, BVMT-R, and the B-Test and/or Dot Test will be measured pre-exercise and may be measured up to four times following CPET.

4.6.16 : Holter monitoring

Heart rate variability will be assessed using standardized Clinical Center EKG/Holter Monitor data, which will be collected per NIH Cardiology standard routines. The EKG monitoring will last less than 24 hours and will be done pre-exercise and post-exercise.

4.6.17 : fMRI

fMRI will be completed during the exercise stress visit and will occur on two separate days, once before and once after the exercise challenge. The following imaging paradigm will be utilized: The subject will lie in the scanner operating a dynamometer to measure the force generated by the muscles of the distal upper limb in a first phase of the scan, and a button box for answering a cognitive test in the second phase of the scan. A structured set of imaging tasks are planned. An optional MRI-compatible electroencephalogram cap may be installed on subject's head before installing the subject in the MRI scanner in order to monitor the cortical activity during fMRI scanning and to allow the measurement of the cortico-muscular coupling during the muscular and cognitive fatiguing exercises. This may add about 30 min to the setup time.

Each scanning session will begin with a localizing image, an anatomical scan, and resting-state BOLD acquisition (20 minutes).

A test of cognitive fatigue will be performed within the MRI environment before the muscular fatigue test. In this study, there will be a sequence of two-choice reaction times. The correct choice will be based on a simple cognitive operation and the difficulty of the operation will not change over the series. Cognitive fatigue will be deemed present if the reaction time increases over the sequence. The test will continue for 10 minutes. fMRI will be done for 10 minutes, and the task analyzed as an event related design. The time between reaction time trials will be jittered, so that the MR signal can be extracted. The behavioral data and the MR data will be related parametrically. Another resting BOLD acquisition will be performed after the cognitive task. Even if no significant learning is expected related to the task, the subjects will get some training on the task prior to the MRI study so the novelty and/or any amount of learning will be further minimized. Subjective appraisals of cognitive fatigue will be measured at intervals during the cognitive fatigue task (20 minutes).

A test of muscular fatigue will be performed afterwards within the MRI environment. The last resting BOLD acquisition for the prior test can be considered the baseline for this test. We will determine maximum voluntary contraction (MVC) of the forearm muscles, expressed in newtons, from the best of three brief presses on the dynamometer. After a resting period, the subject will perform repeated 30-second blocks of isometric muscle contractions at half MVC until fatigued, which is defined as the inability to maintain at least 40% MVC force for more than 3sec. The subject and the investigators will have visual feedback from a computer to monitor force generation. The approximate interval between successive exercise periods will be 30 seconds. The period after the last exercise will be the recovery period, during which resting BOLD will be acquired. Acquisition time will be the same length for all subjects, with fatigued subjects simply stopping doing the task for the block. Subjective appraisals of muscular fatigue will be measured at intervals during the muscular fatigue task (30 minutes).

If MPRAGE, DWI, and T2w images have not been previously collected on a study dedicated MRI machine at the NMR Center, they will be obtained at this time. If an MRI-

compatible cap was installed and further DWI scans are necessary, the cap will be removed prior to the DWI scan. (25 minutes).

The fMRI procedures will all be completed on the same scanner at the NMR center and should take approximately 90-120 minutes to complete. All women of childbearing potential will have a urine pregnancy test performed no more than 24 hours before each fMRI session.

4.6.18 : Effort-Expenditure for Rewards Task (EEfRT)

The Effort-Expenditure for Rewards Task[109] is a multi-trial game in which participants are offered a choice between two task difficulty levels for a reward. The task begins with a 1 second fixation cross, followed by a 5s choice period in which the participant is informed of the probability of "winning" if the task is completed successfully. After the participant chooses the task, a one second "ready" screen is displayed. Next, the participant either completes thirty button presses in seven seconds with the dominant index finger if they have chosen the easy task, or one-hundred button presses in 21 seconds using the non-dominant little finger if they have chosen the hard task. Next, the participant receives feedback on whether or not they completed the task successfully. Finally, the participant learns if they have "won", based upon the probability of winning and the successful completion of the task. This process repeats in its entirety for twenty minutes. This task will evaluate the theory of value-based decision making in the pathophysiology of fatigue by looking for associations between MRI brain measurements implicated in reward and measures of fatigue and motivation in our subjects. In the initial validation study, trait anhedonia as measured by well-validated self-report measures significantly predicted an overall reduced likelihood of making a hard-task choice ($b = -.015$, $p<0.005$).[110]

Participants are told at the beginning of their task that they will win the dollar amount they receive from two of their trials, chosen at random from the computer program (range of total winnings is $2.00-$8.60).

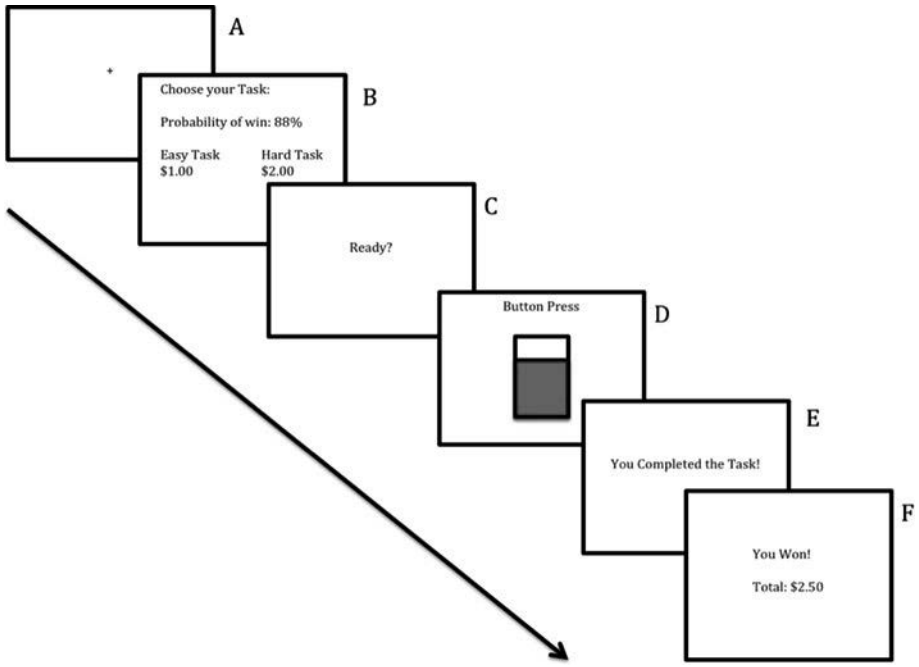

Figure 1. Schematic diagram of a single trial of the modified Effort Expenditure for Rewards Task ('EEfRT'). A. Fixation cross for participant to view, B. 5s choice period in which subjects are presented with information regarding the reward magnitude of the hard task for that trial and probability of receiving any reward for that trial, C. 1s "ready" screen, D. Subjects make rapid button presses to complete the chosen task for 7s (easy task) or 21s (hard task), E. Subjects receive feedback on whether they have completed the task, F. Subjects receive reward feedback as to whether they received a score increase for that trial (adapted from Treadway, 2009).

4.6.19 : Lumbar puncture

All participants will undergo an LP to collect cerebrospinal fluid (CSF) for research. The LP will be done at the Clinical Center at the bedside or in Radiology if done under fluoroscopy. The NIH Radiation Safety Committee guidelines will be followed for participants undergoing LP under fluoroscopy. Analysis of CSF may include (but will not be limited to): opening pressure, cell count, total protein, glucose, PCR for pathogens, cytokine assays, lipid profile, and flow cytometry for phenotyping of immune cells (CD4 and CD8 T cells including central memory, central effector, regulatory and naïve T cell subsets, B cells and monocytes), cytokine/chemokine profile, growth factors, proteome and metabolome, autoantibodies to brain antigens and neurotransmitters. CSF will also be aliquoted and stored for future use. Additionally, specialized laboratory testing such as detection of autoantibodies to novel antigens will be performed as needed. The upper limit of CSF withdrawn will be 24 ml. LP will be performed during post-exercise day 3. (30-60 minutes)

4.6.20 : Stool Sample Collection

All stool produced during the 12 hours prior to CPET and for 72 hours after CPET will be collected fresh in a cup and placed in an ice bath or refrigerator immediately. These

samples will be retrieved, processed, and frozen within 24 hours. Stool samples will be used for microbiome and micronutrient profiling.

4.6.21 : Sleep Electrocephalogram

Sleep architecture will be measured before and up to three times after the exercise stress test using a portable EEG unit. The portable EEG unit is compatible with use in the metabolic chamber. The EEG cap will be placed on the scalp by qualified personnel. Collodion or gel may be used in conjunction with the cap. The collected data will be used for sleep staging and determining within participant and between group alterations in sleep macro- and microarchitecture.

5 Descriptions of Optional Produres

5.1.1 Sedation during LP

Sedation with low-dose benzodiazepines may be used for participants with anxiety during the LP. All participants receiving sedation during the study will be monitored for side effects.

5.1.2 Fluoroscopic-guided LP

If the study investigators determine that the LP should be done under fluoroscopy because of a participant's body habitus, the procedure will be done in Radiology at the Clinical Center. The fluoroscopic-guided LP involves exposure to radiation. The radiation exposure is not required for medical care and is for research purposes only.

5.1.3 Medical, Psychiatric, and Rehabilitation Consultation

Medical, psychiatric, and/or rehabilitation consultation may be requested if it is clinically required or desirable in order to provide for the inpatient needs of study participants.

5.1.4 Whole genome sequencing

All participants will be offered the option of consenting to whole genome sequencing. Sequencing will be performed on stored blood samples. A separate informed consent form will be used for whole genome sequencing. Samples may or may not be tested, even if informed consent for testing is provided.

5.1.5 Skin Biopsies

Participants may be offered the option of undergoing a two-site skin biopsy procedure. One skin biopsy will be taken from the distal lateral leg, 10cm proximal to the superior margin of the lateral malleolus along the midaxillary line. The second skin biopsy will be taken from the distal thigh, 10 cm from the superior margin of the patella. Either the left or right leg may be selected for each biopsy. Aseptic technique is to be utilized throughout the sampling procedure.

The designated area will be prepared with alcohol wipes or another suitable antiseptic. Local anesthetic (i.e. 2% lidocaine with epinephrine) will be infiltrated into the area of planned biopsy. Once adequate anesthesia is obtained, a sterile disposable 3mm skin punch will be used to obtain a punch skin sample approximately 6mm deep. Using sterile

disposable forceps, the dermis will be gently lifted and the sample freed using sterile disposable scissors. The biopsy sample will then immediately be placed in a sample vial containing a fixative agent.

No more than two passes will be attempted at each site. Gentle pressure with sterile gauze will be used to address any bleeding. Persistent bleeding (seen in <1:1000 biopsies) may be addressed with other hemostatic measures, such as placing gelfoam into the biopsy site. Once bleeding stops, the area will be bandaged with sterile bandages.

Only a single two-site skin biopsy will be performed as an optional part of the protocol. The skin biopsy may be performed during Visit 1, during Visit 2, or as an additional outpatient visit.

5.1.6 Muscle Biopsy

All participants may be offered the option of undergoing a muscle biopsy. The vastus lateralis is the site of choice for muscle biopsy. Aseptic technique is to be utilized throughout the sampling procedure.

The designated area will be prepared with Betadine, chlorhexidine, or another suitable antiseptic. Local anesthetic (i.e. 2% lidocaine without epinephrine) will be infiltrated into the area of planned incision with a short #25 needle. Once adequate anesthesia is obtained, an incision approximately 0.5 cm long and 2 cm deep will be created. Deeper tissue layers can be infiltrated with the local anesthetic utilizing a #20-22 needle. Orientation of biopsy sample will be in a cross-sectional fashion.

A 5 mm or 6 mm Bergstrom biopsy needle will be used. The procedure goal is to obtain at least 200 mg of wet tissue. The number of biopsy 'passes' depends on the sample size. No more than four passes will be attempted. An ice pack and pressure will be applied to the biopsy site for at least 15 minutes. The incisions will be closed with steri-strips and a sterile pressure bandage will be applied. If hemostasis remains an issue after the procedure is completed, ice may be applied or the area may be infiltrated with 2% lidocaine with epinephrine.

Only a single muscle biopsy will be performed as an optional part of the protocol. The muscle biopsy may be performed during Visit 1, during Visit 2, or as an additional outpatient visit.

5.1.7 Functional Magnetic Resonance Imaging (fMRI)

Functional MRI may be offered as an optional procedure during Visit 1 or as an additional outpatient visit. The fMRI procedure is identical to that being performed during Visit 2 and is detailed in Section 4.6.17: functional MRI but will be only be performed a single time (rather than before and after an exercise stress). The optional fMRI procedure will only be performed after a medication washout (if required) as detailed in Section 4.2.21.

5.1.8 Transcranial Magnetic Stimulation (TMS)

TMS may be offered as an optional procedure for all participants. It may be performed a maximum on one time, either during Visit 1, prior to CPET during Visit 2, or as an additional outpatient visit. The optional TMS procedure will be performed only after a medication washout (if required) as detailed in Section 4.2.21. All women of childbearing potential will have a urine pregnancy test performed no more than 24 hours before each TMS session. The following paradigm will be utilized:

The subject will sit comfortably on a chair, with the right forearm placed in a rigid-frame dynamometer to measure the force generated by the muscles of the distal upper limb. The output of the dynamometer will be fed to a computer. We will determine MVC as the best of three wrist extensions. We will record the EMG by placing surface electrodes on the right forearm. A TMS-compatible EEG cap may be installed, in order to monitor the cortical activity during the TMS recordings and to allow the measurement of the cortico-muscular coupling during the muscular fatigue exercise.

Using a magnetic stimulator and figure-8 coil, we will determine the optimal position for evoking motor potentials (MEP) by holding the coil tangential to the scalp and slightly displacing it until the highest MEP amplitude will be recorded from the target muscle. The positions of subject's head and TMS coil will be tracked with a neuronavigation system, in order to maintain the stimulation position over the hotspot. We will then record the TMS Input-Output curve. For all subsequent TMS experiments, we will use the S50 stimulus level for the reference MEPs. Also, in this preparatory phase, we will record the maximum M-wave by applying electrical stimulation with surface electrodes on the nerve innervating the target muscle. All MEP amplitudes will be normalized to each subject's Mmax amplitude.

The TMS evaluation will begin with a block of 100 single pulses delivered every 5 seconds in order to allow the subsequent analysis of the EEG responses. This battery will be followed by a short set of double-pulses for measuring short intracortical inhibition (SICI) and intracortical facilitation (ICF).

Then, the subject will perform repeated 30-second periods of isometric muscle contractions at half the MVC until fatigued, which is defined as the inability to maintain at least 40% MVC force for more than 3 seconds. A short train of single-pulse MEPs (elicited every 5 seconds) will be recorded immediately after each 30-second exercise period, at 30 seconds after the last MEP recorded in the final exercise period, and at fixed intervals thereafter for up to 30 minutes. These single-pulse trains will be alternated with blocks of SICI, ICF, and Mmax, that will monitor the effects of muscular fatigue on these parameters. The subject and the investigators will have visual feedback from the computer to monitor force generation and audio feedback from the electromyograph to monitor muscle relaxation and contraction. The period after the last exercise will be the recovery period. Because post-exercise MEP facilitation can last a few minutes after the last exercise set, post-exercise MEP depression will be defined as the mean of the lowest MEP amplitudes recorded either 30 seconds, 2 minutes, or 4 minutes into the subject's recovery period. (2 – 3 hours)

5.1.9 Metabolic Chamber wearing accelerometers

Spending one night in the metabolic chamber will be offered as an optional procedure during Visit 1 or during an additional inpatient visit. Activity monitors (Section 4.6.7) will be placed on participants prior to being placed in the metabolic chamber. The metabolic chamber procedure is identical to that detailed in Section 4.6.8: Metabolic Chamber. As part of this optional procedure, participants will have a DEXA scan (Section 4.6.9).

6 End of participation

Participants will complete the study after discharge from the NIH Clinical Center. If eligible, individuals enrolled in the study may be offered the possibility of enrolling in other NIH protocols as appropriate; however, this is not a requirement for participation in this protocol. Participants will be asked if they would be willing to be re-contacted by the study investigators for new study opportunities, including additional optional procedures added to this protocol in approved Amendments or as separate, related Protocols.

Participants will continue care with the primary care provider throughout the study and after end of study participation.

Results of the study procedures available in the participant's electronic medical record will be shared with the patient and with the subject's physician in a timely fashion via written communication in the form of a letter. Should there be a clinical need to communicate urgently with the patient or his/her physician, this will be pursued via telephone communication in addition to the written communication.

Participants will receive a thank you letter after completing their participation in any of the aspect of the research study. These letters are detailed in Appendix 26.8.

7 Management of Data and Samples

7.1 Storage

All collected samples including serum, plasma, PBMCs, CSF, and saliva will be coded and stored in secured freezers on the NIH campus. All patients enrolled in this protocol will be assigned a sequential code and all biological samples collected for this patient will be labeled with the patient's code, type of the sample, volume, number of cells (if indicated) and date of freezing. All study data will be kept on password-protected computers on the NIH campus. Only study investigators will have access to the stored data and samples. The study investigators will have access to the code key.

Any loss or destruction of samples will be reported to the IRB.

7.2 Data (including genomic data) and sample sharing plan

Samples and data including genomic data may be shared with other NIH protocols, other investigators, or databases/repositories, under the following guidelines:

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

As no large-scale genomic data will be acquired during the course of this protocol, this protocol is not subject to the NIH Genomic Data Sharing (GDS) policy.

Under the Human Data Sharing (HDS) policy, in addition to the procedures for sharing genomic data, as stated above, de-identified non-genomic study data will be shared with the NINDS data repository (data.ninds.nih.gov). Furthermore, applicable (e.g., generated via CRIS) identified data will be shared via BTRIS following standard Clinical Center operating procedures including BTRIS data access policies. Data to be shared include baseline characteristics as well as key study outcome variables in a tabulated format, after adequate data cleaning, processing, and quality control. Data sharing will be performed at or prior to the time of publication.

8 Additional Considerations

8.1 Research with investigational drugs or devices

MRI evaluations of brain may be performed at field strengths of 1.5T, 3T, and/or 7T, under conditions designated by the FDA as constituting nonsignificant risk. MRI scans are performed on FDA-approved scanners with approved radiofrequency coils, and their use conforms to the corresponding FDA labels. These studies may involve software modifications as allowed under 21 CFR 812.2(b)(4), which stipulates that any modification "is not for the purpose of determining safety or effectiveness and does not put subjects at risk."

Conventional TMS studies in this protocol are considered non-significant risk (NSR) devices and will only be used within published guidelines.21CFR812.2 SR states that a significant device

1. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject

TMS is not an implantable device.

2. is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject
TMS is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

3. is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject
TMS, as used under this protocol is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

4. otherwise presents a potential for serious risk to the health, safety or welfare of a subject

TMS (single and pair pulses) has been in use for over three decades and have been cleared by the FDA for treatment of several disorders. Safety guidelines have been developed and updated allowing its dissemination to a wide range of clinical and non-clinical settings.[111,112] For example, brief, self-limited, seizures were seen in early studies, before limits were established for combinations of delivery parameters. However, this risk has been reduced to the order of one in every 40,000 sessions, including for paradigms like theta-burst stimulation not included in the guidelines.[113] In the past 20 years, the FDA has generally waived pre-IDE inquiries for TMS/rTMS studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for TMS studies within these limitations.

8.2 Gene therapy
Gene therapy will not be used in this study.

9 Risks and Discomforts

The potential risks and discomforts of the study include the following:

9.1 Risks of history and physical examination
There is minimal medical risk or discomfort during the history and physical examination.

9.2 Risks of IV insertion
The risks of an IV include bleeding, infection, or inflammation of the skin and vein with pain and swelling. Some people may feel lightheaded.

9.3 Risks of blood collection
Participants may have some discomfort and bruising at the site of needle or IV entry. There is a very small risk of fainting. Infection in the area of the needle insertion is rare.

9.4 Risks of HIV testing

All subjects who test positive for HIV during this study and did not know they were positive will be informed of the result. There is a risk of distress for the subject and/or the subject's family from this. Subjects who test positive will be provided additional information regarding the diagnosis and management of HIV.

9.5 Risks of urine collection

There is minimal risk or discomfort associated with giving a urine sample. Participants who have a positive drug test will be told promptly and will be excluded from the study. The results of the drug testing will be noted in the NIH medical record. Participants who do not want this information in their medical record should not participate in this study. Medical records will only be released with the participant's written agreement. However, insurance companies may require participants to release these records and may not give participants insurance if they refuse.

9.6 Risks of symptom assessment/patient reported outcome measures

There is minimal medical risk or discomfort from providing information about ME/CFS symptoms and in completing the questionnaires. Some of the questions may make participants feel uncomfortable or anxious. Participants may refuse to answer any question or to stop a test at any time and for any reason.

9.7 Risks of psychological assessments and psychological inventories

The nature of the questions may be sensitive to some of the participants, in particular questions about sexual and personal trauma and suicidality. Participants may choose not to answer any question that makes them uncomfortable. If required or requested, appropriate referrals will be made as recommended by the psychologists and psychiatrists involved with this protocol.

During this study, any psychiatric diagnoses determined as part of this study will be discussed along with any recommendations with the participant by a member of the study team. A member of the study team may contact the participant's primary care physician to obtain or provide information in some cases.

Participants whose responses to tests and questionnaires suggest non-emergent psychiatric or medical issues may be referred to a provider in the community for appropriate psychiatric or medical care, unless they are already receiving such care. Emergent psychiatric evaluation and treatment will be performed under the guidance of personnel from the National Institutes of Mental Health (NIMH) Psychiatry Consultation Service. Emergent medical evaluation and treatment will be managed by the NIH Rapid Response Team or the NIH Code Blue Team.

If a participant discloses information during completion of the assessment form that suggests suicidal or homicidal ideation or intent (i.e., that he or she may be a danger to themselves or others) or otherwise suggests a need for psychiatric intervention, the PI of the protocol will be notified. If the participant is in imminent danger of harming himself/herself or someone else, arrangements will be made for hospitalization outside of NIH. If the participant insists upon leaving NIH, we may need to arrange an involuntary

hospital admission to another facility. If outside hospitalization is necessary, this will occur at the participant's expense.

In other situations where the participant expresses psychiatric or psychological distress that is not suicidal or homicidal in nature, the PI or another licensed member of the research staff can provide the participant with a referral for psychiatric or other evaluation with a clinician and/or for psychiatric assistance with a provider in their home community. Moreover, should the questionnaires in this study raise issues a participant would like to discuss further; the PI will be available to him or her.

9.8 Risks of neurocognitive testing

The neuropsychological tests may be frustrating or stressful. Participants may refuse to answer any question or to stop a test at any time and for any reason. The nature of the questions may be sensitive to some of the participants, in particular questions about sexual and personal trauma and suicidality.

Participants whose responses to tests and questionnaires suggest non-emergent psychiatric or medical issues may be referred to a provider in the community for appropriate psychiatric or medical care, unless they are already receiving such care. Emergent psychiatric evaluation and treatment will be performed under the guidance of personnel from the National Institutes of Mental Health (NIMH) Psychiatry Consultation Service. Emergent medical evaluation and treatment will be managed by the NIH Rapid Response Team or the NIH Code Blue Team.

If a participant discloses information during completion of the assessment form that suggests suicidal or homicidal ideation or intent (i.e., that he or she may be a danger to themselves or others) or otherwise suggests a need for psychiatric intervention, the PI of the protocol will be notified. If the participant is in imminent danger of harming himself/herself or someone else, arrangements will be made for hospitalization outside of NIH. If the participant insists upon leaving NIH, we may need to arrange an involuntary hospital admission to another facility. If outside hospitalization is necessary, this will occur at the participant's expense.

In other situations where the participant expresses psychiatric or psychological distress that is not suicidal or homicidal in nature, the PI or another licensed member of the research staff can provide the participant with a referral for psychiatric or other evaluation with a clinician and/or for psychiatric assistance with a provider in their home community. Moreover, should the questionnaires in this study raise issues a participant would like to discuss further; the PI will be available to him or her.

9.9 Risks of Occupational therapy evaluation

There is minimal medical risk or discomfort related to the occupational therapy evaluation. Some of the questions may make participants feel uncomfortable or anxious. Participants may refuse to answer any question or to stop a test at any time and for any reason.

9.10 Risks of MRI

Participants may be at risk for injury from the MRI magnet if they have some kinds of metal in their body. It may be unsafe for participants to have an MRI scan if they have a pacemaker or other implanted electrical device, brain stimulator; some types of dental implants, aneurysm clips, and metallic prostheses (including metal pins and rods, heart valves, and cochlear implants); permanent eyeliner; implanted delivery pump; or shrapnel fragments. Welders and metal workers may have small metal fragments in the eye. Participants will be screened for such metal before having any scan. If they have any, you will not receive an MRI scan. If they have a question about metal in their body, they should inform the study investigators. Participants will be asked to complete an MRI screening form before each MRI scan they have.

All magnetic objects must be removed before entering the MRI scan room. This includes items like watches, coins, jewelry, and credit cards.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women who are able to get pregnant will have a pregnancy test done no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, participants should let us know right away.

Symptoms from the contrast infusion are usually mild and may include feeling hot, burning, or
coldness in the arm during the injection, a metallic taste, headache, allergic reactions and nausea. In an extremely small number of individuals, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Unless specifically allowed by the protocol, participants will not receive gadolinium-based contrast agents for research purposes if they have previously had an allergic reaction to them. Individuals with a history of anaphylaxis to other agents or chronic asthma requiring treatment will not receive gadolinium under this protocol unless they have previously received gadolinium and tolerated it well. Participants will be asked about such allergic reactions and history of asthma before a contrast agent is administered.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called “nephrogenic systemic fibrosis,” which has resulted in a very small number of deaths. If subjects are 60 years old or greater or have diabetes, kidney disease or liver disease, blood work to assess kidney function will be performed within 4 weeks before any MRI scan with gadolinium contrast. Participants may not receive gadolinium for a research MRI scan if kidney function is not normal. There is no evidence for the potential of gadolinium-related toxicity in people with normal kidney function. This protocol follows

NIH Clinical Center guidelines for kidney-function screening related to gadolinium administration.

Most of the gadolinium contrast is eliminated in the urine. However, recent studies have found very small amounts of residual gadolinium in the body, including the brain, by imaging and at autopsy. Macrocyclic gadolinium-containing contrast agents are substantially less likely to leave gadolinium behind than linear agents. The use of macrocyclic vs. linear agents in this study is delineated in the procedures section above. There is presently no evidence that the retained gadolinium is associated with any adverse effects.

9.11 Risks of muscle strength testing

There is minimal medical risk or discomfort for the muscle strength testing.

9.12 Risks of activity monitoring and fatigue diary

There is minimal medical risk or discomfort from the physical activity monitoring and recording fatigue levels in a diary.

9.13 Risks of nutritional assessment and metabolic diet

There is minimal medical risk or discomfort from the nutritional assessments and maintaining a metabolic diet.

9.14 Risks of saliva collection

There is minimal medical risk or discomfort for providing saliva samples.

9.15 Risks of buccal swab collection

There is minimal medical risk or discomfort for providing buccal swabs.

9.16 Risks of stool sample collection

There is minimal medical risk or discomfort from stool sample collection.

9.17 Risks of Holter monitoring

There is minimal medical risk or discomfort from the Holter monitoring.

9.18 Risks of LP

Participants may have a brief pain or tingling sensation in their legs during the lumbar puncture if the needle brushes against a nerve. Participants will be instructed to let the study team member know immediately and the needle will be adjusted. Some people get a mild backache at the site of needle insertion. About one-third of people have a headache for a few days after a lumbar puncture. Usually the headache is not severe and improves without treatment other than a mild pain reliever. Headaches lasting longer than 7 days develop with one in 50 to 200 lumbar punctures and usually improve gradually over 2 weeks. In rare cases, headaches persist longer. Prolonged headaches may be due to persistent leakage of CSF from the area of the lumbar puncture. If the headache is prolonged, participants may get a blood patch. Collecting ~20 ml of CSF per procedure represents negligible risk (Evans, et al. 2000). In adults the rate of CSF synthesis is approximately 21.5 ml/hour, or approximately 500 ml/24 hours, which represents roughly 4 times the total volume of CSF in adult patients. Therefore, the maximum volume of 24

ml of CSF that would be collected will be replenished in its entirety within approximately 1-1.5 hours after collection.

9.19 Risks of sedation for LP

Sedation with low-dose benzodiazepines is associated with a small risk of respiratory depression and an even smaller risk of cardiac arrhythmias. More common risks are: sleepiness, dizziness, weakness, unsteadiness, hallucinations, confusion, and rarely an allergic reaction causing rash, hives, or swelling.

9.20 Risks of fluoroscopic-guided LP

Fluoroscopy may be used during LP to facilitate placement of the needle into the subarachnoid space. Participants who undergo fluoroscopy will be exposed to radiation in the amount of 23 mrem, (or 46 mrem for two procedures) which is within the safety limit of 5 rem (5000 mrem) per year set by the NIH Radiation Safety Committee for adult research subjects. If participants would like more information about radiation and examples of exposure levels from other sources, they will be provided with the flyer, "An Introduction to Radiation for NIH Research Subjects."

9.21 Risks of autonomic testing

Dynamic heart rate and blood pressure monitoring during respiratory, positional and valsalva maneuvers: The blood pressure monitoring consists of blood pressure sensor placed on the finger, this has a squeezing sensation which can be slightly uncomfortable. The heart rate monitor, similar to a standard EKG recording, will have three surface leads placed on the chest and torso area to noninvasively monitor heart rate. The chest expansion monitor will consist of a strap wrapped around the low chest to record the breathing pattern. It can be cumbersome but otherwise is painless. The testing maneuvers of deep breathing, Valsalva or tilt test may cause lightheadedness, syncope, and arrhythmias in some people. Though rhythm disturbances have been noted, the most common being extra systoles, the dysrhythmias appear to resolve without medical intervention. The Valsalva maneuver can also cause an increase in intraocular and intracranial pressure but there has not been any reported complications related to intraocular hemorrhage or lens displacement.

9.22 Risks of cytapheresis

Cytapheresis can cause lightheadedness or fainting. Some people have tingling in the fingers or around the mouth. The citrate blood thinner used to prevent blood clots during apheresis can cause mild muscle cramps. We will slow down the blood flow and/or administer calcium carbonate (Tums or similar oral formulation) if participants complain of symptoms. If the symptoms do not go away, we will stop the cytapheresis.

9.23 Risks of immune cell blood draw

Participants may have some discomfort and bruising at the site of needle entry. There is a very small risk of fainting. Infection in the area of the needle insertion is rare.

9.24 Risks of medication washout

There is a more than minimal risk for the planned tapering off of medications that potentially interfere with brain excitability. Participants may experience an increase in

their typical symptoms or experience symptoms related to the cessation of medications. Patients may decide to discontinue with the medication taper if it is too difficult for them to tolerate.

9.25 Risks of metabolic chamber

Besides inconveniences that can reasonably be expected as a result of spending an extensive time (overnight) in the live-in room calorimeter, there is no risk to subjects' physical health. Claustrophobia is an exclusionary criterion. All subjects will be given an opportunity to experience the metabolic chamber prior to enrollment in the study. Risks of dual energy x-ray absorptiometry

9.26 Risks of DEXA

For each scan (around 12 minutes), the amount of radiation (effective dose) during the DEXA scan is less than one mrem to the whole body. This radiation exposure is below the guideline of 5000 mrem per year allowed for research subjects by the NIH Radiation Safety Committee The use of the DEXA scan apparatus may cause some minimal discomfort in claustrophobic subjects and may cause some minimal back pain in a small minority of the individuals.

9.27 Risks of resting energy expenditure measurements by ventilated hood

The use of the clear plastic ventilation hood may cause some minimal discomfort in claustrophobic subjects

9.28 Risks of peak exercise testing

Exercise testing may be risky for participants with cardiac disease so participants with known cardiac disease will be excluded from the study. Participants who do not have known cardiac disease are believed to be at low risk from exercise tests using a bike or a treadmill. The major risk of this type of exercise in general is that someone may have unknown or asymptomatic cardiac disease. Even though participants will be examined before the study to try to detect the presence of cardiac disease, it is possible to have unknown cardiac disease that can lead to angina, arrhythmia, or death during the test. The risk of death for healthy adults who undergo maximum exercise is between 0 and 3 deaths/100,000 tests. During the test, the participant's heart rhythm will be constantly monitored by the study team. A participant will stop the test at the earliest sign of cardiac problems whether those signs are from the monitor or based on symptoms. Other risks of exercise include the risk of falls while using the treadmill or short-term myalgia or arthralgia caused by exercising that could last several days. Participants will be instructed to tell the study team if myalgia or arthralgia becomes bothersome as these may be treated with as needed mild pain relievers.

9.29 Risks of Qualitative Interviews

Answering questions about one's illness could elicit negative feelings or emotions. Interviewers will be cognizant of this during interviewing and make every attempt to put the patient at ease and remind respondents that they can decline to answer questions and/or terminate the interview.

9.30 Blood measurements before and after CPET

There is minimal medical risk from blood sampling from the IV line.

9.31 Risks of Effort-Expenditure for Rewards Task

This computer test of effort expenditure for perceived reward is similar to other computer based neuropsychological tests. This task is not harmful but may be frustrating or stressful. Participants may stop this task or withdraw from the study at any time and for any reason.

9.32 Risks of TMS

TMS may cause strong contractions of scalp muscles leading to discomfort or a headache. If participants find TMS too uncomfortable, they may stop it any time. Headaches usually go away by themselves or with nonprescription medication. The noise of the TMS magnet can damage hearing, so participants will be fitted with earplugs which must be worn during TMS. TMS can interfere with implanted medical devices. Participants will not be able to have TMS if they have a pacemaker, implanted pump, a stimulator (such as a cochlear implant) or metal objects inside the eye or skull.

Risk of skin irritation at the site of the EMG electrode may be minimal in some subjects. There are no medical risks associated with surface EMG.

9.33 Risks of sleep electroencephalogram

There is no known risk concerning the EEG technique itself. The electrode cap can be uncomfortably tight. There may be mild irritation of the skin and scalp from the mild abrasion needed to assure good contact or from the collodion, paste, or gels used.

9.34 Risks of Medical, Psychiatric, Sleep Service and Rehabilitation Consultation

There is minimal medical risk or discomfort from medical, psychiatric, sleep, and rehabilitation consultation.

9.35 Risks of genetic testing

Genetic testing can provide information about how illness is passed on within a family. This knowledge may affect participants' emotional wellbeing. They might feel differently about their life if they learned that they or their children were at increased risk of a disease, especially if there were no treatments. Their children, brothers or sisters may find out that they are at risk for health problems because of information found out about the participants, which might affect the participants' relationships with family members. Other family members may also be affected by uncovering risks they have but did not want to know about. This information can cause stress, anxiety, or depression.

Some genetic testing can also determine if people are directly related. These tests sometimes show that people were adopted or that their biological parent is someone other than their legal parent. If these facts were not known previously, they could be troubling. Genetic counseling is available at the NIH to help participants understand the nature and implications of genetic findings.

Because of the emotional risk, some participants do not want to know the results of genetic testing. It is our policy to not disclose the results of genetic testing unless it may have direct medical or reproductive implications for participants or the participants'

families. Any genetic results disclosed to participants will be done in the context of genetic counseling.

Results of genetic testing obtained at NIH are often preliminary and difficult to interpret because the testing is being done for research purposes only and the laboratories are not CLIA-certified. If a reportable genetic change is noted on a non—CLIA genetic test, the protocol will cover the costs of confirming the result using a CLIA genetic test.

Genetic information will be kept confidential to the extent possible. The results of genetic testing will be kept in a locked and secured manner at the NIH.

Problems, such as with insurance or employment discrimination, may occur if they disclose information about themselves or agree to have their research records released. We will not release any information about participants to any physician, insurance company or employer unless they sign a document allowing release of the information.

9.36 Risks of Skin Biopsies

The skin punch-biopsies have potential risks including infection, bleeding, pain, and potential scarring.

9.37 Risks of Muscle Biopsy

The muscle punch-biopsy procedure has potential risks including infection, bleeding, pain, and post-procedure anesthesia or paresthesia if a cutaneous nerve is damaged. The risk of these events is minimized by having the procedure performed only by experienced physicians under sterile conditions with adequate local anesthesia.

10 Subject Safety Monitoring

Study personnel, overseen by the PI, will closely monitor participant safety during study procedures. If, during the course of interviewing, assessment procedures and the research procedures, study staff identifies a condition that should require immediate clinical intervention, all necessary steps will be taken. Parameters to be monitored for safety reasons include level of consciousness. All MRI scans will be monitored in accordance with NIH Clinical Center policies. Toxicity will be assessed according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). Participants will be removed from the study if they are unable to cooperate with the study procedures, if a medical exclusionary condition develops, or if the PI judges discontinuation to be in the participant's best medical interest.

11 Outcome Measures:

The primary purpose of this protocol is to perform exploratory analysis of collected samples for the generation of new hypotheses regarding ME/CFS. The types of analyses to be performed will be wide ranging. Planned areas of focus include:

1. Characterization of the immune system and inflammatory signaling in collected samples at baseline and following maximal exercise exertion.
2. Characterization of the pattern of microbiome in collected samples at baseline and following maximal exercise exertion.
3. Characterization of bioenergetics, autonomic, and metabolic function in collected samples at baseline and following maximal exercise exertion.
4. Characterization of physical and cognitive fatigue using functional magnetic resonance imaging and transcranial magnetic stimulation at baseline and following maximal exercise exertion.
5. Characterization of neurocognition at baseline and following maximal exercise exertion.

12 Statistical Analysis

12.1 Analysis of data/ study outcomes

The studies performed under this protocol are exploratory. Quantitative research data will be acquired and logged into a database. The PI or designated investigator will monitor data integrity. Because a variety of types of data will be obtained in the study, the statistical methods most appropriate for the nature of the data will be used.
Some of the relevant statistical approaches are as follows:

We are primarily interested in evaluating whether various types of data obtained are significantly different between the three groups as measured in cross-section during the phenotyping visit as well as between HV and PI-ME/CFS groups before and after an exercise stress. Each of these groups will be characterized using standard descriptive statistical methods.

As noted in Section 4.5, ME/CFS participants that are found no to meet the strict inclusion/exclusion of this study all have been diagnosed with ME/CFS by health providers and represent ME/CFS in the community. HVs and COVID-19 HVs that do not have fatigue or PEM may also be found to medical issues or other findings that would exclude them from meeting the strict inclusion/exclusion criteria of being healthy, but still represent a non-fatigue comparator to ME/CFS patients. The primary focus of the analyses detailed below will be between adjudicated ME/CFS participants and HVs and COVID-19 HVs that meet all inclusion/exclusion criteria. If the result of a primary analysis is positive, a secondary analysis will be repeated using all of participants. Findings that remain robust even when less stringent inclusion/exclusion criteria are used suggest that they are more likely to be replicable in community cohorts and perhaps worthy of further exploration.

For each continuous-scale outcome, repeated measures analysis of variance (ANOVA) will be used to test whether there are significant differences in cross-sectional measurements as well as in the pre and post measurements among two groups by using F tests. These initial tests will be performed using the adjudicated PI-ME/CFS group (i.e. participants determined to have PI-ME/CFS by the protocol's adjudication process) compared to HV and COVID-19 HV participants meeting all inclusion and exclusion criteria. The cross-sectional analyses may then be repeated using all HV and COVID-19 HV participants that did not report substantial clinical fatigue as noted above.

If the overall multivariate test is significant, we will follow up with several post-hoc tests. Specifically, we will first conduct multivariate tests to compare the adjudicated PI-ME/CFS group (i.e. participants determined to have PI-ME/CFS by the protocol's adjudication process) vs the other two control groups, and the PI-ME/CFS group vs each of the two control groups. These analyses will initially performed using only HV and COVID-19 HV participants meeting all inclusion and exclusion criteria. As noted above, for analyses that are statistically significant, the analysis will be repeated using all HV and COVID-19 HV participants that did not report substantial clinical fatigue to determine if the results remain statistically significant when less stringent inclusion/exclusion criteria are applied.

Second, univariate ANOVA will be used for both cross-sectional measurements and pre and post exercise measurements separately to identify differences among groups. A

similar univariate analysis will be conducted for the measurements of change from pre- to post exercise using the univariate ANOVA.

Additionally, descriptive statistics will be used to characterize the entire group of ME/CFS participants that completed the phenotyping evaluation regardless of adjudication. Comparisons will be made between the adjudicated PI-ME/CFS group and the phenotyping ME/CFS group. If there is no significant difference between the above two groups, it is not necessary to compare a ME/CFS group to our two control groups. Where statistically appropriate, the ANOVA methods described above will be used to compare the ME/CFS group with the two control groups.

Statistical approaches such as chi-square tests and multinomial logistic regression models will be used to examine the group differences for categorical outcomes.

A level of significance is set at 0.05. Multiple comparison test procedures such as Bonferroni and Dunnett's methods will be used to examine the differences in each outcome measure among groups.

The primary MRI imaging aim is to test whether PI-ME/CFS group has significantly different connectivity patterns compared to the HV control group.

To this end, functional MRI measures will be taken at three conditions: 1) performing a force task, 2) performing a cognitive task, to define a network of interest, and 3) at rest to compute functional connectivity. At each condition, fMRI will be measured at two time points: pre exercise (preEx) and post exercise (postEx).

We will use a multivariate regression model where the response variable is connectivity strength among regions of interest pre- and post- exercise. Predictors include group status and fatigue scores.

Region of interest for the connectivity analysis will be defined based on tasks 1) and 2). For this, data will be pre-processed with standard techniques using AFNI. Imaging quality assurance will be performed (i.e. motion correction parameters). We will fit a multivariate regression model to detect the differences in activity patterns of each task. Behavior and scales of fatigue will be entered as covariates.

Significance of the results will be set at $p = 0.05$ corrected for multiple comparisons for all analyses.

Lastly, we will conduct receiver operating characteristic (ROC) analysis for selected markers that are potentially important, which will be identified by exploratory analysis and several statistical approaches mentioned above. We will evaluate the accuracy of each marker for its ability to classify subjects in the PI-ME/CFS group from those in the HV control group. For example, if MRI imaging measurements are significantly different among groups, next, we will calculate the nonparametric area under the ROC curve (AUC) for each imaging marker and its 95% confidence interval.

12.2 Power analysis

The accrual ceiling for participants in the phenotyping and exercise stress arms of this study is 150 participants (50 per group) and dropouts will be replaced. The sample sizes were chosen based on convenience and equals about 1-2 participants per week. This is a phase I pilot study intended to explore a variety of clinical and biological data from the three different groups. For neuroimaging studies of somatosensory stimulation, it is estimated to require 12 subjects to achieve 80% power at the threshold of 0.05 to determine differences at the single voxel level for typical activations.[114] The proposed sample size of 20 participants per group should be adequate for distinguishing group differences in fMRI activity during the proposed fatiguing tasks. The study is exploratory and its results will be used to estimate effect sizes to design a larger clinical study.

12.3 Analytic Plan

To analyze the vast, multidimensional data collected in this protocol, the research team has organized four Working Each Working Group has been assigned a post-baccalaureate IRTA to assist in organization and with conducting a literature review. Groups to focus on four central themes: Clinical, Pathophysiology, Immunology, and Bioenergetics:

Clinical: Brian Walitt (Chair)
Data for analysis:

- Life Narratives
- Medical History
- Physical Exam
- Clinical laboratory measurements of blood and urine
- Neuropsychological Testing data
- SCID - 5
- Patient Reported Outcome Measures
- Polysymptom Interview
- Activity Card Sort
- Cardiopulmonary Exercise Test
- Clinical Tilt Table Result
- Clinical Sleep Study Result
- Muscle Strength Grip Testing
- Nutritional data
- Microbiome
- ME/CFS Criteria Adjudication

Pathophysiology: Mark Hallett (Chair)
Data for analysis:

- Neurological exams
- Structural Brain MRI
- Functional Brain MRI (Resting State, Diffuse Tensor Imaging, Hand Grip Task, Math Task)
- Dynamic Tilt-table Study data
- Single time point blood and CSF catecholamines measures

- 24 Hour Holter monitor data
- Overnight sleep EEG data
- Neuropsychological Testing data
- Transcranial Magnetic Stimulation data
- Salivary Cortisol measures (morning, afternoon, evening)
- Patient Reported Outcome Measures
- Cutaneous small fiber measurements of skin

Immunology: Avindra Nath and Steve Jacobson (Co-chairs)
Data for analysis:

- Clinical laboratory measures of inflammation
- Brain MRI with gadolinium
- Somalogic proteomics on blood and CSF
- Flow cytometry on blood and CSF
- RNASeq on PBMCs
- GDF-15 measures
- Muscle biopsy pathology (limited)
- Muscle RNASeq
- NK Cell functional assay
- Tryptase
- Toxicity studies on PBMCs
- Metabolomics of plasma and CSF
- Iron levels of CSF
- Exosomes of plasma
- Neurofilament analysis of plasma and CSF

Bioenergetics: Kong Chen (Chair)
Data for analysis:

- Metabolic Chamber
- Physical Activity Monitoring
- DEXA measurement
- Dietary History and Food Records
- Cardiopulmonary Exercise Test
- RNASeq on PBMCs
- Seahorse mitochondrial assay on PBMCs
- Muscle Strength Testing
- Muscle biopsy pathology(limited)
- Muscle RNASeq
- Limited mitochondrial genetics data
- Metabolomics of plasma and CSF
- Microbiome

Each Working Group will begin by performing a literature review of their topic to ascertain the state of the ME/CFS field and guide their analyses. Analyses will be performed to both interrogate published findings to determine reproducibility and to make novel insights. Group members will first analyze the data that they are responsible

for collecting as part of the protocol. These individual data analyses will each be appropriate for the type of data collected and performed by Working Group members with experience in analyzing the data. Each Working Group will meet periodically to present their findings to each other, discuss issues of honing analyses, the need to expand data collection or perform additional experiments, identify areas of data consilience, and assemble a general consensus of how to interpret the results.

Once each Working Group completes its analysis and evaluation of the data, a series of combined Working Group meetings will be convened. Each Working Group will present their methods and results to each other. Break-out sessions will be organized that will mix the members of the Working Groups together to discuss how findings in one domain may relate to those in other domains. An open discussion for all Working Group members will be conducted to discuss issues of honing analyses, the need to expand data collection or perform additional experiments, identify areas of data consilience, and assemble a general consensus of how to interpret the results.

With the analyses of each Working Group completed, the research team will identify computational biologist collaborators to determine how to appropriately apply network analytics and machine learning approaches to the combined dataset. The goal of such analysis would to be elucidate observable linkages between the four research themes. These linkages would be areas of high interest in generating new hypotheses and also suggest potential therapeutic targets for future interventional studies.

These Working Groups have already been initiated to perform an interim analysis on the ME/CFS and HV cohorts completed as of October 2020.

13 Human Subjects Protection

13.1 Subject selection

Subject selection will be equitable among eligible participants. ME/CFS is reported to happen most commonly in persons aged 40-59.[115] Increasing age also has significant effects of both on the experience of daily fatigue and on brain physiology. For this reason, individuals over the age of 60 will be excluded. Several of the neurocognitive instruments being used in this protocol require a 7th grade education for proper administration. These include the MMPI2, the Controlled Oral Word Association, and Grooved Pegboard tests. For this reason, persons with less than a 7th grade education will be excluded.

13.2 Justification for exclusion of children

Children less than 18 years old are excluded from the trial. Because ME/CFS is rare in children, pediatric cases may not be representative of the typical pathophysiology of the condition.

13.3 Justification for exclusion of pregnant women

Pregnancy-related physiological changes and fatigue would impact the interpretability of the results. Also, pregnancy is a contraindication for MRI. For these reasons, pregnant women are excluded from the study.

13.4 Justification for exclusion of women who have given birth in the past year

Post-partum fatigue is likely to confound the results of the study.

13.5 Justification for exclusion of those who lack consent capacity

Persons who lack consent capacity will be excluded from this study. This study provides no direct benefit to the participant, involves varied and complex testing, and is more than minimal risk.

Cognitive impairment severe enough to affect consent capacity would be unexpected in our target population. Such patients also would be unable to properly perform several study procedures, in particular patient-reported outcome scales and neurocognitive testing. For these reasons, persons with severe cognitive impairment will be excluded. If necessary, a CC-credentialed LIP will assess if a person is too cognitively impaired to participate in the protocol. While unexpected, a consultation to assess consent capacity will be obtained from the NIH Ability to Consent Assessment Team (301-496-9675) if necessary.

13.6 Justification of exclusion of non-English speakers

Persons who are not fluent in the English language will be excluded. Such persons would be unable to properly perform several study procedures that are only valid in the English language, in particular patient-reported outcome scales and neurocognitive testing.

13.7 Justification for inclusion of employees

NIH staff will not be solicited for participation, but will not be excluded if they express the desire to enroll. Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. If the individual obtaining consent is a co-worker, an independent person (either from Bioethics or NIMH Human Subjects Protections Unit) will monitor the consent process for any NIH employees that desire to participate. Employees that participate in this protocol during work hours will be informed that they must obtain their supervisor's permission and that per NIH guidelines they will not be paid.

13.8 Justification for sensitive procedures

Drug tapering is a sensitive procedure that may be performed on a subset of patients. Allowing for the use of central-acting medications would create problematic heterogeneity in the study groups. The impact of central-acting medications on biological measurements, in particular those made in blood, cerebrospinal fluid, fMRI, and neurocognitive testing, are also significant. As these medications do not treat the underlying cause of ME/CFS, do not substantially reduce morbidity or mortality, and have not been shown to lead to substantial clinical improvements, the period of tapering withdrawal will not likely to have substantial impact on health outcomes. As it is anticipated that a majority of participants will be taking at least one of these medications, it is necessary to allow for medication withdrawal rather than automatically excluding the potential participants. Participants taking stable doses of medications for medical diseases or psychiatric diagnoses will not be withdrawn from their medications. All withdrawal plans will require the agreement and full cooperation of the participant's primary care physician. If psychiatric medications prescribed by the participant's psychiatrist are being withdrawn, agreement and full cooperation from the psychiatrist will also be obtained. Participants unable to tolerate drug tapering always have the option to remain or restart their medications and withdraw from the study.

13.9 Safeguards for vulnerable populations and sensitive procedures

Urine pregnancy testing will be performed on all persons of pregnancy potential at the time of admission for both Visit 1 and Visit 2. This pregnancy test will be considered valid for the entire period of the inpatient admission. Further, additional pregnancy testing will be performed 24 hours prior to MRI scans performed in the NIH NMR center and prior to the use of ionizing radiation, such as DEXA, and fluoroscopy-guided LP.

Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. 4) Independent consent monitoring will be provided by the NIH HSPU or the Bioethics Department.

To ensure that research staff are adequately trained to respect the privacy and confidentiality of NIH employees and staff, study investigators and staff are required to complete the CITI "Just in Time" Training: Vulnerable Subjects – Workers/Employees.

This study collects sensitive information on drug use, alcohol use, and psychiatric diagnoses. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. Information on drug and alcohol use will be in the participant's NIH medical record.

To ensure that sensitive information collected as part of this protocol remain as private as possible under the law, a Certificate of Confidentiality was obtained from the Department of Health and Human Services (DHHS).

14 Anticipated Benefit: Indirect

This study does not offer direct benefit to participants but is likely to yield generalizable knowledge about ME/CFS.

15 Consent Documents and Process

The following section describes the informed consent process for participating in scientific protocol at NIH. The informed consent process for focus group participants is described fully in Section 4.3.

15.1 Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

Participants who have completed participation (prior to Amendment 11) will be offered the opportunity to complete missing research procedures and undertake optional procedures made available in prior Amendments. These individuals will complete a written re-consenting process with the most recent version of the informed consent form. Persons who have completed participation (prior to approval of Amendment I) will also be offered the option of undergoing whole genome sequencing. These individuals will be contacted by email or telephone to determine if they are interested. Interested participants will be mailed a copy of the whole genome sequencing informed consent form. The individual will be instructed to read the consent form and be ready at the agreed upon date and time to review the consent document with an investigator authorized to obtain consent.
The consenting investigator will document the date and time oral consent was obtained on a consent procedure note. The participant will sign and date the consent form in the presence of a witness. The witness will also sign and date the consent form. The subject will be instructed to mail the consent form back to the site, where the investigator will sign and date the form. The investigator will then send a copy of the form with all signatures to the subject. The original consent document will be placed in the research chart.

15.2 Consent documents

The consent form contains all required elements. One consent form will be used for ME/CFS and HV participants in the main study (ME/CFS, HV Consent Form). A separate consent form will be used for COVID-19 HV participants in the main study (COVID-19 HV Consent Form). Two separate consent forms will be used for participants in Focus Group A and for Focus Group B. A separate consent form will be used for participants in the technical development sub-study (Technical Development Substudy Consent Form). A separate consent for will be used for obtaining consent for whole genome sequencing (Whole Genome Sequencing Consent Form).

16 Data and Safety Monitoring

16.1 Data and safety monitor

Data and safety will be monitored by the Principal investigator in conjunction with an independent medical monitor. The independent medical monitor is Dr. Camilo Toro.

16.2 Data and safety monitoring plan

Study investigators will evaluate the safety of study subjects throughout the conduct of the study and respond to adverse events (AEs) in a timely manner. The PI in collaboration with associate investigators will review any cases associated with serious adverse outcomes and advise on whether or not any changes in the research plan are warranted. Data and safety monitoring will occur annually,

16.3 Criteria for stopping the study or suspending enrollment or procedures

If, in the judgment of the PI and the adjunct PI, a specific study procedure is yielding frequent unexpected or adverse outcomes, that procedure will be suspended until a review can be undertaken in consultation with the IRB. Depending on that consultation, the procedure may be dropped from the protocol via an amendment, or specific language may be added to the protocol and consent forms to reflect the changing risk level.

17 Quality Assurance

17.1 Quality assurance monitor

The NINDS Quality Assurance Audit Committee will monitor the study.

17.2 Quality assurance plan

This protocol will undergo periodic review by the NINDS Quality Assurance (QA) Audit Committee as outlined in the NINDS QA Standard Operating Procedure. The purpose of the QA audit is to assess compliance with applicable regulatory requirements, good clinical practice guidelines, NINDS policy, as well as to provide recommendations for improving the management of clinical research data. The protocol will be audited a minimum of one time a year.

18 Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Reportable events for this protocol will be tracked and reported in compliance with policy 801.

19 Alternatives to Participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate."

20 Privacy

All research activities will be conducted in as private a setting as possible.

21 Confidentiality

21.1 For research data and investigator medical records

Each participant will receive a unique study ID number at the time of enrollment. A master list correlating the unique numbers with personal health information such as names, addresses, and contact information will exist but will only be accessible to investigators on this protocol in order to minimize the risk of compromising confidentiality. These data will be kept in password-protected computers on the NIH campus.

The results of the research tests will not be shared with participants, other than those that are important for the participant's health and well-being. Clinically-relevant information will be shared with the participant and the participant's physician, with the participant's

permission or upon request. Genetic results will be confirmed at NIH expense in CLIA lab prior to returning to participants or providers as detailed in Section 9.35.

21.2 For stored samples

Data and samples will be stored in secured areas. Samples kept at the NIH will be stored in a coded fashion in a locked room in the laboratories of the study investigators.

21.3 Special precautions

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

22 Conflict of Interest

22.1 Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

22.2 Conflict of interest

There are no conflicts-of-interest to report.

23 Technology Transfer

No technology transfer agreements are needed for this study.

24 Research and Travel Compensation

Participants will be compensated for research-related inconveniences. Compensation will be prorated for parts completed if subjects do not complete the study. Payments will be sent after each visit. The schedule for procedure-based payments is below:

Phenotyping Visit

- $10 for physical exam
- $20 for IV line
- $20 for blood draw
- $10 for a urine sample
- $10 for symptom questionnaires (13)
- $10 for the mental health assessment/SCID
- $10 for psychiatric inventories (10)
- $25 for neurocognitive testing
- $25 for occupational therapy evaluation
- $50 for the structural brain MRI scan
- $30 for MRI contrast
- $10 for muscle strength testing
- $10 for activity level monitoring training
- $10 for nutritional assessment

- $10 for saliva collection
- $10 for buccal swab
- $10 for stool sample
- $20 for autonomic testing
- $200 for lumbar puncture
- $2-$8.60 for each Effort for Reward Task (1)
- Immune cell collection:
- (Option A): $110 for cytapheresis (2 pass)
- (Option B): $60 for immune cell blood draw

Exercise Stress Visit

- $10 for physical exam
- $20 for IV line
- $20 for admission blood draw
- $10 for a urine sample
- $10 for saliva collection (2)
- $30 for stool samples
- $10 for admission questionnaires
- $10 for activity level monitoring
- $25 for DEXA
- $50 for exercise testing (CPET)
- $100 for CPET blood draws (4-6)
- $10 for CPET qualitative interviews (4-6)
- $10 for CPET symptom questionnaires (4-6)
- $10 for CPET neurocognitive testing
- $20 for Holter monitor
- $2-$8.60 for each Effort for Reward Task (2)
- $70 for each fMRI scan session (2)
- $200 for lumbar puncture
- $100 for sleep electroencephalograms
- Resting energy expenditure measurements:
- (Option 1): $250 for inpatient stay in metabolic chamber and one ventilated hood measurement
- (Option 2): $40 for ventilated hood measurements (4-6)

Optional Procedures

- $100 for the skin biopsies
- $250 for a muscle biopsy
- $70 for fMRI scan session
- $50 for each transcranial magnetic stimulation session
- $150 for sleep electroencephalograms + Metabolic Chamber + DEXA

Compensation for completing the phenotyping visit for PI-ME/CFS and HV participants is $500. Compensation for completing the phenotyping visit for persons choosing to have cytapheresis will be $610. Compensation for completing the phenotyping visit for persons choosing to have immune cell blood draw will be $560.

Compensation for completing the phenotyping visit for DLI-A participants is $465.

Compensation for completing all procedures during the exercise stress visit will be $915. Participants receiving ventilated hood measurements in lieu of staying in the metabolic chamber will receive $705.

The total compensation for completing the protocol will be $1,415. Maximum compensation for participation in additional procedures will be $1,795. Participants may earn up to an addition $6.00 - $25.80 if they complete the Effort for Reward Task three times.

Compensation for the optional skin biopsies will be $100. Compensation for the optional muscle biopsy will be $250. Compensation for the optional fMRI session will be $70. Compensation for the optional TMS session will be $50. Compensation for the optional sleep electroencephalogram + Metabolic Chamber + DEXA will be $150. Participants undergoing additional immune cell collections will be compensated ($110 for cytaphersis, $60 for large volume blood draw).

Compensation for travel and lodging will be provided for each participant. Travel, lodging and meal vouchers will be provided for a single companion for ME/CFS participants. Only local travel will be compensated for participants in the technical development sub-study.

Focus group participants will not be compensated.

The schedule for payment for participants in the technical development sub-study is described below:

- $10 for each hour of time (up to 8 hours)
- $40 for fMRI scan session
- $40 for transcranial magnetic stimulation session
- $20 for EEG testing
- $10 for questionnaires

The maximum compensation for technical development participants that complete both the TMS and fMRI tasks is $190. The payment for the technical development study was increased since the start of the protocol. Retrospective payments were made to these participants. A letter to this effect is detailed in Appendix 26.9.

NIH employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

25 Attachments/ Appendices

25.1 Schedule of Events

Study Overview: PI-ME/CFS, Healthy Volunteers

Procedures	Pre-Screening	Phenotyping Visit (V1)	Between Visits	Exercise Stress Visit (V2)
Telephone Contact	**X**		**X**	
Obtain Medical Records	**X**			
Contact Participant Primary Physician	**X**		**X**	
Informed Consent		**X**		
History and Physical Exam		**X**		**X**
Intravenous Line		**X**		**X**
Screening Blood Assessment		**X**		**X**
Urine Collection		**X**		**X**
Symptom Assessment		**X**		**X**
Psychological Assessment		**X**		
Neurocognitive Testing		**X**		**X**
Occupational Therapy Evaluation		**Only PI-ME/CFS**		
Clinical Brain MRI		**X**		
Muscle Strength Testing		**X**		
Activity Monitoring/Fatigue Diary		**Instructions Given**	**X**	**X**
Nutritional Assessment		**X**	**X**	**X**
Saliva Sample Collection		**X**		**X**
Buccal Swab Sample Collection		**X**		
Stool Sample Collection		**X**		**X**
Holter Monitoring		**X**		**X**
Lumbar Puncture		**X**		**X**
Autonomic Testing		**Optional**		
Immune Cell Collection		**X**	**Optional**	
Medication Review/Wash-out planning		**X**		
Medication Wash-out Monitoring			**X**	**X**
Clinical Center Consultation		**Optional**		**Optional**
Neuropsychological Measurements		**X**		**X**
Case Adjudication			**X**	
Metabolic Chamber		**Optional with Sleep EEG**	**Optional with Sleep EEG**	**X**
Ventilated Hood Measurements				**X**
Dual Energy X-ray Absorptiometry		**Optional with Sleep EEG**	**Optional with Sleep EEG**	**If not previously completed**
Peak Exercise Testing				**X**
Qualitative Measurement of PEM				**X**
Functional Magnetic Resonance Imaging		**Optional**	**Optional**	**X**
Transcranial Magnetic Stimulation		**Optional**	**Optional**	**Optional**
Effort to Reward Task		**X**		**X**
Sleep EEG		**Optional**	**Optional**	**X**
Skin Biopsy		**Optional**	**Optional**	**Optional**
Muscle Biopsy		**Optional**	**Optional**	**Optional**

Study Overview: COVID-19 Healthy Volunteers

Procedures	Pre-Screening	Phenotyping Visit (V1)
Telephone Contact	**X**	
Obtain Medical Records	**X**	
Contact Participant Primary Physician	**X**	
Informed Consent		**X**
History and Physical Exam		**X**
Intravenous Line		**X**
Screening Blood Assessment		**X**
Urine Collection		**X**
Symptom Assessment		**X**
Psychological Assessment		**X**
Neurocognitive Testing		**X**
Clinical Brain MRI		**X**
Muscle Strength Testing		**X**
Nutritional Assessment		**X**
Saliva Sample Collection		**X**
Buccal Swab Sample Collection		**X**
Stool Sample Collection		**X**
Holter Monitoring		**X**
Lumbar Puncture		**X**
Autonomic Testing		**Optional**
Immune Cell Collection		**X**
Neuropsychological Measurements		**X**
Effort to Reward Task		**X**
Skin Biopsy		**Optional**
Muscle Biopsy		**Optional**
Functional MRI		**Optional**
Transcranial Magnetic Stimulation		**Optional**

Exercise Stress Visit Overview

Procedures	Pre-Exercise	Exercise Day 0	Post-Exercise Days 1-4
Informed Consent Review	X		
History and Physical Exam Update	X		
Intravenous Line	X		
Blood Draw	Optional		
Urine Collection	X	X	X
Saliva Collection	X		X
Stool Sample Collection	X	X	X
Symptom Assessment	X	X	X
Activity Monitoring/Fatigue Diary	X	X	X
Nutritional Assessment	X		
Metabolic Diet	X	X	X
Neurocognitive Testing		X	X
Metabolic Chamber	X	X	X
Ventilated Hood Measurements	X		Optional
Dual Energy X-ray Absorptiometry			If not performed previously
Holter Monitoring	X		X
Peak Exercise Testing		X	
Functional Magnetic Resonance Imaging	X		X
Effort to Reward Task	X		X
Serial Qualitative Interviews of PEM		X	
Serial Blood Draws		X	X
Lumbar Puncture			X
Sleep EEG	X	X	X
Clinical Center Consultation	Optional	Optional	Optional
Skin Biopsy	Optional		Optional
Muscle Biopsy			Optional

25.2 Questionnaires

Attached in PTMS.

25.3 Case Report Forms (CRFs)

Clinical data will be captured using the NIH Clinical Research Information System (CRIS) as is required per NIH policy. We will work with CTDB to develop forms to capture all required research data.

25.4 Focus Group Forms

All materials required to recruit, screen, and perform the Focus Group interviews are available in PTMS.

25.5 Advertisements

Flyers and Public Service Announcements have previously been approved. There are three flyers (ME/CFS, COVID-19 HV, and HV) and three PSAs (ME/CFS, COVID-19 HV, and HV).

25.6 Website Language

The most current version of the website language is attached in PTMS.

25.7 Screening Questionnaires

The protocol's screening questionnaire for Patient Recruitment Office has been previously approved.

25.8 Thank you letters

Thank you letters will be sent to each participant on the completion of their participation. A thank you letter for participating in the main study and the focus groups study are attached.

25.9 Letter for Retroactive Increase in Compensation for Technical Development Substudy

A letter will be sent to prior participants that completed the technical development substudy to inform that they will be receiving additional compensation for their involvement.

25.10 Adjudication Board Members

The list of the current members of the Adjudication Board has been previously approved.

26 Consent Forms

Al consents attached in PTMS:
ME/CFS, HV Consent Form
COVID-19 HV Consent Form
Focus Group Study A Consent Form
Focus Group Study B Consent Form

Technical Developments Consent Form
Genetic Consent Form

27 References

1. AN OUTBREAK of encephalomyelitis in the Royal Free Hospital Group, London, in 1955. *British medical journal.* 1957;2(5050):895-904.
2. Acheson ED. Benign myalgic encephalomyelitis. *Lancet.* 1957;272(6973):834-835.
3. Holmes GP, Kaplan JE, Gantz NM, et al. Chronic fatigue syndrome: a working case definition. *Annals of internal medicine.* 1988;108(3):387-389.
4. Epidemic myalgic encephalomyelitis. *British medical journal.* 1978;1(6125):1436-1437.
5. Wessely S. Old wine in new bottles: neurasthenia and 'ME'. *Psychological medicine.* 1990;20(1):35-53.
6. McEvedy CP, Beard AW. Concept of benign myalgic encephalomyelitis. *British medical journal.* 1970;1(5687):11-15.
7. O'Hern EM. Alice Evans and the brucellosis story. *Annali Sclavo; rivista di microbiologia e di immunologia.* 1977;19(1):12-19.
8. Gilliam AG. Epidemiological study on an epidemic, diagnosed as poliomyelitis, occuring among the personnel of Los Angeles County General Hospital during the summer of 1934. In: Department UST, ed. Vol 240. Washington DC: United States Treasury Department Public Health Service Public Health Bulletin; 1938:1-90.
9. Jones JF, Ray CG, Minnich LL, Hicks MJ, Kibler R, Lucas DO. Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. *Annals of internal medicine.* 1985;102(1):1-7.
10. DeFreitas E, Hilliard B, Cheney PR, et al. Retroviral sequences related to human T-lymphotropic virus type II in patients with chronic fatigue immune dysfunction syndrome. *Proceedings of the National Academy of Sciences of the United States of America.* 1991;88(7):2922-2926.
11. Alberts B. Editorial expression of concern. *Science.* 2011;334(6057):760.
12. Griffin DE. Viral encephalomyelitis. *PLoS pathogens.* 2011;7(3):e1002004.
13. Sacks O. *Awakenings.* New York: HarperPerennial; 1990.
14. Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *Bmj.* 2006;333(7568):575.
15. Hotopf M, Noah N, Wessely S. Chronic fatigue and minor psychiatric morbidity after viral meningitis: a controlled study. *Journal of neurology, neurosurgery, and psychiatry.* 1996;60(5):504-509.
16. Brurberg KG, Fonhus MS, Larun L, Flottorp S, Malterud K. Case definitions for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): a systematic review. *BMJ Open.* 2014;4(2):e003973.
17. Carruthers BM, van de Sande MI, De Meirleir KL, et al. Myalgic encephalomyelitis: International Consensus Criteria. *Journal of internal medicine.* 2011;270(4):327-338.

18. Fark AR. Infectious mononucleosis, Epstein-Barr virus, and chronic fatigue syndrome: a prospective case series. *The Journal of family practice.* 1991;32(2):202, 205-206, 209.
19. Cameron B, Bharadwaj M, Burrows J, et al. Prolonged illness after infectious mononucleosis is associated with altered immunity but not with increased viral load. *J Infect Dis.* 2006;193(5):664-671.
20. White PD, Thomas JM, Amess J, et al. Incidence, risk and prognosis of acute and chronic fatigue syndromes and psychiatric disorders after glandular fever. *The British journal of psychiatry : the journal of mental science.* 1998;173:475-481.
21. Kawai K, Kawai A. Studies on the relationship between chronic fatigue syndrome and Epstein-Barr virus in Japan. *Internal medicine.* 1992;31(3):313-318.
22. Lerner AM, Beqaj SH, Deeter RG, Fitzgerald JT. IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome. *In vivo.* 2004;18(2):101-106.
23. Loebel M, Strohschein K, Giannini C, et al. Deficient EBV-specific B- and T-cell response in patients with chronic fatigue syndrome. *PloS one.* 2014;9(1):e85387.
24. Manian FA. Simultaneous measurement of antibodies to Epstein-Barr virus, human herpesvirus 6, herpes simplex virus types 1 and 2, and 14 enteroviruses in chronic fatigue syndrome: is there evidence of activation of a nonspecific polyclonal immune response? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 1994;19(3):448-453.
25. Sairenji T, Yamanishi K, Tachibana Y, Bertoni G, Kurata T. Antibody responses to Epstein-Barr virus, human herpesvirus 6 and human herpesvirus 7 in patients with chronic fatigue syndrome. *Intervirology.* 1995;38(5):269-273.
26. Koelle DM, Barcy S, Huang ML, et al. Markers of viral infection in monozygotic twins discordant for chronic fatigue syndrome. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2002;35(5):518-525.
27. Fremont M, Metzger K, Rady H, Hulstaert J, De Meirleir K. Detection of herpesviruses and parvovirus B19 in gastric and intestinal mucosa of chronic fatigue syndrome patients. *In vivo.* 2009;23(2):209-213.
28. Hellinger WC, Smith TF, Van Scoy RE, Spitzer PG, Forgacs P, Edson RS. Chronic fatigue syndrome and the diagnostic utility of antibody to Epstein-Barr virus early antigen. *Jama.* 1988;260(7):971-973.
29. Mawle AC, Nisenbaum R, Dobbins JG, et al. Seroepidemiology of chronic fatigue syndrome: a case-control study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 1995;21(6):1386-1389.
30. Swanink CM, van der Meer JW, Vercoulen JH, Bleijenberg G, Fennis JF, Galama JM. Epstein-Barr virus (EBV) and the chronic fatigue syndrome: normal virus load in blood and normal immunologic reactivity in the EBV regression assay. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 1995;20(5):1390-1392.
31. Jason LA, Katz BZ, Shiraishi Y, Mears CJ, Im Y, Taylor R. Predictors of Post-Infectious Chronic Fatigue Syndrome in Adolescents. *Health psychology and behavioral medicine.* 2014;2(1):41-51.

32. Montoya JG, Kogelnik AM, Bhangoo M, et al. Randomized clinical trial to evaluate the efficacy and safety of valganciclovir in a subset of patients with chronic fatigue syndrome. *Journal of medical virology.* 2013;85(12):2101-2109.
33. Lerner AMB, S; Fitzgerald, J.T.; Gill, K; Gill, C; Edington J. Subset-directed antiviral treatment of 142 herpesvirus patients with chronic fatigue syndrome. *Virus Adaptation and Treatment.* 2010;2:47-57.
34. Diaz-Mitoma FTEKALWLLHBM. Clinical Improvement in Chronic Fatigue Syndrome Is Associated with Enhanced Natural Killer Cell-Mediated Cytotoxicity: The Results of a Pilot Study with Isoprinosine®. *Journal of Chronic Fatigue Syndrome.* 2003;11(2).
35. Straus SE, Dale JK, Tobi M, et al. Acyclovir treatment of the chronic fatigue syndrome. Lack of efficacy in a placebo-controlled trial. *The New England journal of medicine.* 1988;319(26):1692-1698.
36. Stringer EA, Baker KS, Carroll IR, et al. Daily cytokine fluctuations, driven by leptin, are associated with fatigue severity in chronic fatigue syndrome: evidence of inflammatory pathology. *Journal of translational medicine.* 2013;11:93.
37. Vernon SD, Whistler T, Cameron B, Hickie IB, Reeves WC, Lloyd A. Preliminary evidence of mitochondrial dysfunction associated with post-infective fatigue after acute infection with Epstein Barr virus. *BMC infectious diseases.* 2006;6:15.
38. Fluge O, Bruland O, Risa K, et al. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. *PloS one.* 2011;6(10):e26358.
39. Irwin MR. Inflammation at the intersection of behavior and somatic symptoms. *The Psychiatric clinics of North America.* 2011;34(3):605-620.
40. Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. *Nature reviews Immunology.* 2011;11(9):625-632.
41. Solomon L, Reeves WC. Factors influencing the diagnosis of chronic fatigue syndrome. *Archives of internal medicine.* 2004;164(20):2241-2245.
42. Peterson TM, T. W. Peterson, S. Emerson, E. Regalbuto, M. A. Evans, L. A. Jason. Coverage of CFS within U.S. medical schools. *Universal Journal of Public Health.* 2013;1(4):177-179.
43. Jason LA, Paavola E, Porter N, Morello ML. Frequency and content analysis of chronic fatigue syndrome in medical text books. *Aust J Prim Health.* 2010;16(2):174-178.
44. Jason LA, J. A. Richman. How science can stigmatize: The case of chronic fatigue syndrome. *Journal of Chronic Fatigue Syndrome.* 2008;14(4):85-103.
45. Brimmer DJ, Fridinger F, Lin JM, Reeves WC. U.S. healthcare providers' knowledge, attitudes, beliefs, and perceptions concerning Chronic Fatigue Syndrome. *BMC family practice.* 2010;11:28.
46. Fossey M, Libman E, Bailes S, et al. Sleep quality and psychological adjustment in chronic fatigue syndrome. *J Behav Med.* 2004;27(6):581-605.
47. Bayliss K, Goodall M, Chisholm A, et al. Overcoming the barriers to the diagnosis and management of chronic fatigue syndrome/ME in primary care: a meta synthesis of qualitative studies. *BMC family practice.* 2014;15:44.

48. Anderson JS, Ferrans CE. The quality of life of persons with chronic fatigue syndrome. *J Nerv Ment Dis.* 1997;185(6):359-367.
49. Twemlow SW, Bradshaw SL, Jr., Coyne L, Lerma BH. Patterns of utilization of medical care and perceptions of the relationship between doctor and patient with chronic illness including chronic fatigue syndrome. *Psychol Rep.* 1997;80(2):643-658.
50. Marshall R, Paul L, Wood L. The search for pain relief in people with chronic fatigue syndrome: a descriptive study. *Physiother Theory Pract.* 2011;27(5):373-383.
51. Jason LA, Benton MC, Valentine L, Johnson A, Torres-Harding S. The economic impact of ME/CFS: individual and societal costs. *Dynamic medicine : DM.* 2008;7:6.
52. Reynolds KJ, Vernon SD, Bouchery E, Reeves WC. The economic impact of chronic fatigue syndrome. *Cost effectiveness and resource allocation : C/E.* 2004;2(1):4.
53. Andersen MM, Permin H, Albrecht F. Illness and disability in Danish Chronic Fatigue Syndrome patients at diagnosis and 5-year follow-up. *J Psychosom Res.* 2004;56(2):217-229.
54. Tiersky LA, DeLuca J, Hill N, et al. Longitudinal assessment of neuropsychological functioning, psychiatric status, functional disability and employment status in chronic fatigue syndrome. *Appl Neuropsychol.* 2001;8(1):41-50.
55. Twisk FN. Underperformance of myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) patients at neurocognitive tests should be assessed objectively without an a priori judgment about the etiology. *J Psychosom Res.* 2014;76(4):339.
56. Wiborg JF, van der Werf S, Prins JB, Bleijenberg G. Being homebound with chronic fatigue syndrome: A multidimensional comparison with outpatients. *Psychiatry Res.* 2010;177(1-2):246-249.
57. Wiborg JF, Knoop H, Stulemeijer M, Prins JB, Bleijenberg G. How does cognitive behaviour therapy reduce fatigue in patients with chronic fatigue syndrome? The role of physical activity. *Psychological medicine.* 2010;40(8):1281-1287.
58. Jason LA, Corradi K, Gress S, Williams S, Torres-Harding S. Causes of death among patients with chronic fatigue syndrome. *Health Care Women Int.* 2006;27(7):615-626.
59. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2006;43(9):1089-1134.
60. Koedel U, Fingerle V, Pfister HW. Lyme neuroborreliosis-epidemiology, diagnosis and management. *Nature reviews Neurology.* 2015;11(8):446-456.
61. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study.

International Chronic Fatigue Syndrome Study Group. *Annals of internal medicine.* 1994;121(12):953-959.
62. Carruthers BMJAKD, K.L.; Peterson, D.L.; Klimas, N.G.; Lerner, A.M.; Bested, A.C.; Flor-Henry, P.; Joshi, P; Peter Powels, A.C.; Sherkey, J.A.; van de Sande, M.I. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Clinical Working Case Definition, Diagnostic and Treatment Protocols. *Journal of Chronic Fatigue Syndrome.* 2003;11(1):7-96.
63. Clayton EW. Beyond myalgic encephalomyelitis/chronic fatigue syndrome: an IOM report on redefining an illness. *Jama.* 2015;313(11):1101-1102.
64. Keech A, Sandler CX, Vollmer-Conna U, Cvejic E, Lloyd AR, Barry BK. Capturing the post-exertional exacerbation of fatigue following physical and cognitive challenge in patients with chronic fatigue syndrome. *J Psychosom Res.* 2015;79(6):537-549.
65. Vaismoradi M, Turunen H, Bondas T. Content analysis and thematic analysis: Implications for conducting a qualitative descriptive study. *Nurs Health Sci.* 2013;15(3):398-405.
66. Braun V, Clarke V. What can "thematic analysis" offer health and wellbeing researchers? *Int J Qual Stud Health Well-being.* 2014;9:26152.
67. Medicine) IIo. *Beyond myalgic encephalomyelitis/chronic fatigue syndrome: Redfining an illness.* Washington, D.C. 2015.
68. Ware JE, Jr., Kosinski M, Bayliss MS, McHorney CA, Rogers WH, Raczek A. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: summary of results from the Medical Outcomes Study. *Medical care.* 1995;33(4 Suppl):AS264-279.
69. Wagner D, Nisenbaum R, Heim C, Jones JF, Unger ER, Reeves WC. Psychometric properties of the CDC Symptom Inventory for assessment of chronic fatigue syndrome. *Population health metrics.* 2005;3:8.
70. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* 1995;39(3):315-325.
71. Lin JM, Brimmer DJ, Maloney EM, Nyarko E, Belue R, Reeves WC. Further validation of the Multidimensional Fatigue Inventory in a US adult population sample. *Population health metrics.* 2009;7:18.
72. Hays RD, Bjorner JB, Revicki DA, Spritzer KL, Cella D. Development of physical and mental health summary scores from the patient-reported outcomes measurement information system (PROMIS) global items. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation.* 2009;18(7):873-880.
73. Saligan LN, Luckenbaugh DA, Slonena EE, Machado-Vieira R, Zarate CA, Jr. Development of a clinician-administered National Institutes of Health-Brief Fatigue Inventory: A measure of fatigue in the context of depressive disorders. *Journal of psychiatric research.* 2015;68:99-105.
74. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. *Pain.* 1975;1(3):277-299.

75. Galer BS, Jensen MP. Development and preliminary validation of a pain measure specific to neuropathic pain: the Neuropathic Pain Scale. *Neurology.* 1997;48(2):332-338.
76. Wolfe F, Clauw DJ, Fitzcharles MA, et al. Fibromyalgia criteria and severity scales for clinical and epidemiological studies: a modification of the ACR Preliminary Diagnostic Criteria for Fibromyalgia. *The Journal of rheumatology.* 2011;38(6):1113-1122.
77. Kroenke K, Spitzer RL, Williams JB. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. *Psychosomatic medicine.* 2002;64(2):258-266.
78. Buysse DJRCFMTHB, S.R.; Kupfer, D.J. The Pittsburgh Sleep Quality Indez: A New Instrument for Psychiatric Practice and Research. *Psychiatry Res.* 1988;28:193-213.
79. Jacobsen PB, Hann DM, Azzarello LM, Horton J, Balducci L, Lyman GH. Fatigue in women receiving adjuvant chemotherapy for breast cancer: characteristics, course, and correlates. *Journal of pain and symptom management.* 1999;18(4):233-242.
80. Seidenberg M, Haltiner A, Taylor MA, Hermann BB, Wyler A. Development and validation of a Multiple Ability Self-Report Questionnaire. *J Clin Exp Neuropsychol.* 1994;16(1):93-104.
81. Rimes KA, Chalder T. The Beliefs about Emotions Scale: validity, reliability and sensitivity to change. *J Psychosom Res.* 2010;68(3):285-292.
82. Bernstein DP, Stein JA, Newcomb MD, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child abuse & neglect.* 2003;27(2):169-190.
83. Foa EB, Cashman L, Jaycox L, Perry K. The Validation of a Self-Report Measure of Posttraumatic Stress Disorder: The Posttraumatic Diagnostic Scale. *Psychological Assessment* 1997;9(4):445 - 445`.
84. Bernstein DF, L. *Childhood Trauma Questionnaire: A retrospective self-report manual.* San Antonio, TX: The Psychological Corporation; 1998.
85. Kooiman CG, Ouwehand AW, ter Kuile MM. The Sexual and Physical Abuse Questionnaire (SPAQ). A screening instrument for adults to assess past and current experiences of abuse. *Child abuse & neglect.* 2002;26(9):939-953.
86. Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *Journal of personality assessment.* 1996;67(3):588-597.
87. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *Journal of consulting and clinical psychology.* 1988;56(6):893-897.
88. McCullough ME, Emmons RA, Tsang JA. The grateful disposition: a conceptual and empirical topography. *Journal of personality and social psychology.* 2002;82(1):112-127.
89. Corporation TP. *Wechsler Test of Adult Reading Manual.* San Antonio, TX2001.
90. Wechsler D. *Wechsler Adult Intelligence Scale-III.* San Antonio, TX: The Psychological Corporation; 1997.

91. Greenberg L, Kindschi, C., Dupuy, T., & Corman, C. . *Test of Variables of Attention: Clinical guide.* Los Alamitos, CA: Universal Attention Disorders; 1996.
92. Brandt J, & Benedict, R. H. B. . *Hopkins Verbal Learning Test-Revised.* Odessa, Fla: PAR; 2001.
93. Benedict RHB. *Brief Visuospatial Memory Test-Revised: Professional Manual.* Lutz, FL: Psychological Assessment Resource; 1997.
94. Kongs SK, Thompson, L. L., Iverson, G. L., & Heaton, R. K. *Wisconsin Card Sorting Test - 64 Card Computerized Version.* Odessa, FL: Psychological Assessment Resources; 2000.
95. Benton AL, & Hamsher, K. D. . *Multilingual Aphasia Examination.* Iowa City: AJA Associates; 1989.
96. Gronwall DMA. Paced auditory serial-addition task: A measure of recovery from concussion. *Perceptual and Motor Skills.* 1977;44:367-373.
97. Kløve H. Clinical neuropsychology. . In: Forster FM, ed. *The medical clinics of North America.* New York: Saunders.
98. Green P. *Word memory test for windows. User's manual and program (Revised 2005).* . Edmonton, Alberta: Green Publishing; 2005.
99. Boone KB LP, Sherman D, Palmer B, Back C, Shamieh E, Warner-Chacon K, Berman NG. . Validation of a new technique to detect malingering of cognitive symptoms: the b Test. *Archives of Clinical Neuropsychology.* 2000;15(3):227-241.
100. Boone KB LP, Back C, King C, Lee A, Philpott L, Shamieh E, Warner-Chacon K. . Sensitivity and specificity of the Rey Dot Counting Test in patients with suspect effort and various clinical samples. *Archives of Clinical Neuropsychologist.* 2002;17(7):625-642.
101. Ben-Porath YS, & Tellegen, A. *MMPI-2-RF (Minnesota Multiphasic Personality Inventory-2 Restructured Form): Manual for administration, scoring, and interpretation.* Minneapolis: University of Minnesota Press; 2008.
102. Baum CME, D. *Activity Card Sort, 2nd Edition.* AOTA Press; 2008.
103. O'Shea SD, Taylor NF, Paratz JD. Measuring muscle strength for people with chronic obstructive pulmonary disease: retest reliability of hand-held dynamometry. *Archives of physical medicine and rehabilitation.* 2007;88(1):32-36.
104. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Annals of the Academy of Medicine, Singapore.* 1994;23(2):129-138.
105. Van Dam NT, Earleywine M. Validation of the Center for Epidemiologic Studies Depression Scale--Revised (CESD-R): pragmatic depression assessment in the general population. *Psychiatry Res.* 2011;186(1):128-132.
106. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr.* 1990;51(2):241-247.
107. Fletcher GF, Ades PA, Kligfield P, et al. Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation.* 2013;128(8):873-934.

108. VanNess JM, Stevens SR, Bateman L, Stiles TL, Snell CR. Postexertional malaise in women with chronic fatigue syndrome. *Journal of women's health.* 2010;19(2):239-244.
109. Treadway MT, Buckholtz JW, Schwartzman AN, Lambert WE, Zald DH. Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PloS one.* 2009;4(8):e6598.
110. Chapman LJ, Chapman JP, Raulin ML. Scales for physical and social anhedonia. *Journal of abnormal psychology.* 1976;85(4):374-382.
111. Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr Clin Neurophysiol.* 1998;108(1):1-16.
112. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMSCG. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol.* 2009;120(12):2008-2039.
113. Lerner AJ, Wassermann EM, Tamir DI. Seizures from transcranial magnetic stimulation 2012-2016: Results of a survey of active laboratories and clinics. *Clin Neurophysiol.* 2019;130(8):1409-1416.
114. Desmond JE, Glover GH. Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *Journal of neuroscience methods.* 2002;118(2):115-128.
115. Gallagher AM, Thomas JM, Hamilton WT, White PD. Incidence of fatigue symptoms and diagnoses presenting in UK primary care from 1990 to 2001. *Journal of the Royal Society of Medicine.* 2004;97(12):571-575.
116.
1. Strawbridge R, Sartor ML, Scott F, Cleare AJ. Inflammatory proteins are altered in chronic fatigue syndrome-A systematic review and meta-analysis. *Neurosci Biobehav Rev.* 2019;107:69-83.
2. Milivojevic M, Che X, Bateman L, et al. Plasma proteomic profiling suggests an association between antigen driven clonal B cell expansion and ME/CFS. *PloS one.* 2020;15(7):e0236148.
3. Mandarano AH, Maya J, Giloteaux L, et al. Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations. *J Clin Invest.* 2020;130(3):1491-1505.
4. Nath A. Long-Haul COVID. *Neurology.* 2020.
5. Couzin-Frankel J. The long haul. *Science.* 2020;369(6504):614-617.
6. Lambert NJSC. COVID-19 “Long Hauler” Symptoms Survey Report. 2020.
117.