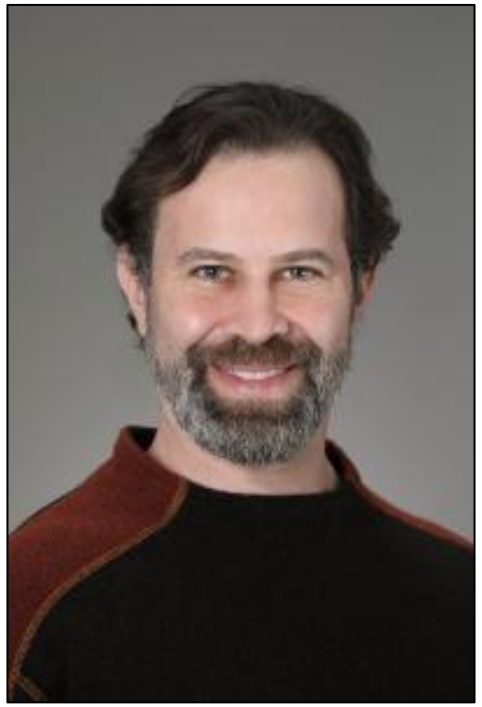
# Deep Phenotyping of Post-Infectious Myalgic Encephalomyelitis/Chronic Fatigue Syndrome



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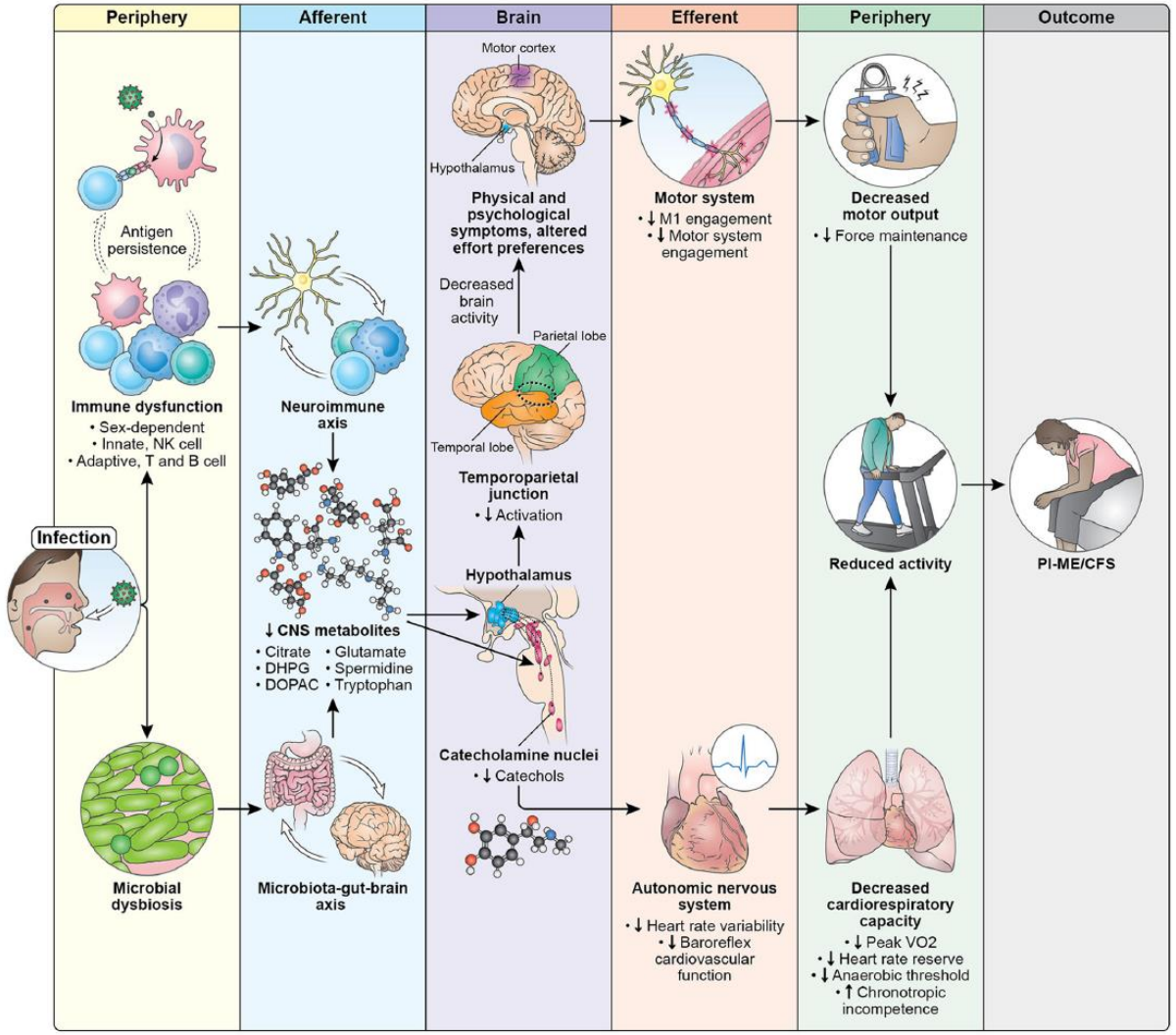


National Institute of Neurological Disorders and Stroke

# Insights into the Mechanisms of Post-Infectious ME/CFS

Brian Walitt MD MPH

National Institute of Neurological Disorders and Stroke

I have no conflicts of interest

The Washington Post

To Your Health

# NIH announces new effort to tackle chronic fatigue syndrome

By Lenny Bernstein October 29, 2015

# Protocol 16-N-0058: Post-Infectious –Myalgic Encephalomyelopathy/Chronic Fatigue Syndrome (PI-ME/CFS) at the National Institutes of Health

Overall Hypothesis: PI-ME/CFS is triggered by an infectious illness that results in immune mediated brain dysfunction

Study Aim

To conduct a cross sectional study for deep phenotyping of PI-ME/CFS to define its pathophysiology

Article | Open access | Published: 21 February 2024

# Deep phenotyping of post-infectious myalgic encephalomyelitis/chronic fatigue syndrome

Brian Walitt, Komudi Singh, Samuel R. LaMunion, Mark Hallett, Steve Jacobson, Kong Chen, Yoshimi Enose-Akahata, Richard Apps, Jennifer J. Barb, Patrick Bedard, Robert J. Brychta, Ashura Williams Buckley, Peter D. Burbelo, Brice Calco, Brianna Cathay, Li Chen, Snigdha Chigurupati, Jinguo Chen, Foo Cheung, Lisa M. K. Chin, Benjamin W. Coleman, Amber B. Courville, Madeleine S. Deming, Bart Drinkard, Li Rebekah Feng, Luigi Ferrucci, Scott A. Gabel, Angelique Gavin, David S. Goldstein, Shahin Hassanzadeh, Sean C. Horan, Silvina G. Horovitz, Kory R. Johnson, Anita Jones Govan, Kristine M. Knutson, Joy D. Kreskow, Mark Levin, Jonathan J. Lyons, Nicholas Madian, Nasir Malik, Andrew L. Mammen, John A. McCulloch, Patrick M. McGurrin, Joshua D. Milner, Ruin Moaddel, Geoffrey A. Mueller, Amrita Mukherjee, Sandra Muñoz-Braceras, Gina Norato, Katherine Pak, Iago Pinal-Fernandez, Traian Popa, Lauren B. Reoma, Michael N. Sack, Farinaz Safavi, Leorey N. Saligan, Brian A. Sellers, Stephen Sinclair, Bryan Smith, Joseph Snow, Stacey Solin, Barbara J. Stussman, Giorgio Trinchieri, Sara A. Turner, C. Stephenie Vetter, Felipe Vial, Carlotta Vizioli, Ashley Williams, Shanna B. Yang, Center for Human Immunology, Autoimmunity, and Inflammation (CHI) Consortium & Avindra Nath ✉ — Show fewer authors

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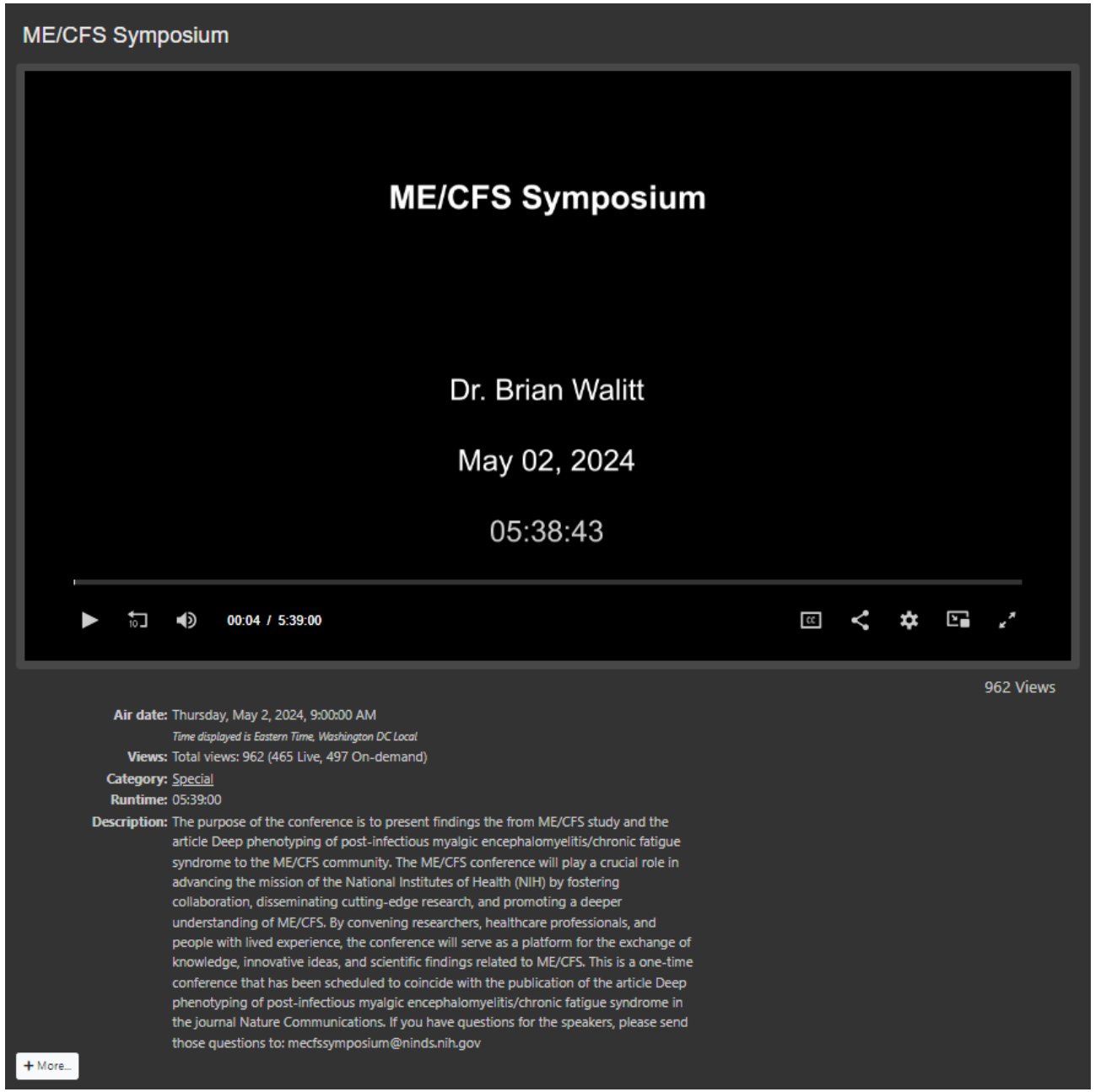



https://videocast.nih.gov/watch=54675

# Study Design

## Deep Phenotyping Visit:

- Detailed clinical evaluation
- Collection of physiological measures
- Collection of biological samples

Case Adjudication

## Exercise Stress Visit:

- Cardiopulmonary Exercise Stress Test
- Serial collection of physiological measures (72 hours)
- Serial collection of biological samples (72 hours)

# Deep Phenotyping Measurements

- History and Physical Examination
  - NINDS (Walitt)
  - CC (Solin/Deming)
- Neurological Examination
  - NINDS (Smith/Reoma/Nath)
- Neuropsychological Assessment:
  - NIMH: SCID-5 (Sinclair)
  - NIMH: Neurocognitive Testing (Snow/Tierney/Madian)
- Patient Reported Outcome Measures
  - NINDS: Questionnaires (Walitt, Calco, Chigurupati, Coleman, Horan, Vetter, Williams)
  - NINR: Symptom Interviews (Walitt, Kreskow)
  - NCCIH: Post-exertional malaise qualitative interviews (Stussman/Gavin)
- Dietary Evaluation:
  - CC Nutrition (Yang/Courville/Turner)
- Neuroimaging
  - CC Radiology: Contrast MRI (Butman)
- Body Composition
  - NIDDK: Dual-energy Xray absorptiometry (Chen/Brychta/Lamunion)
- Sleep
  - NIMH: Polysomnography (Buckley)
- Neurophysiology
  - Transcranial magnetic stimulation (Hallett/Horovitz/Bedard/Popa/McGurrin)
  - Functional magnetic resonance imaging (Hallett/Horovitz/Bedard/Popa/Knutson)
- Autonomic Testing
  - NINDS: Provocative Tilt Table Testing (Goldstein)
  - NHLBI: Heart Rate Variability (Levin/Cathay)

- Blood
  - DTM: Clinical laboratory testing
  - CHI: Proteomics (Apps, Chen, Cheung, Mukherjee, Sellers)
  - NIA: Lipidomics, Cellular Senescence (Ferruci, Moaddel)
  - NIDCR: Autoantibody testing (Burbelo)
  - NK cell function (Cincinnati Children's Hospital)
- Peripheral Blood Mononuclear Cells:
  - NINDS: Neuronal toxicity models (Malik)
  - NINR: Mitochondrial Function (Saligan/Feng)
  - Transcriptomics: (Sack/Hassanzadeh/Singh)
- Cerebrospinal Fluid
  - NINDS: Flow cytometry (Jacobson, Akahata)
  - CHI: Proteomics (Apps, Chen, Cheung, Mukherjee, Sellers)
  - NINDS: Catecholamines (Goldstein)
  - Metabolomics: (Metabolon)
- Stool Measurements
  - NCI: Microbiome (McCulloch, Trincheri), NINR (Vizioli), CC (Barb)
  - NIEHS: Metabolomics (Mueller, Gabel)
- Skin Biopsy
  - JHU: Small fiber and sympathetic fiber density (Polydefkis)
- Muscle Biopsy
  - NIAMS: Pathology (Mammen, Pak, Munoz-Braceras)
  - NINDS: Transcriptomics (Mammen, Pinal-Fernandez)
  - Mitochondrial genetics (GeneDx)
  - NHLBI: Endoplasmic Reticular Stress (Hwang/Wang)

# Case Adjudication

- Briefings summarized participant's history, physical exam, and medical testing performed at NIH
  - Each participant reviewed their history to ensure accuracy
- Convened a panel of clinical experts in ME/CFS to independently review briefings
  - Iterative process
  - Decisions made independently
- Unanimous agreement that a participant has PI-ME/CFS required to be included in the study analyses
  - Met as a panel to discuss disagreements

Thanks to our adjudicators: Lucinda Bateman, Andy Kogolnik, Anthony Komaroff, Benjamin Natelson, Daniel Peterson, and Avindra Nath

# Exercise Stress Measurements

Cardiopulmonary Exercise Test:

- Clinical Center: Rehabilitation

72 hours of serial measurements:

- NIDDK: Metabolic Chamber with Metabolic Diet
- NCCIH: Qualitative Interview
- NINDS: Patient Reported Outcome Questionnaires
- CHI: Blood
- NCI: Stool
- NINDS: Saliva

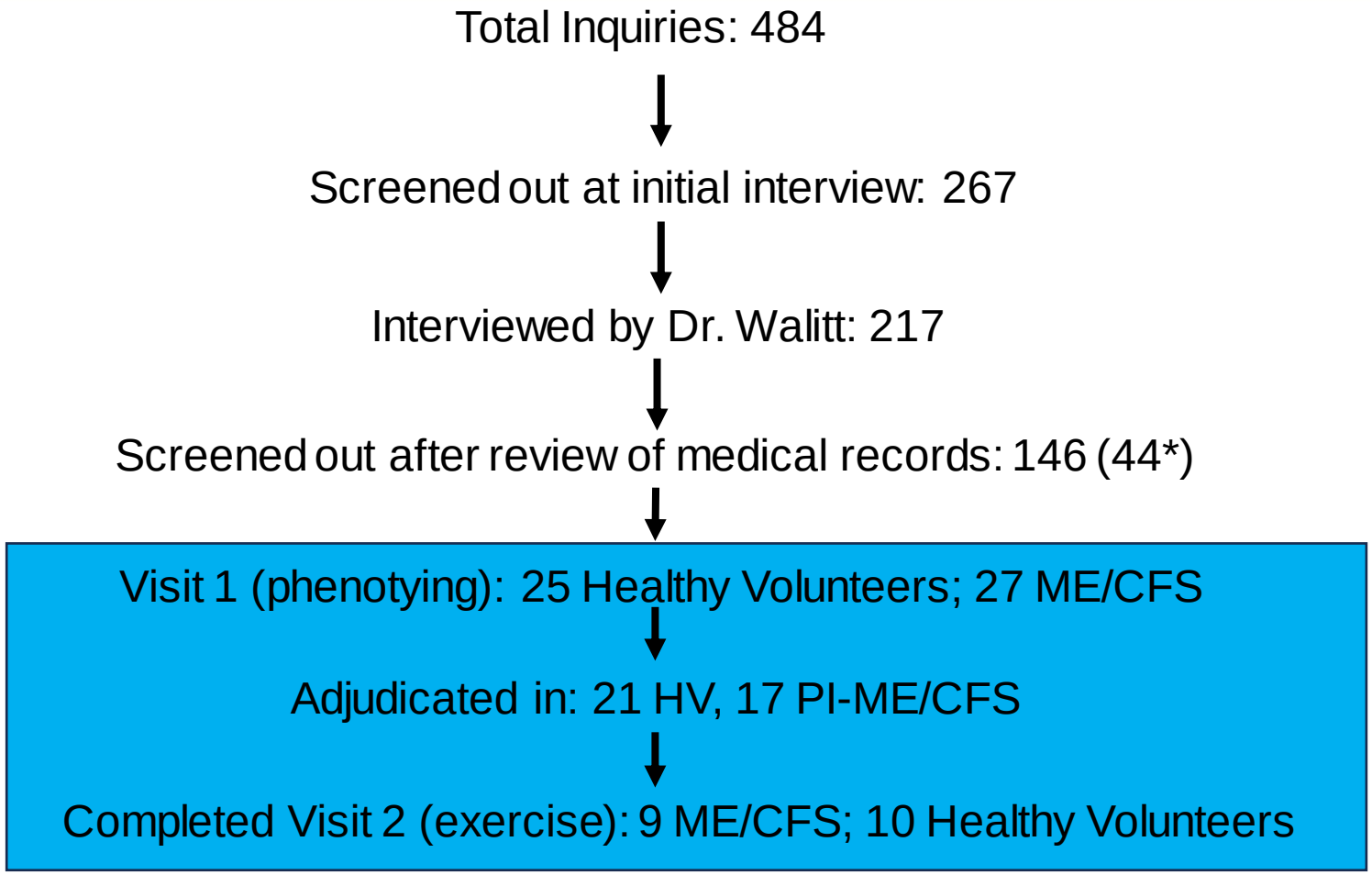
Lumbar Puncture (at 48 hours)

Baseline/Post-Exercise:

- NIMH: Neurocognitive Testing
- NINDS: functional MRI:
  - Muscle fatigue task
  - Cognitive fatigue task
  - Voxel-Based Morphometry, Default Mode Network, Diffuse Tensor Imaging
- NINDS:Transcranial Magnetic Stimulation
  - Evoke motor potentials for extensor carpi radialis and record motor excitatory potential (MEP) amplitudes

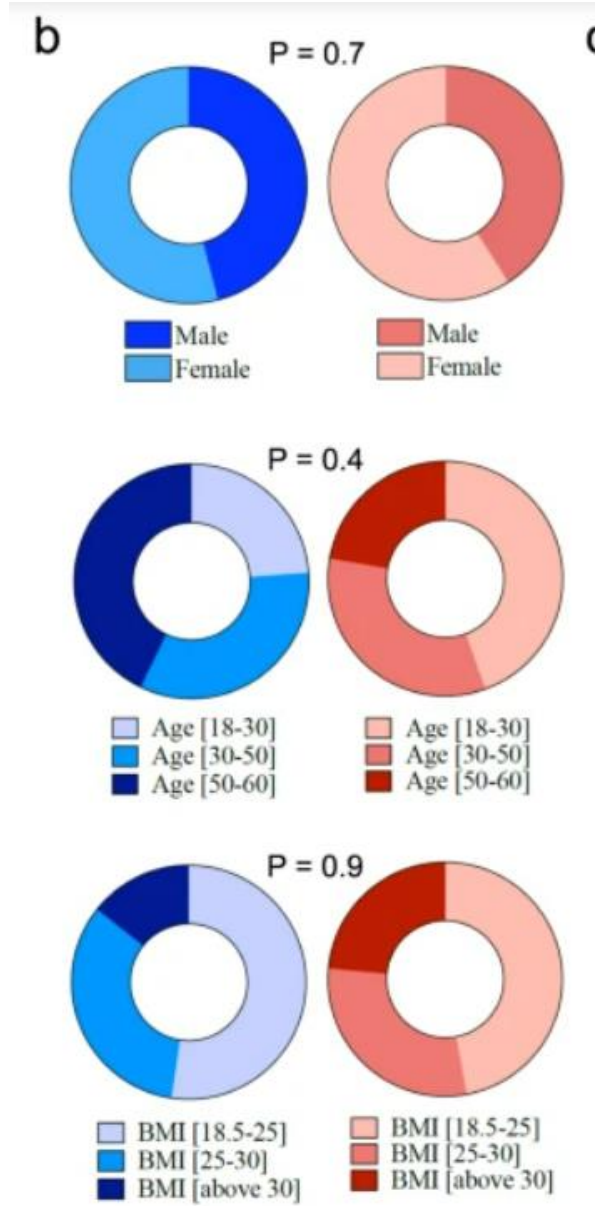
New study: Patients push limits for clues to chronic fatigue syndrome - Chicago Sun-Times (suntimes.com)

# Recruitment



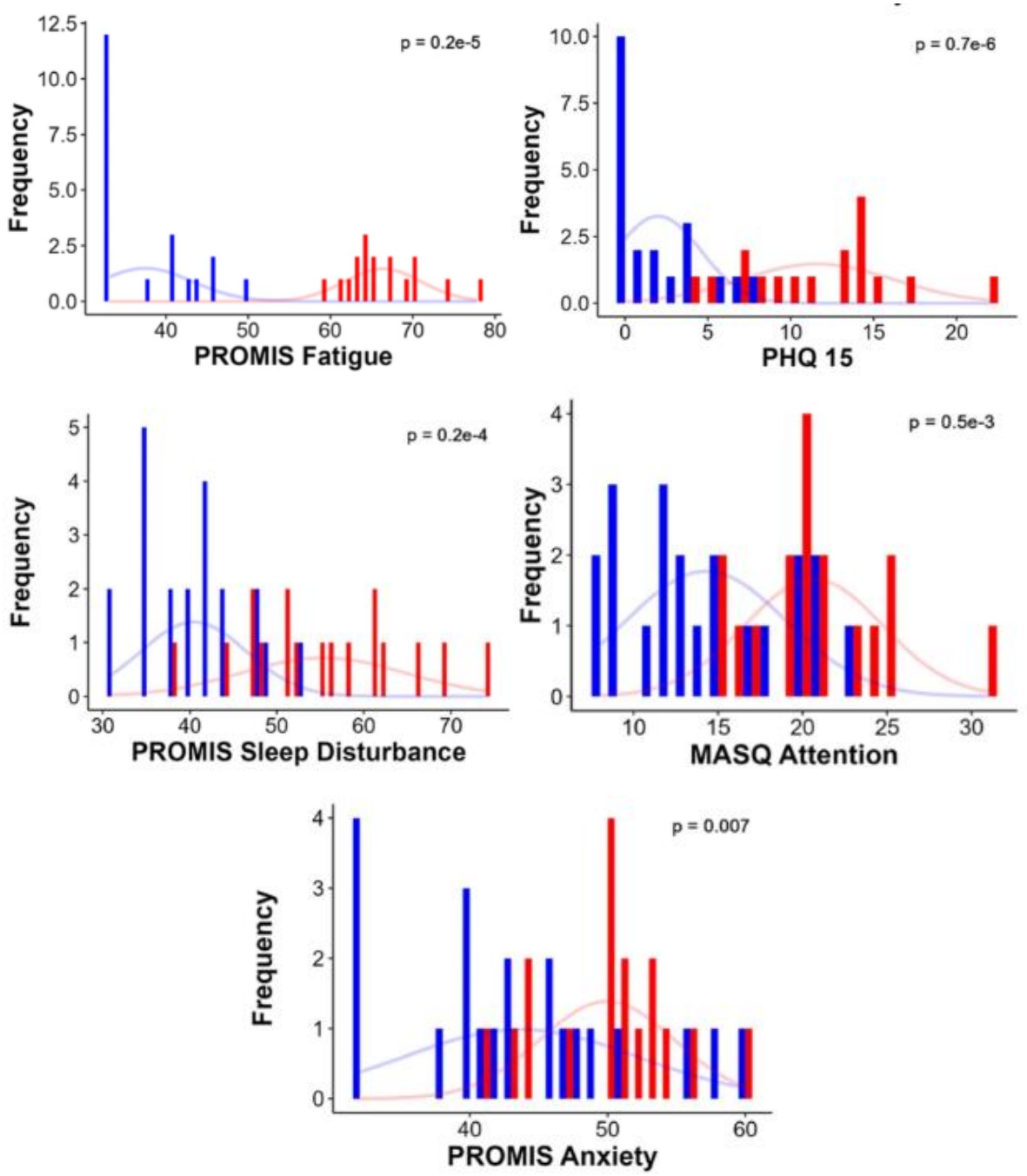
# Demographics

| Characteristic | | HV (n=21) | PI-ME/CFS (n=17) |
|---|---|---|---|
| Age | [mean (SD)] | 42.2 (13.5) | 37.8 (14.7) |
| Sex | [% males] | 48% | 41% |
| Race | [% white] | 86% | 88% |
| | [% asian] | 0% | 6% |
| | [% multiracial] | 14% | 6% |
| Ethnicity | [% Hispanic] | 5% | 12% |
| Education | [% high school or less] | 10% | 0% |
| | [% some college or college graduate] | 52% | 59% |
| | [% advanced degree] | 38% | 41% |
| BMI | [mean (SD)] | 25.8 (3.4) | 25.9 (5.3) |
| Work/School disability | [%] | 0% | 76% |
| Elapsed time since infection | [# months (SD)] | 0 months | 33 (15) months |
| Met 2015 Institute of Medicine Criteria | [%] | 0% | 100% |
| Met 1994 Fukuda Criteria | [%] | 0% | 82% |
| Met 2003 Canadian Consensus Criteria | [%] | 0% | 53% |

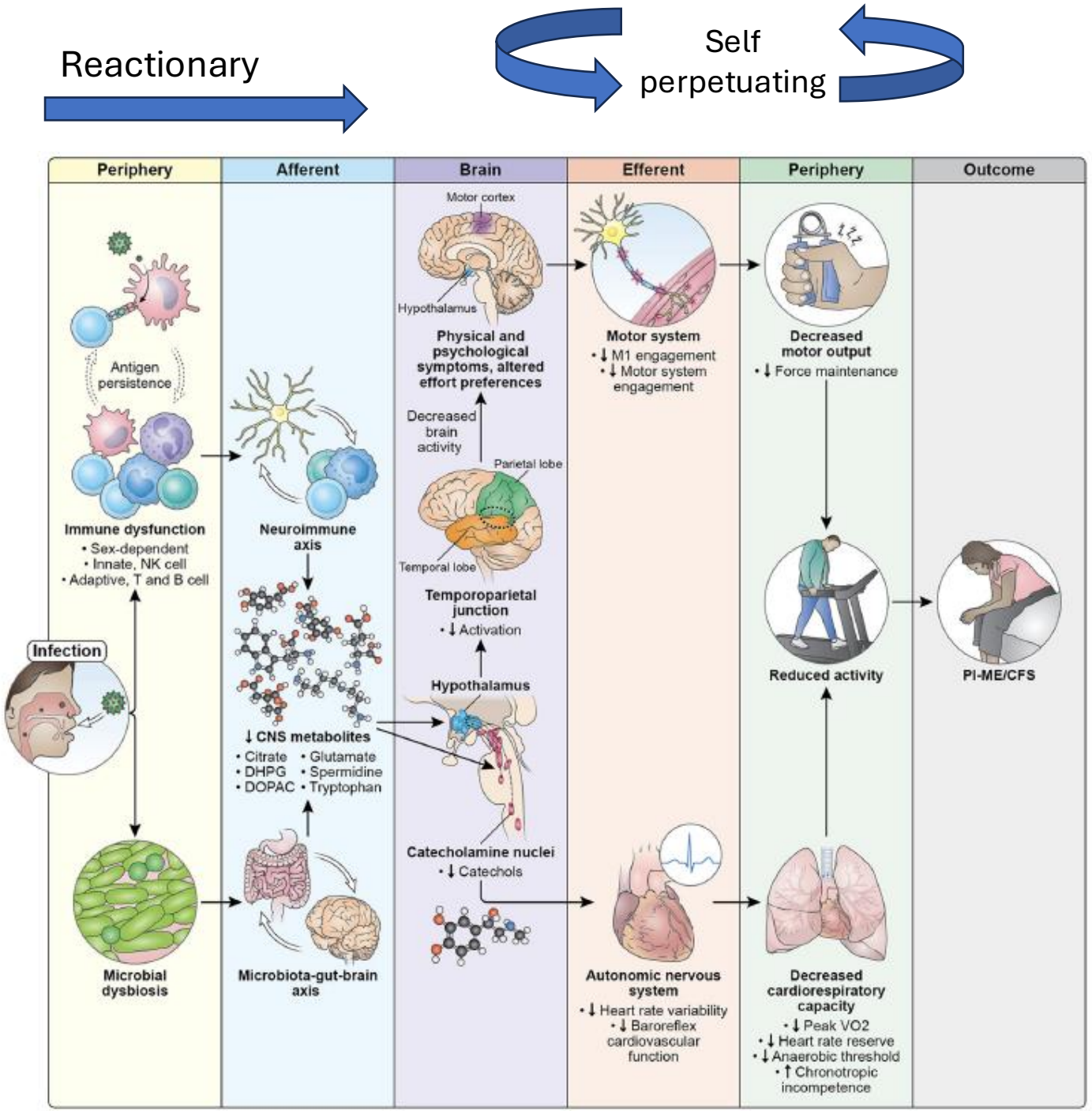


# Symptom Reporting

| Patient Reported Outcome Measure | HV: Mean | PI-ME/CFS: Mean | p-value |
|---|---|---|---|
| SF-36 Physical Component Score | 56.7 | 23.5 | 6.95E-11 |
| SF-36 Mental Component Score | 54.8 | 49.1 | 0.003 |
| MFI-20: Total | 30.0 | 77.3 | 1.71E-07 |
| MFI-20: Reduced Activity | 6.0 | 16.4 | 2.12E-07 |
| MFI-20: General Fatigue | 6.3 | 18.6 | 1.40E-07 |
| MFI-20: Physical Fatigue | 5.5 | 18.4 | 1.26E-07 |
| MFI-20: Mental Fatigue | 6.9 | 13.3 | 0.0001 |
| MFI-20: Reduced Motivation | 5.2 | 10.6 | 0.00016 |
| McGill Pain Questionnaire | 1.0 | 19.9 | 3.04E-05 |
| Multiple Ability Self-Report Qnr: Attention | 14.3 | 20.6 | 0.0005 |
| Multiple Ability Self-Report Qnr : Visual Memory | 13.3 | 16.1 | 0.09 |
| Multiple Ability Self-Report Qnr: Verbal Memory | 15.0 | 20.9 | 0.0002 |
| Multiple Ability Self-Report Qnr: Visuoperceptual | 9.9 | 13.0 | 0.02 |
| Multiple Ability Self-Report Qnr: Language | 12.6 | 18.9 | 4.01E-05 |
| Neuropathy Pain Scale: Unpleasant Overall | 0.6 | 5.1 | 7.95E-06 |
| Physical Symptoms (PHQ-15) | 2.0 | 11.6 | 7.22E-07 |
| Pittsburgh Sleep Quality Index (PSQI) | 3.2 | 7.8 | 0.0001 |
| PROMIS Emotional Distress - Anxiety | 43.7 | 50.0 | 0.007 |
| PROMIS Emotional Distress - Depression | 42.3 | 48.1 | 0.03 |
| PROMIS Fatigue-Short Form | 56.5 | 66.4 | 1.54E-06 |
| PROMIS Global Health: Mental | 57.2 | 45.9 | 6.74E-05 |
| PROMIS Global Health: Physical | 61.3 | 33.8 | 1.16E-07 |
| PROMIS Pain Behavior | 38.6 | 53.3 | 7.07E-05 |
| PROMIS Pain Intensity | 39.7 | 56.2 | 4.90E-05 |
| PROMIS Pain Interference | 42.4 | 58.9 | 3.75E-05 |
| PROMIS Sleep Disturbance | 40.4 | 55.3 | 1.73E-05 |
| PROMIS Sleep Related Impairment | 40.0 | 61.3 | 1.04E-06 |
| Polysymptomatic Distress Scale | 1.6 | 13.2 | 2.22E-07 |
| Beck Depression Inventory -II | 2.1 | 12.0 | 1.65E-06 |
| Beck Anxiety Inventory | 1.9 | 7.8 | 0.007 |
| Center for Epidemiologic Studies Depression Scale - R | 1.5 | 13.3 | 2.88E-07 |
| Childhood Trauma Questionnaire | 39.1 | 35.8 | 0.3 |
| Beliefs About Emotions Scale | 15.1 | 13.9 | 0.2 |



# Proposed Pathophysiology of ME/CFS



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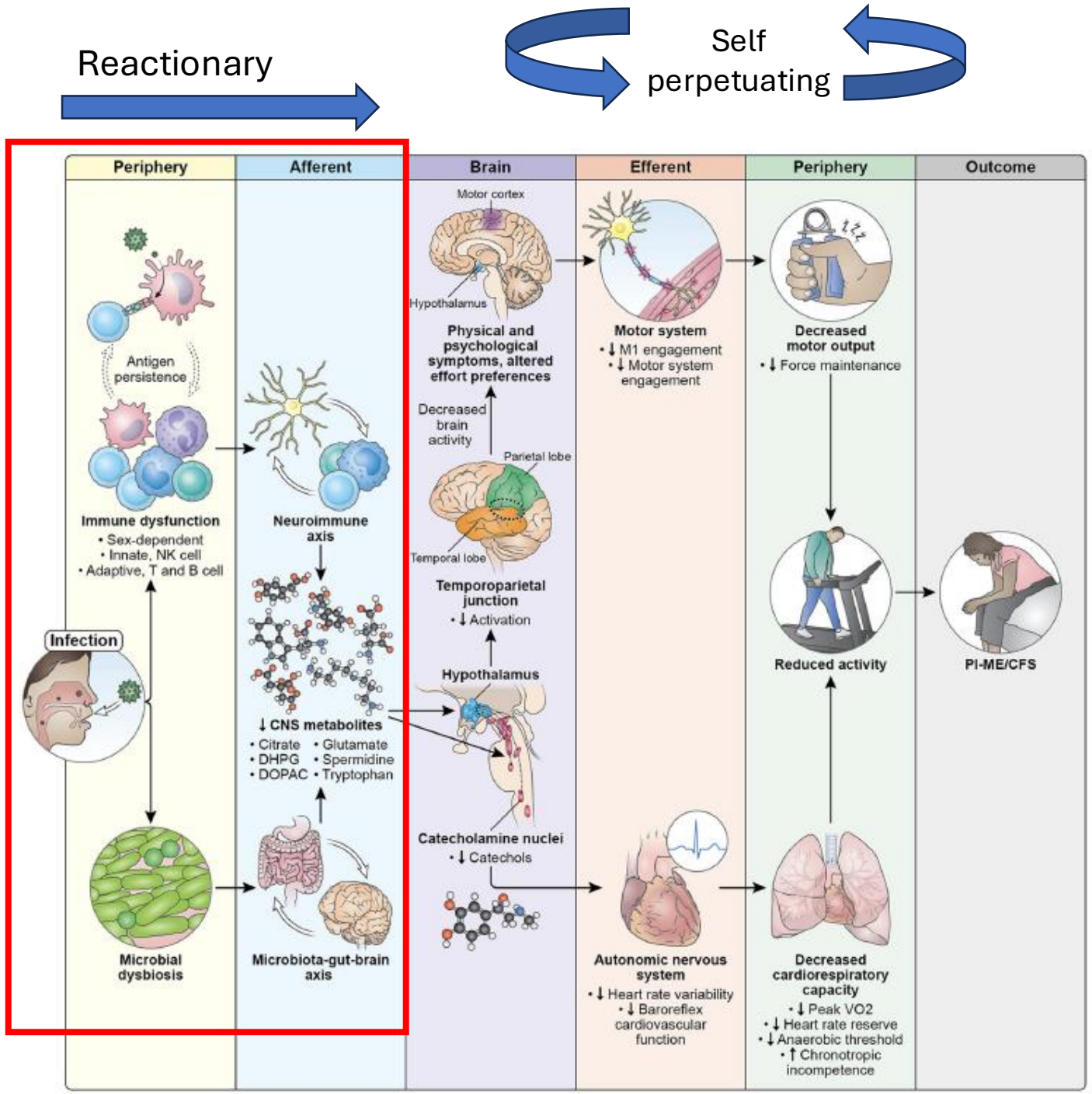
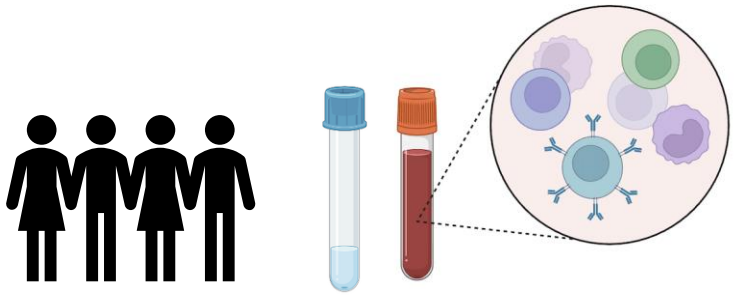
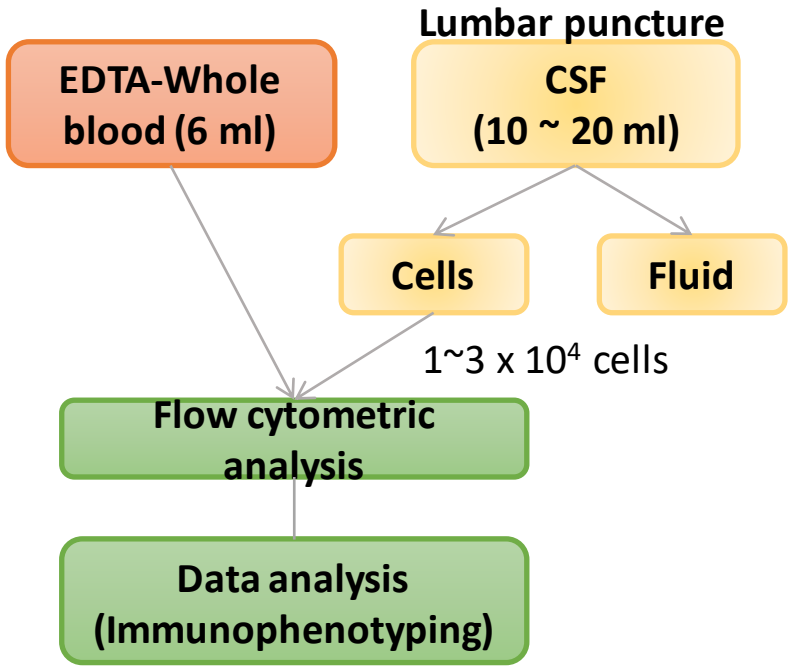
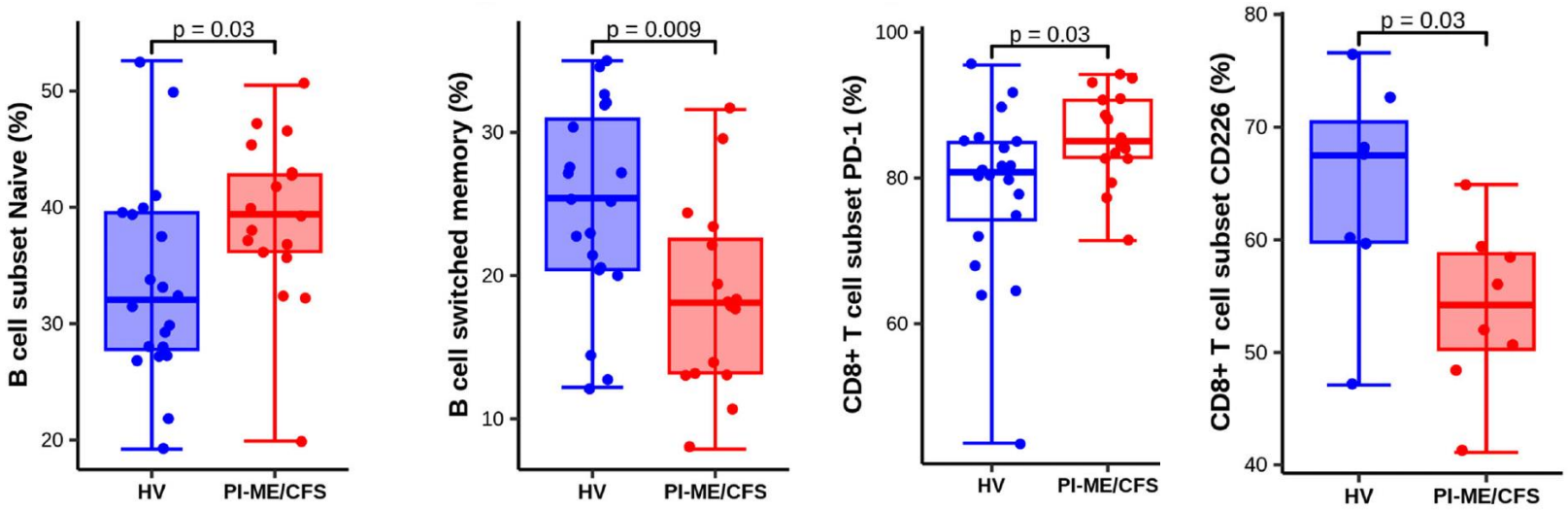


# PI-ME/CFS has disease specific immune signatures



19 Healthy Volunteers
16 PI-ME/CFS

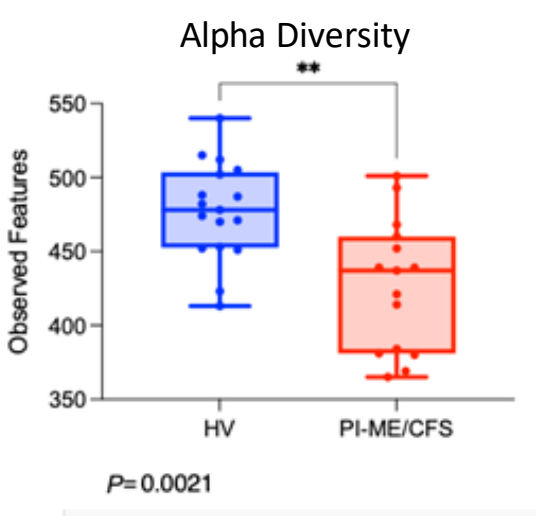


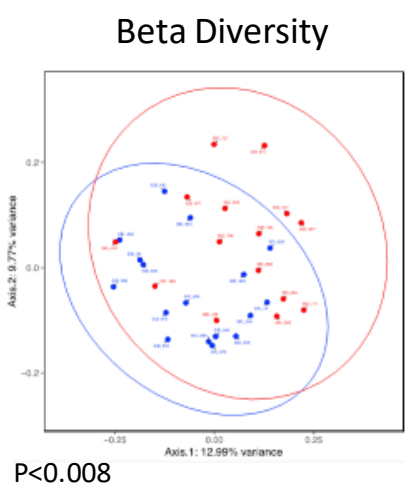




Flow cytometry of blood and cerebrospinal fluid PI-ME/CFS observed:

- Altered B cell phenotypes
- Elevated T-cell exhaustion and activation
- Sex-specific differences

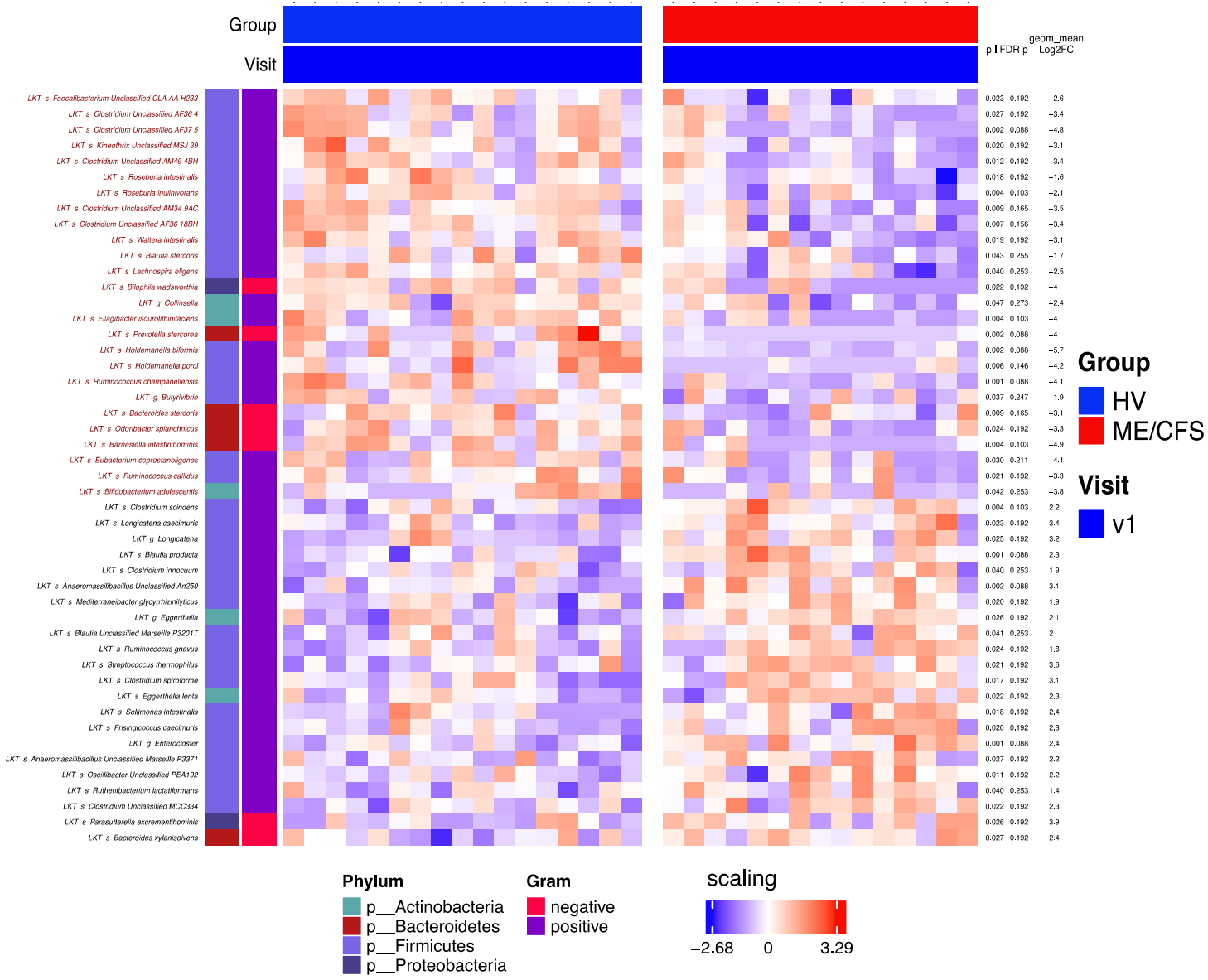
# The gut microbiome is different in PI-ME/CFS



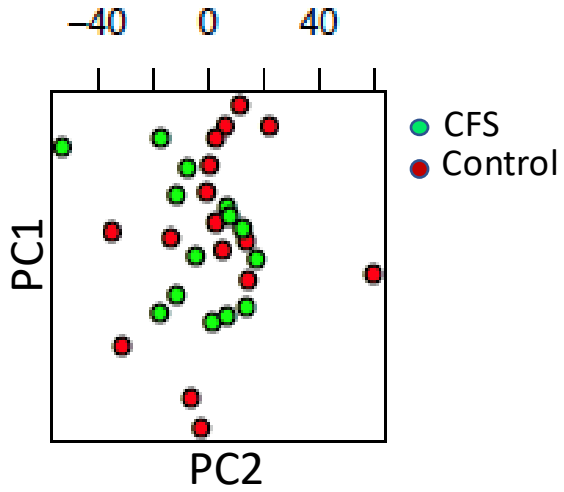




Analysis of stool samples observed:

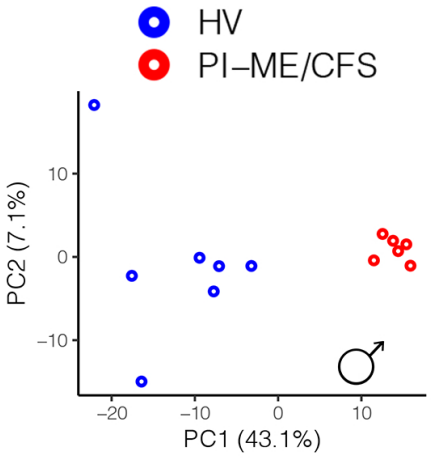
- The microbiome structure of PI-ME/CFS is less diverse and has different proportions of microbial species compared to HV
- Many of the species differences noted confirm findings from other studies
- Some differences noted in butyrate producing bacterial species

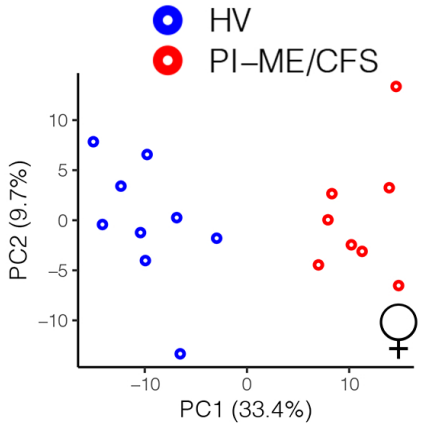


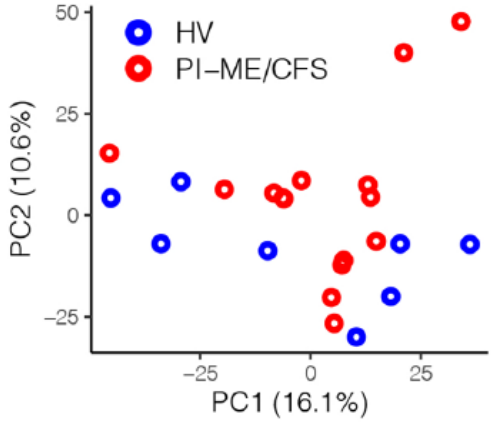
# Birth sex is biologically important in understanding PI-ME/CFS



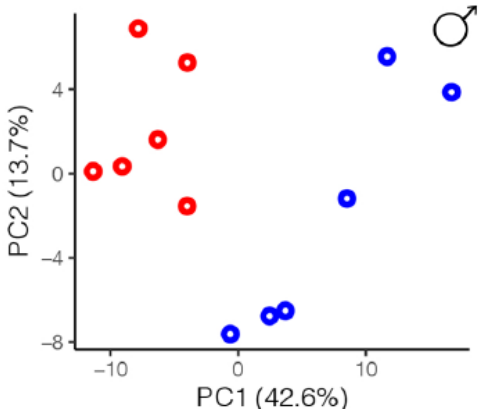

PBMC RNASeq

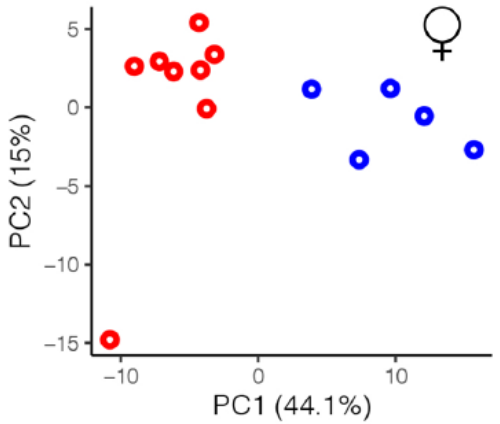



Muscle RNASeq



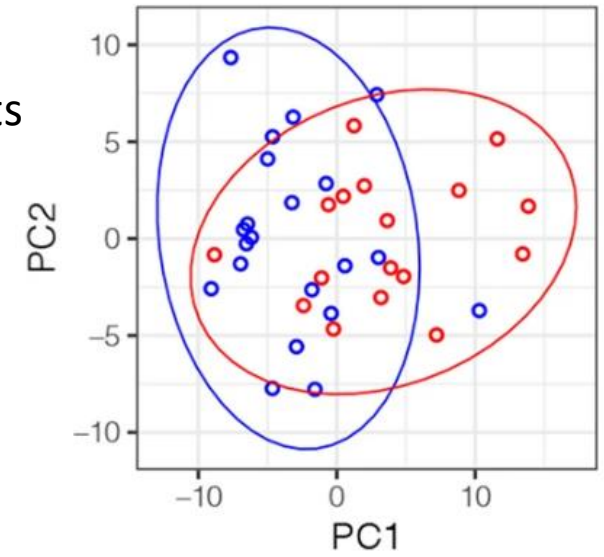



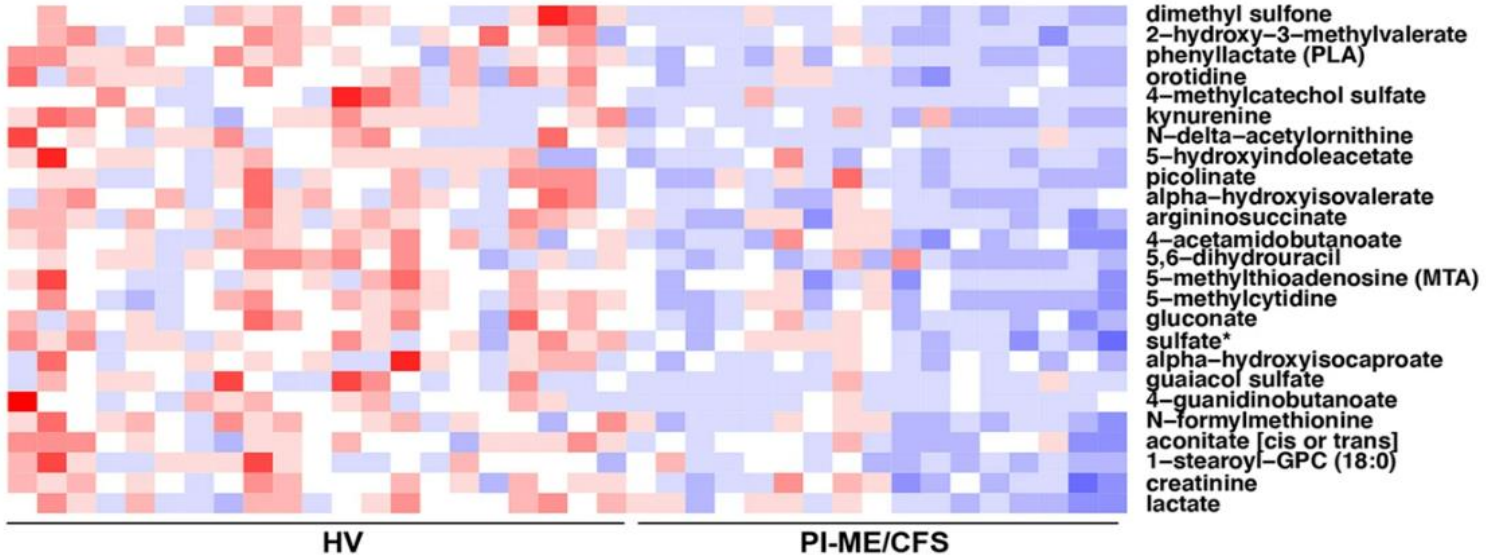

Discriminative power increases despite a decrease in sample size with:

- Gene expression from immune cells and muscle cells
- Proteins in the blood and cerebrospinal fluid
- Lipids in the blood
- Metabolic molecules in the cerebrospinal fluid

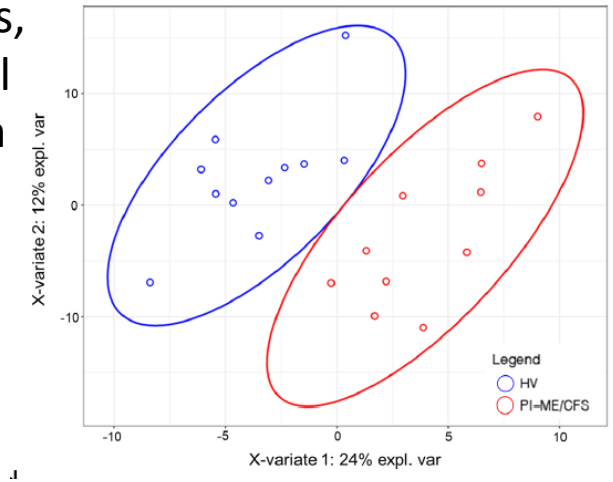
# Cerebrospinal fluid metabolomics could discriminate PI-ME/CFS status

PCA: All participants



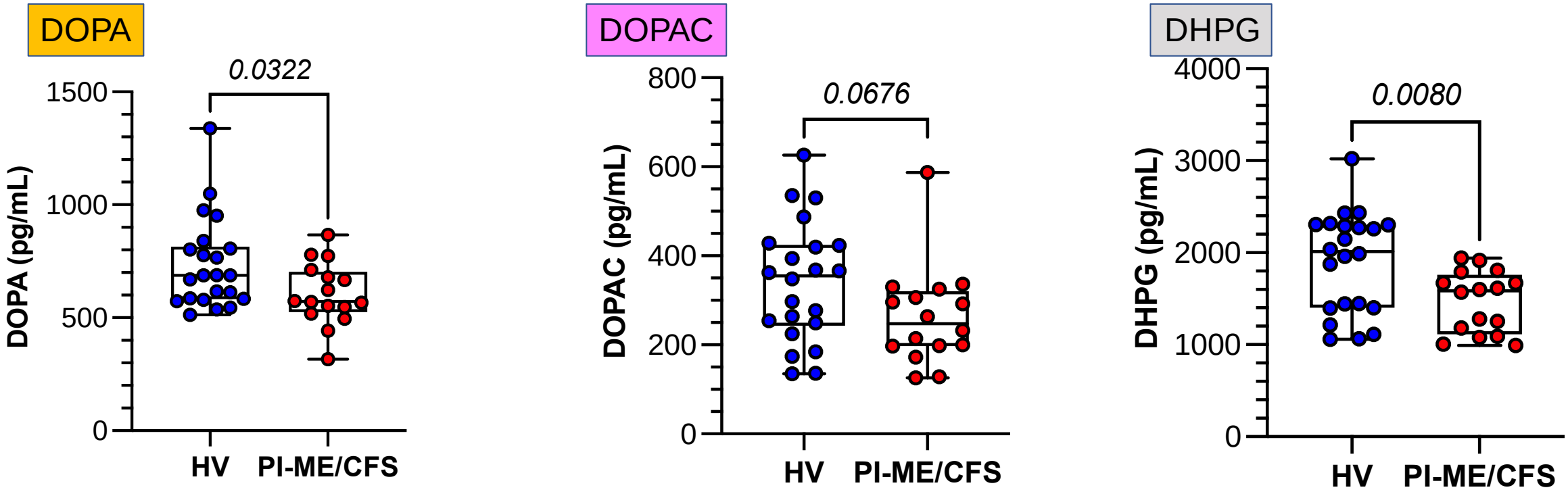

PLS-DA: All participants, Differential Expression




Cerebrospinal fluid metabolomics was best at discriminating PI-ME/CFS from Healthy Volunteers

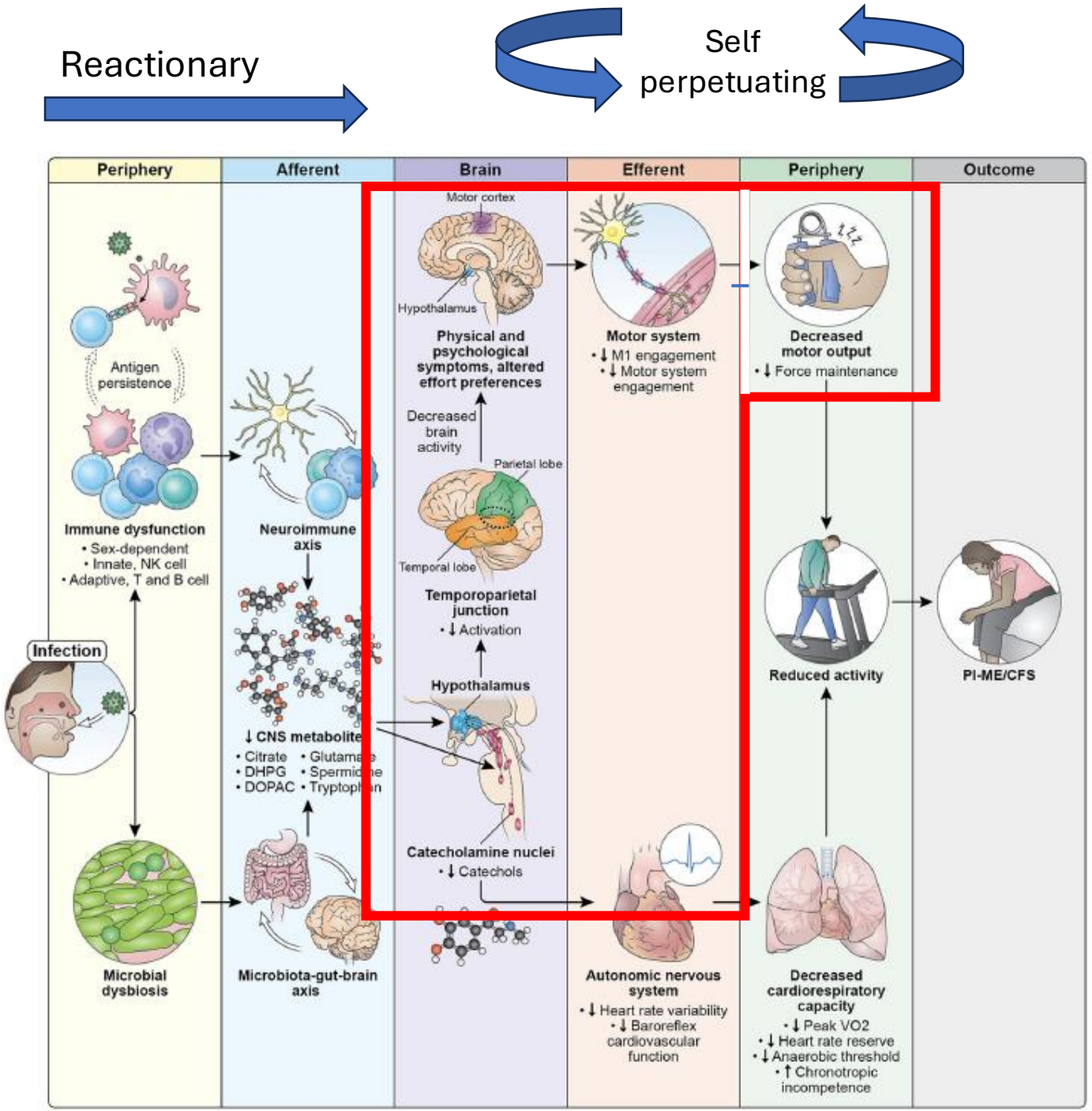
- Was able to discriminate without considering birth sex
- Decreased glutamate, Dopamine 3-O-sulfate, Butyrate, polyamine, and tricarboxylic acid (TCA) pathway metabolites in PI-ME/CFS
- Suggests that the central nervous system is where divergent mechanisms unify in the pathogenesis of PI-ME/CFS

# Catechol neurotransmitters were decreased in PI-ME/CFS

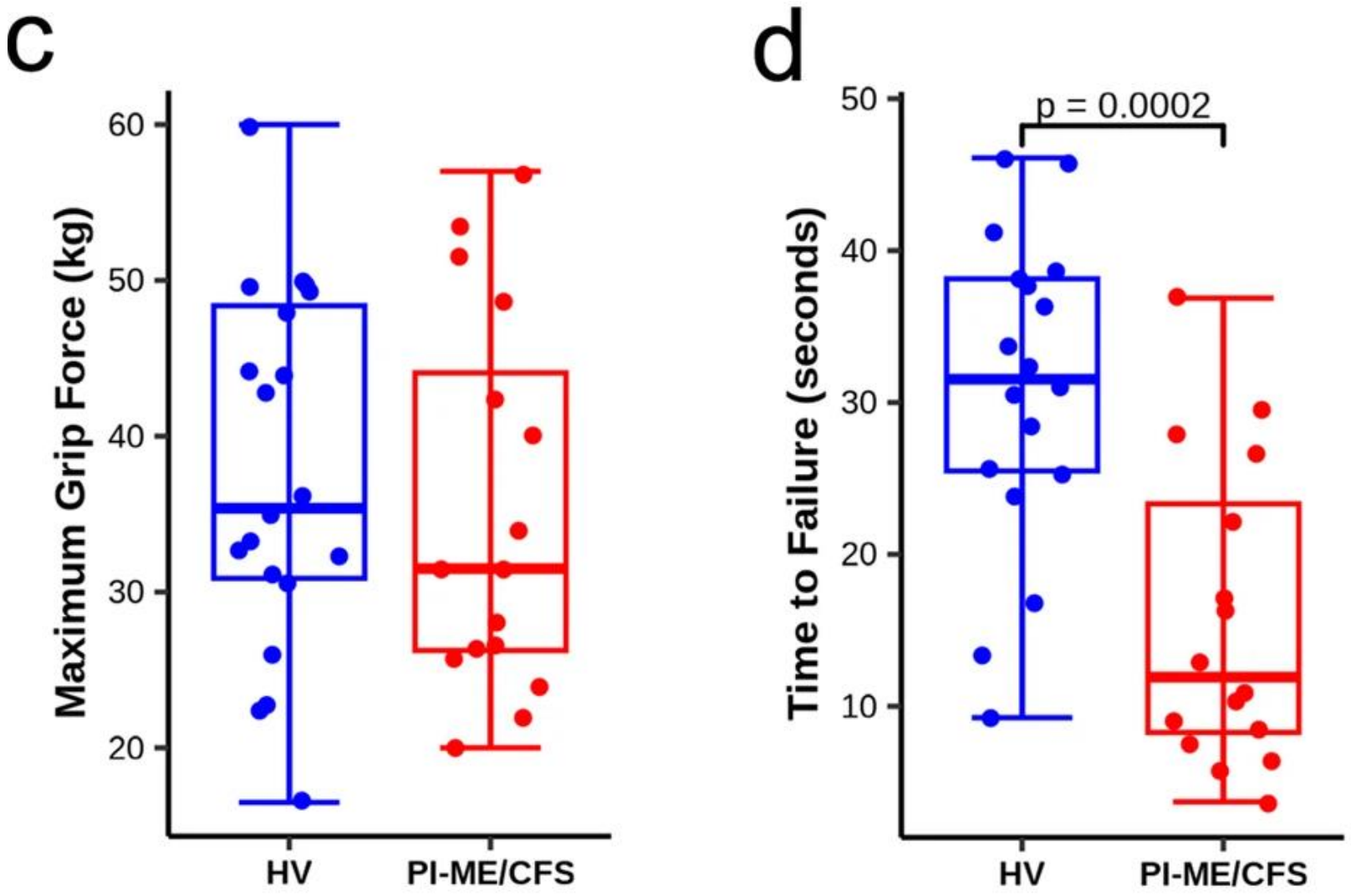



- Catechol neurotransmitters are important compounds in the regulation of autonomic function
- The decreased levels of catechol metabolites suggest decreased central catecholamine biosynthesis

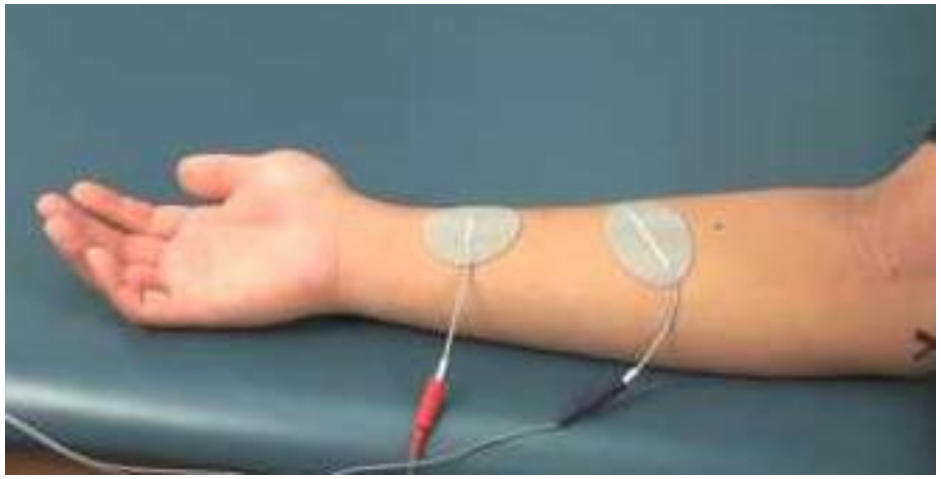
# Proposed Pathophysiology of ME/CFS

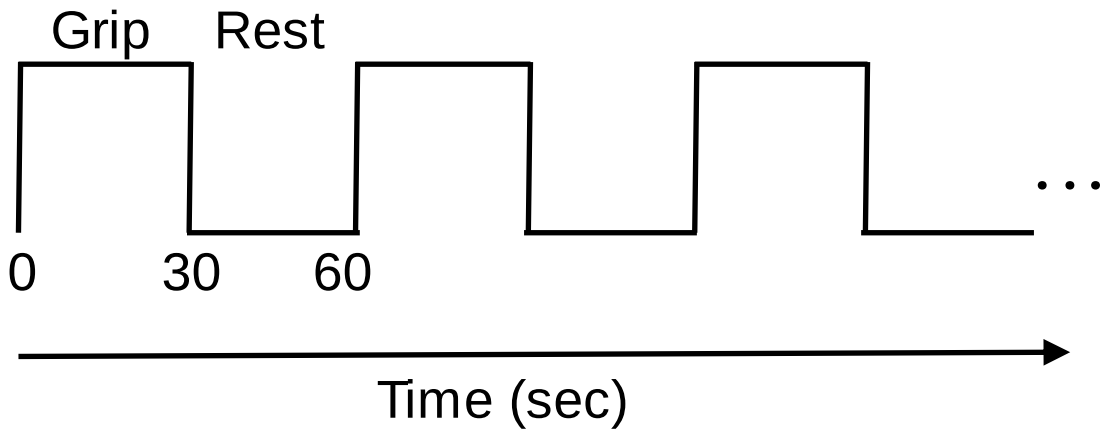


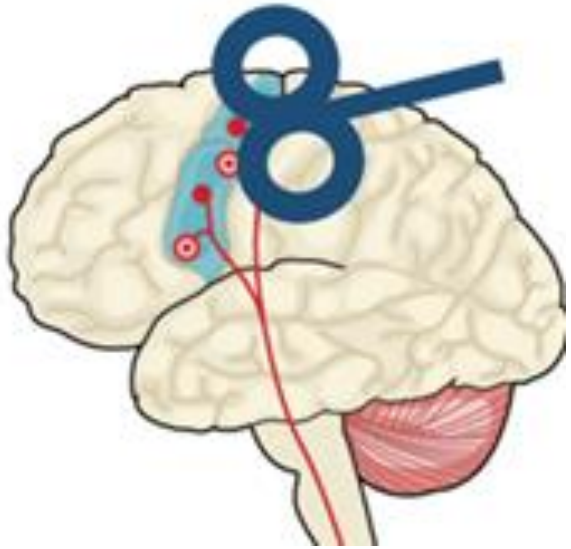
# Grip endurance but not maximum strength impaired in PI-ME/CFS



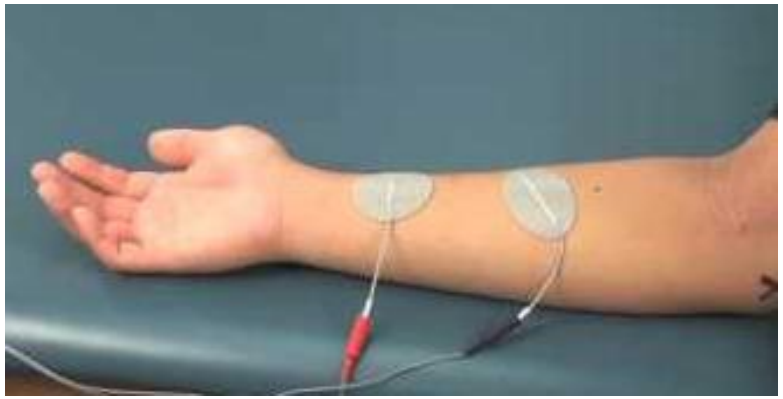
# Decreased grip endurance is not due to muscular or central motor fatigue

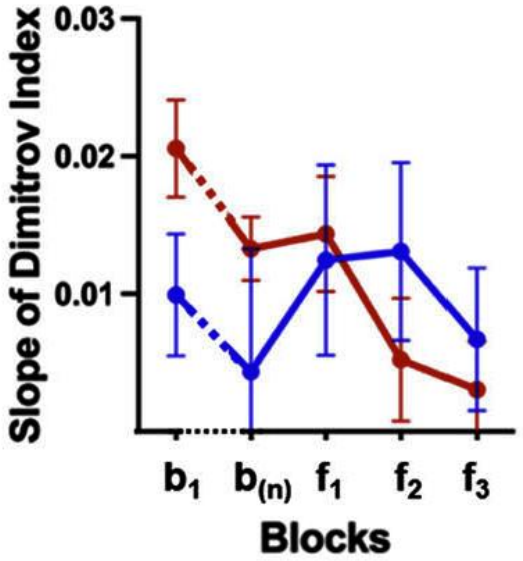




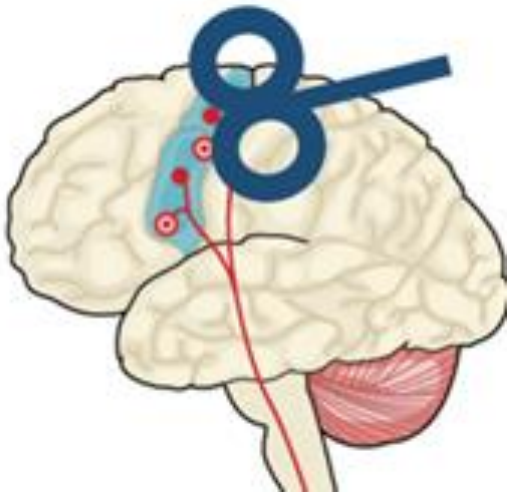



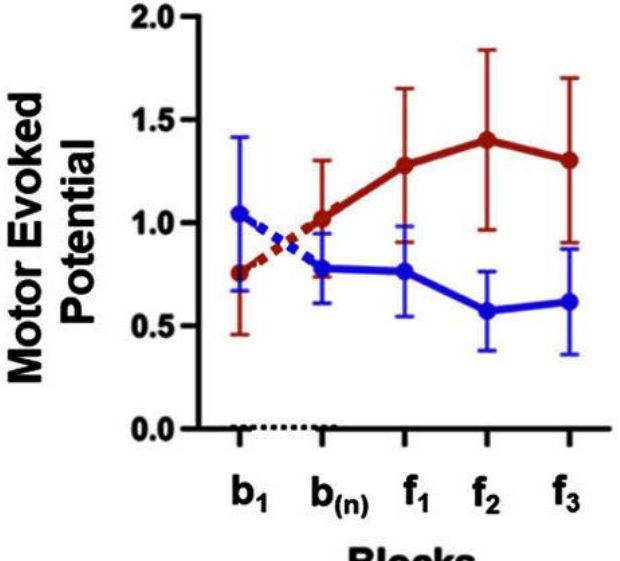
# Decreased grip endurance is not due to peripheral muscle or central motor fatigue





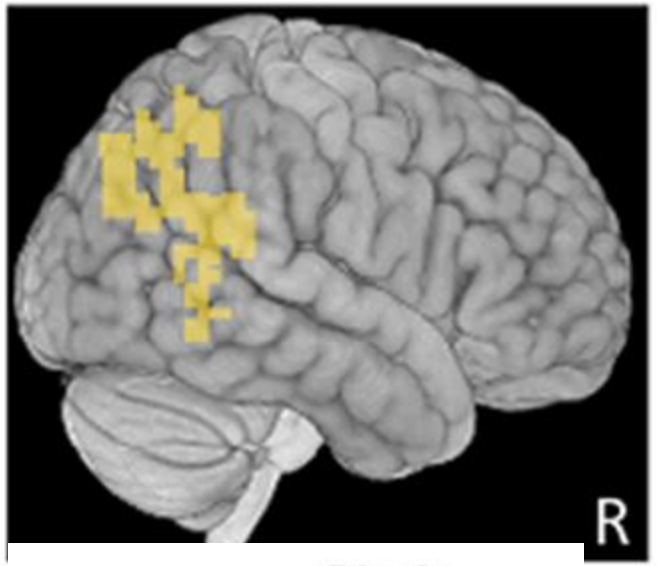

- In PI-ME/CFS, a biomarker of muscle lactic acid decreased, instead of increased, with grip failure
- Peripheral fatigue of the muscle is not the cause decreased grip endurance

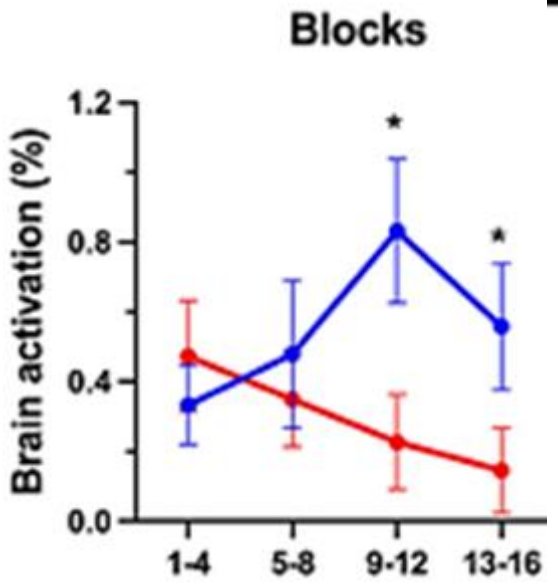





- In PI-ME/CFS, a biomarker of central motor reserve increased, instead of decreased, with grip failure
- Central fatigue of the motor cortex is not the cause decreased grip endurance

# Decreased grip endurance is related to dysfunction of integrative brain regions that drive the motor cortex

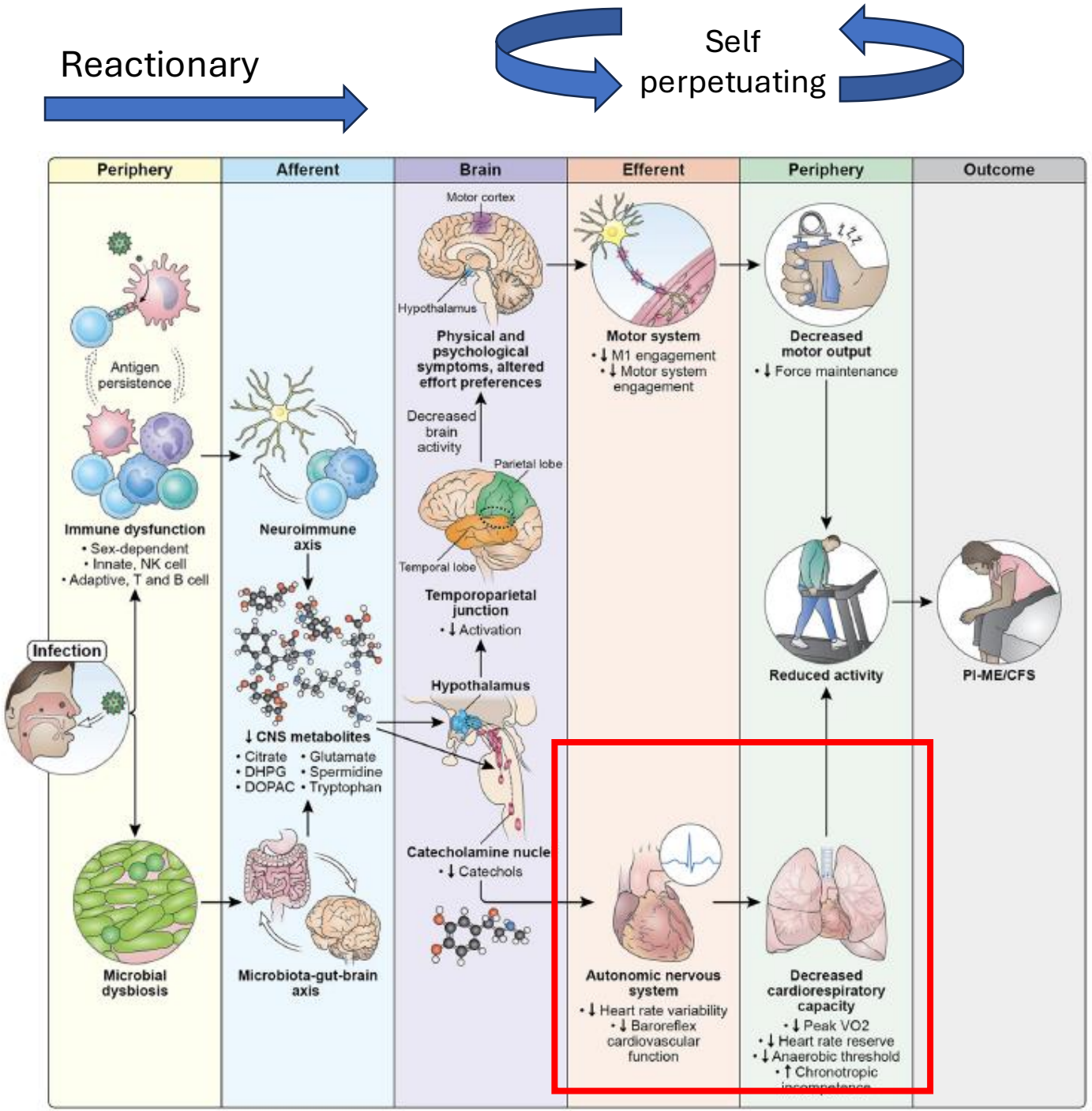


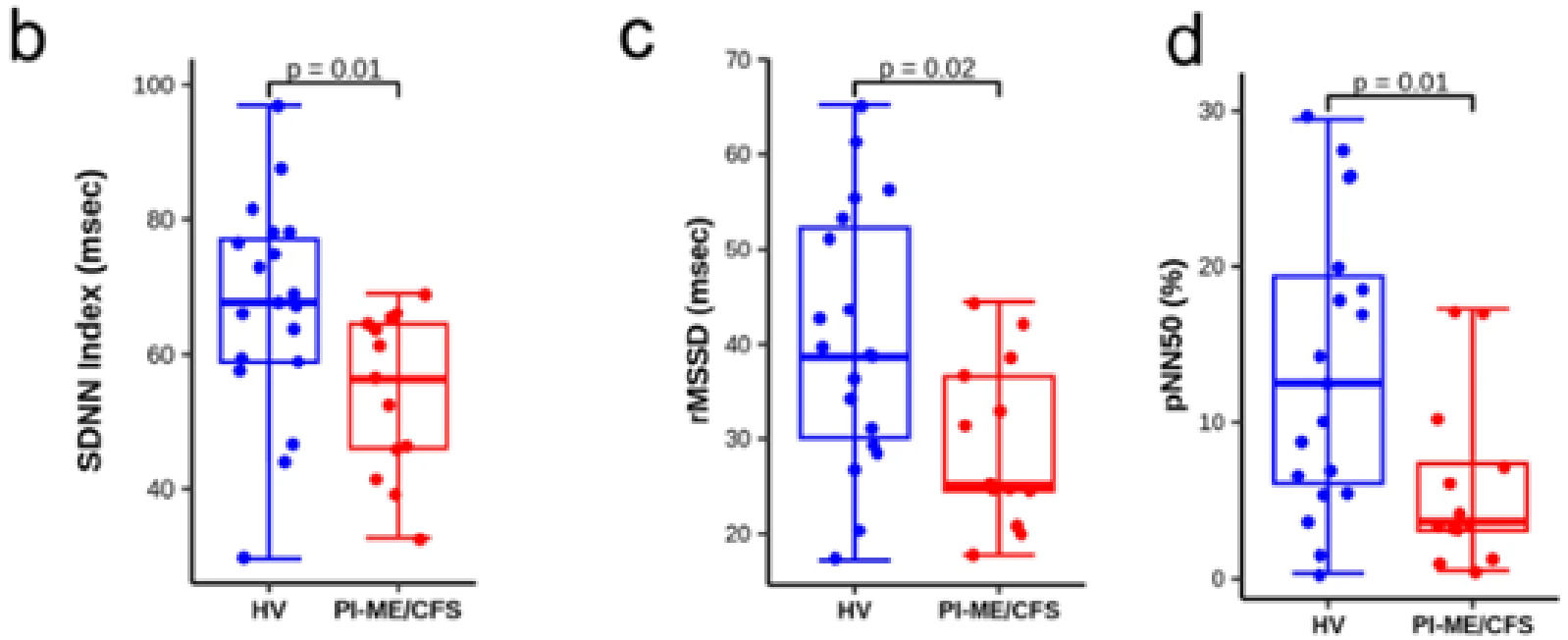

- No difference in motor system activation was noted between groups
- The right temporoparietal junction (TPJ) activated differently between the groups
- The TPJ is responsible for high order integration of brain function and ‘mismatch’ detection

Suggests that there is a defect in the neuronal circuit integration

# Proposed Pathophysiology of ME/CFS

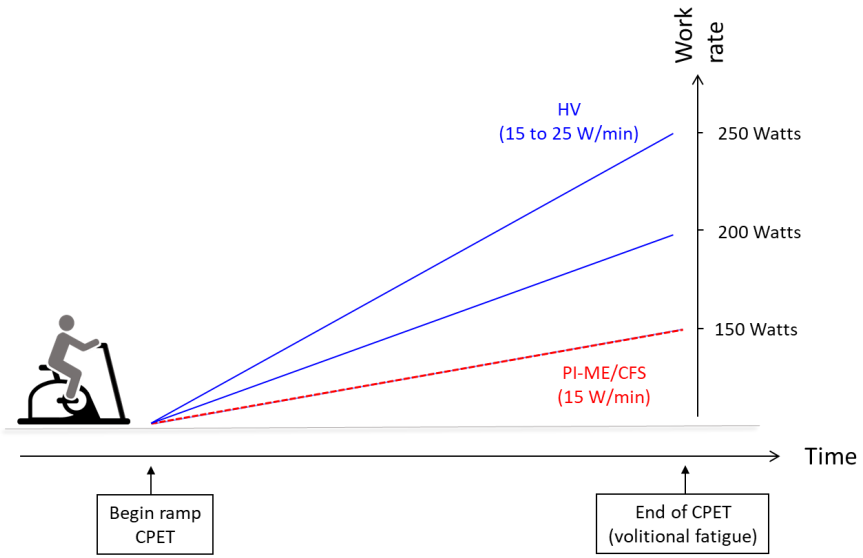


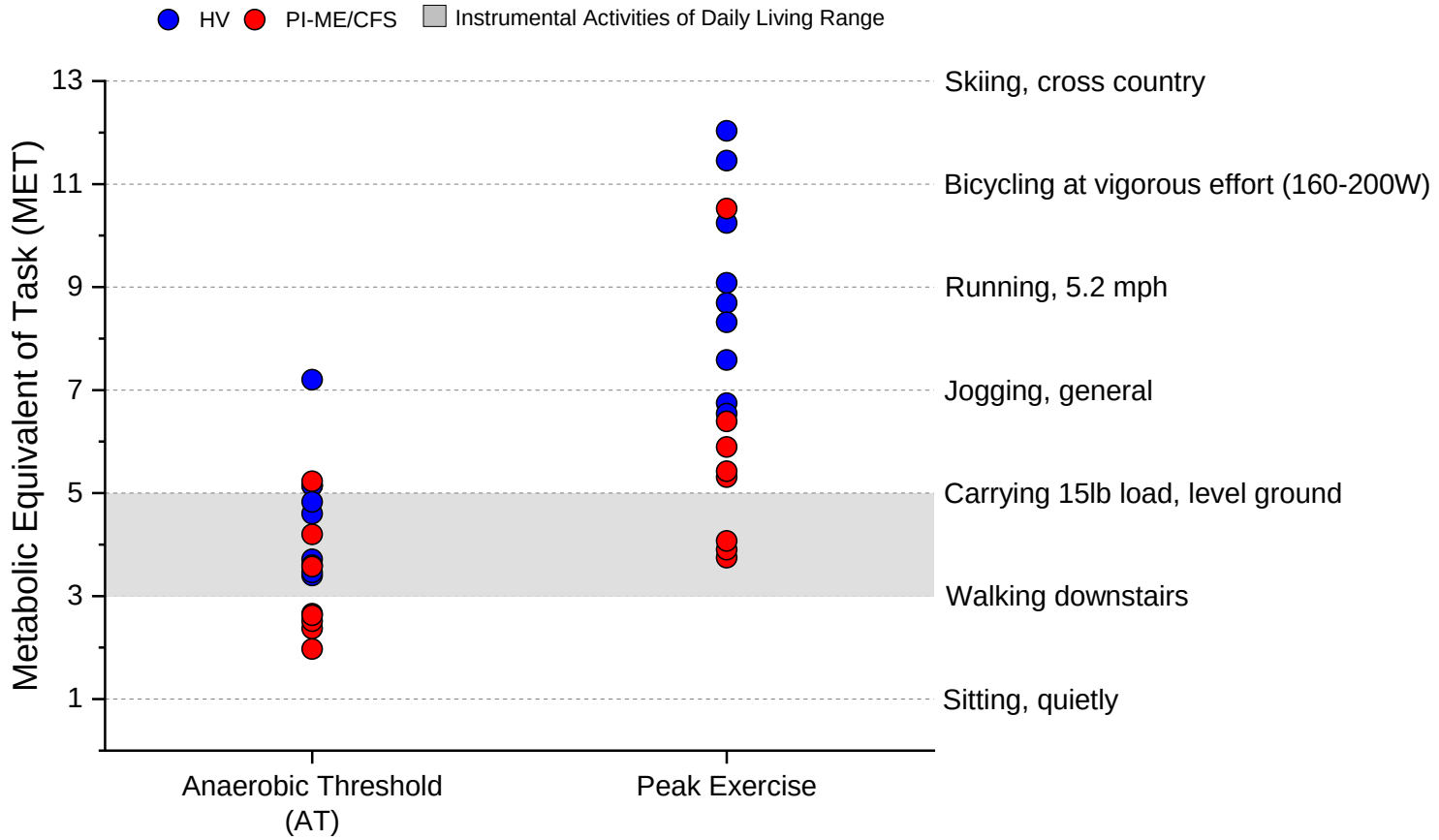
# Autonomic dysfunction was apparent in PI-ME/CFS with multiple measurements




- Abnormal tilt table responses were frequent in both groups
- Heart rate variability measurements demonstrate decreased parasympathetic activity

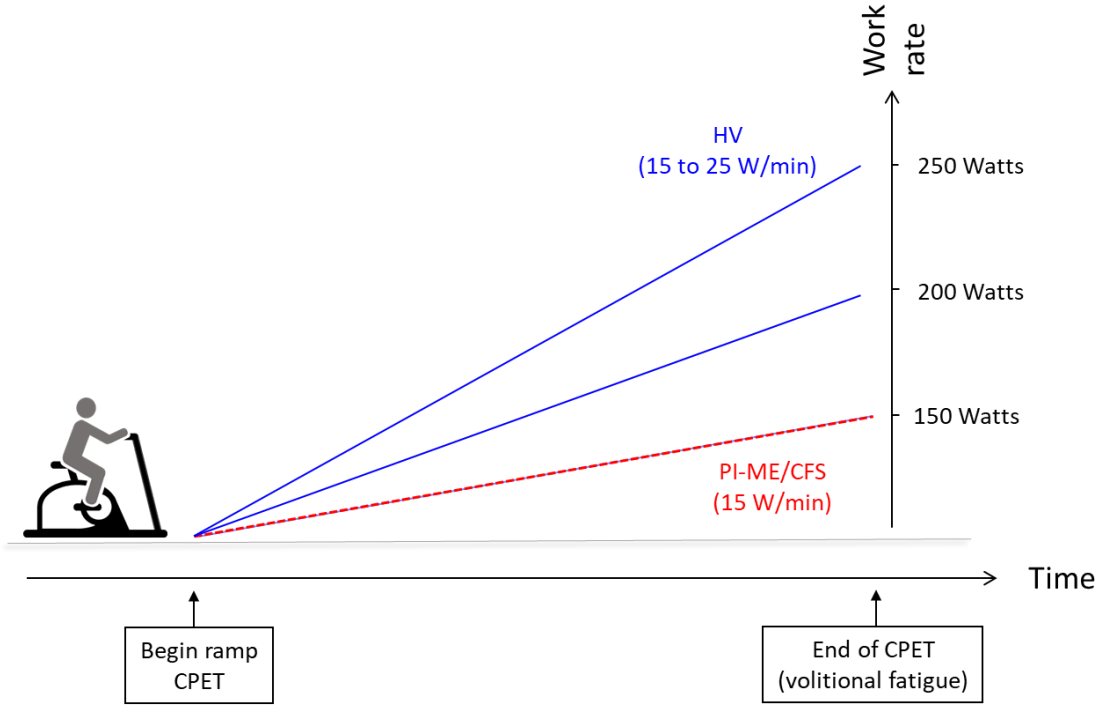
# Cardiopulmonary performance is reduced in PI-ME/CFS

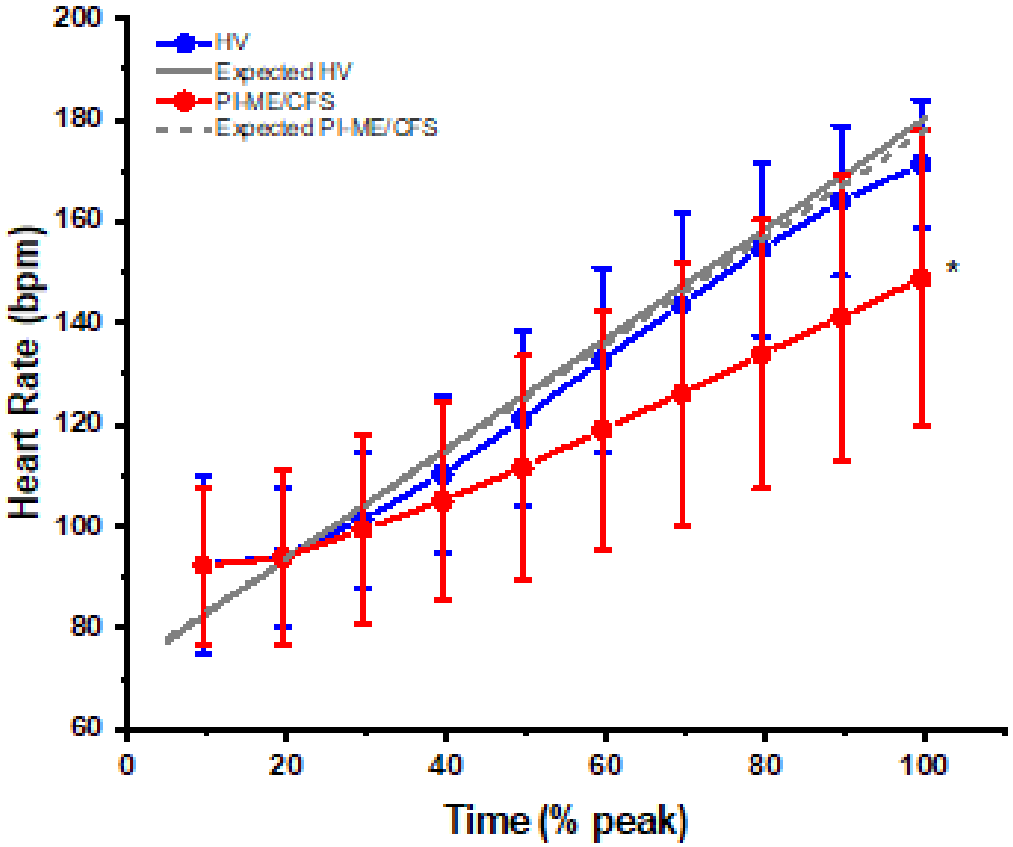



*METs at the anaerobic threshold was lower by 1.4 and at peak exercise by 3.3 in PI-ME/CFS vs. HV*

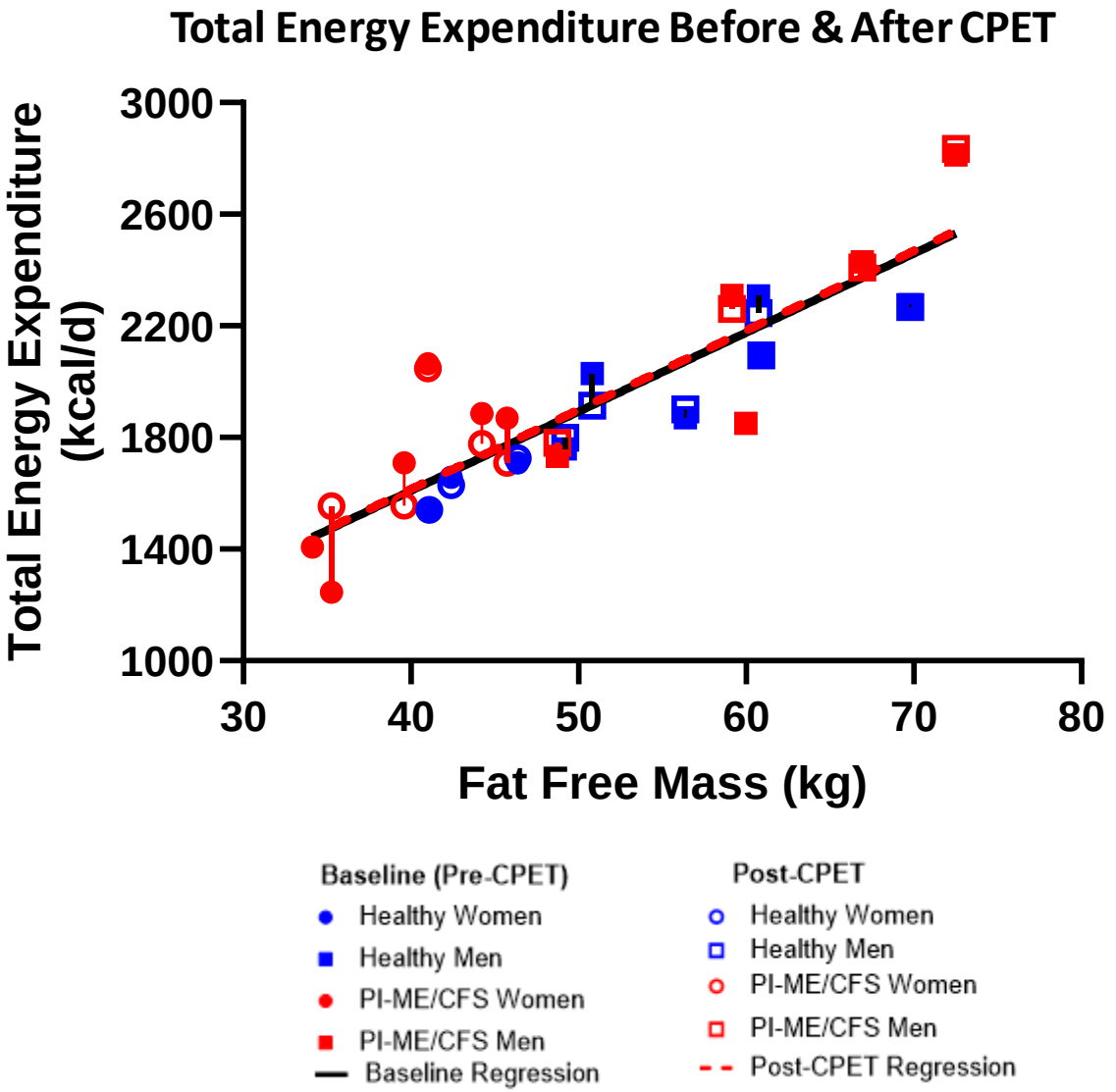
# Cardiopulmonary performance is reduced in PI-ME/CFS



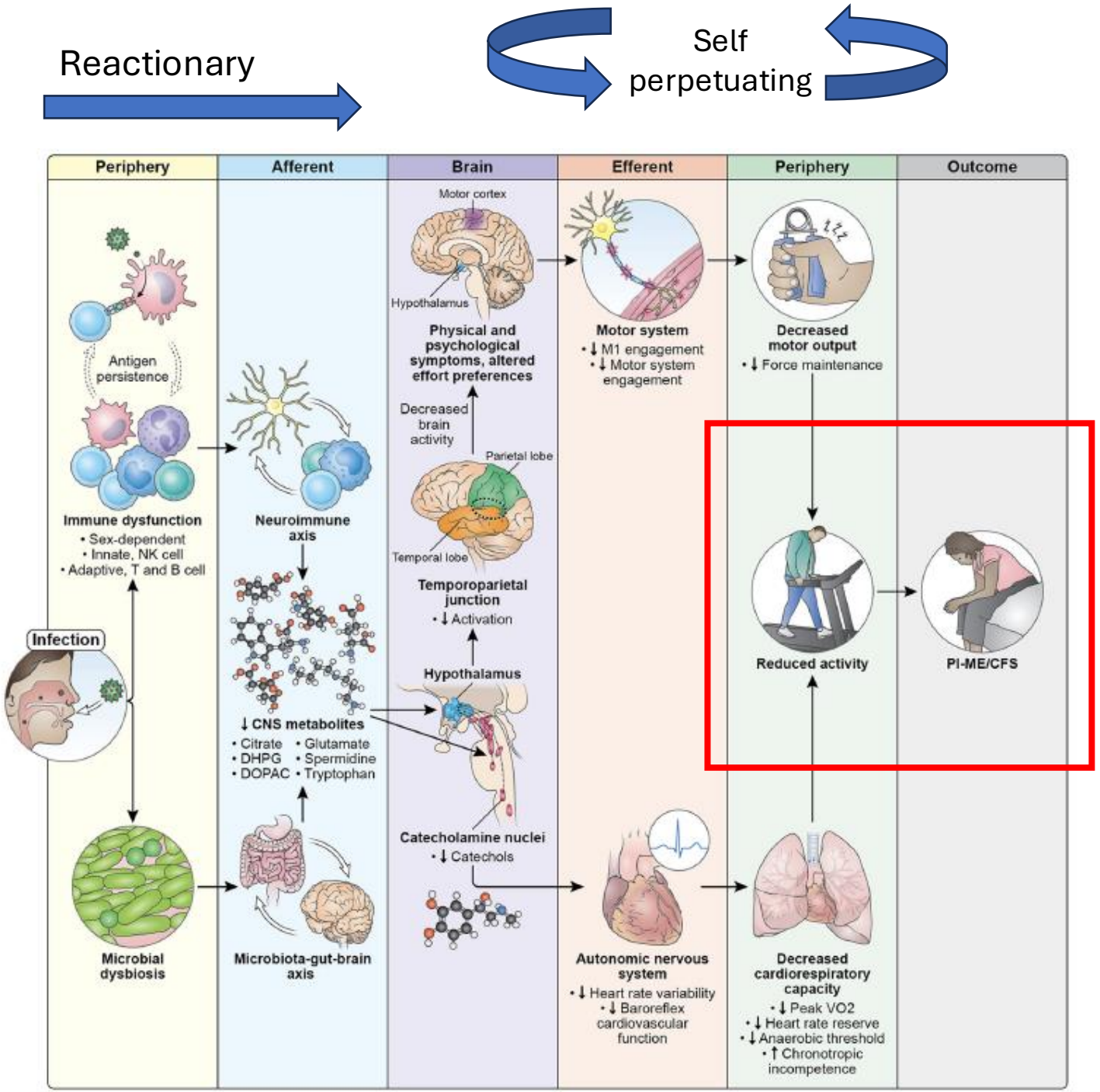




*Chronotropic incompetence was observed, with a lower than expected heart rate response among PI-ME/CFS participants.*

# No alteration in total body energy use in PI-ME/CFS



# Proposed Pathophysiology of ME/CFS



# Summary

| | |
|---|---|
| ME/CFS may have underlying treatable diseases | • They need to be closely followed |
| Some patients spontaneously recover | • Need to understand why |
| Multiple biological systems are involved | • Nervous system plays a critical role |
| Multiple Targets for intervention | • Need to develop sex specific treatments |

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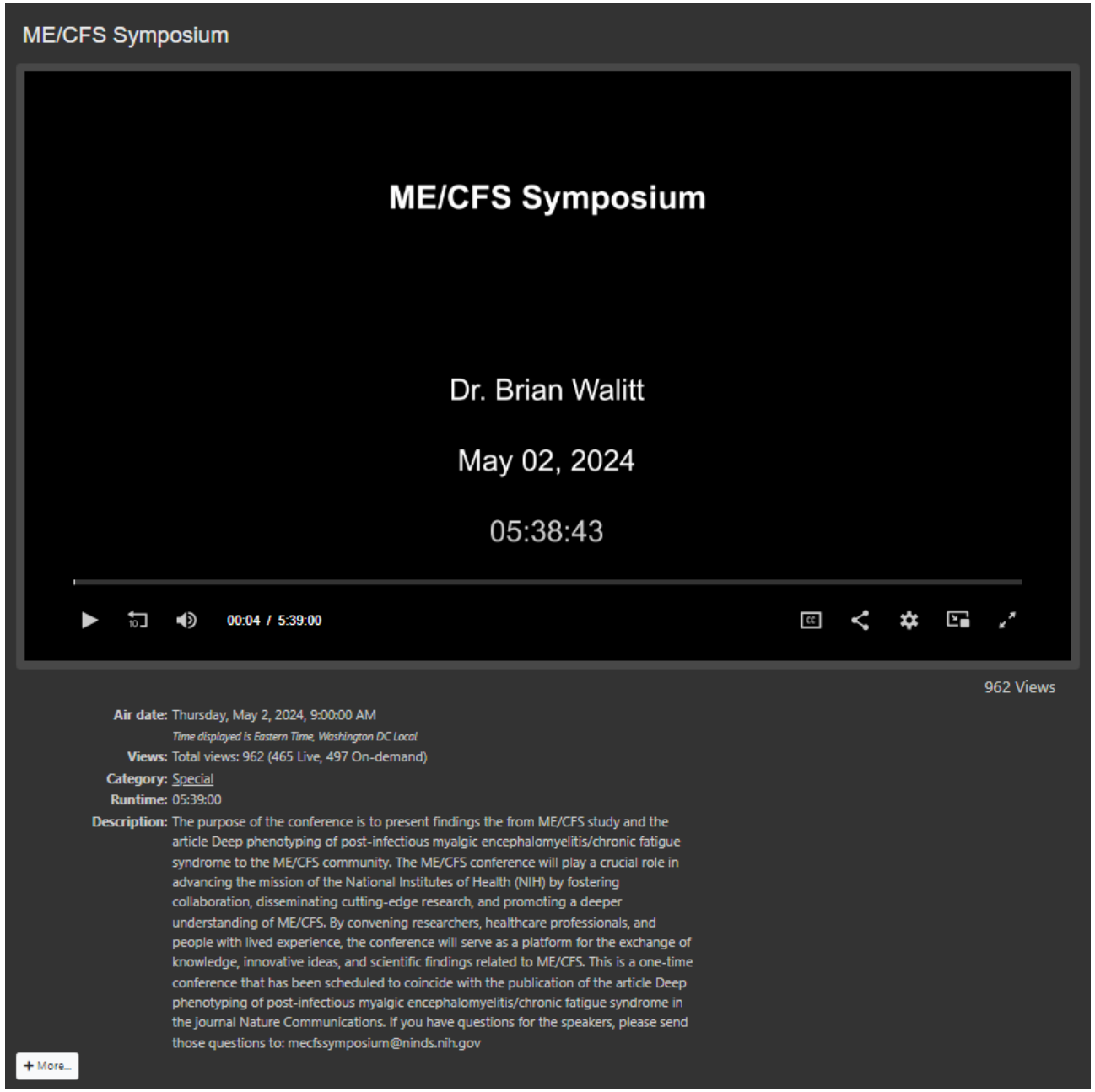


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