



Post exertion malaise in GWI - brain, autonomic, and behavioral interactions

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U.S. Department of Veterans Affairs CSR&D: I01 CX0011329 (Cook/Falvo)

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Outline

Project Overview

Characteristics of post-exertional malaise in Gulf War illness (GWI)

Cardiopulmonary and perceptual predictors of PEM

Gene expression mediation of PEM



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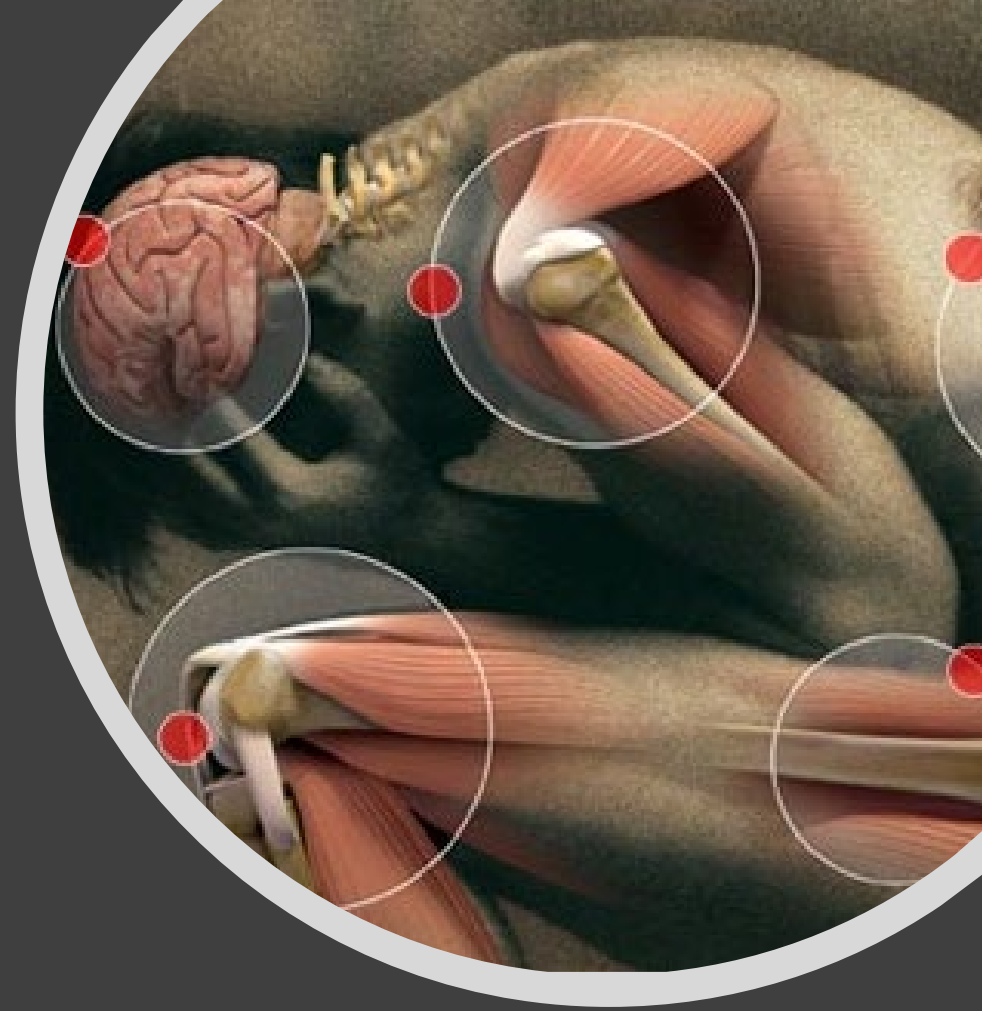
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Introduction

- Post Exertion Malaise (PEM) – symptom worsening following effort
- An understudied aspect of Gulf War Illness (GWI)
 - Symptom type, severity and time-course are not well understood
- Critical towards understanding pathophysiology & safety of exercise



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$$F = G \frac{m_1 m_2}{d^2}$$

$$i\hbar \frac{\partial}{\partial t} \psi = \hat{H} \psi$$

$$\phi(x) = \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$ds \geq 0$$

Project Overview

To determine dysfunction among central, autonomic, and immune systems in GWI using a post-exertional malaise model

$$\frac{df}{dt} = \text{liv} - h -$$

Specific Aims



Determine baseline function across multiple physiological systems (CNS, autonomic, and immune) in GVs with and without GWI.



Determine the impact of an exercise challenge on CNS regulation of pain/fatigue, cardiovascular autonomic function, immune system activity, and symptoms in GVs with and without GWI.



Determine whether interactions among multiple systems explain symptoms of GWI.

Participants (Total)



U.S. Navy photo by Photographer's Mate 1st Class Arlo K. Abrahamson. (RELEASED)

- N = 124 Deployed Gulf War Veterans
 - n=73 GWI
 - n=51 GV CON

GWJ – Kansas Domains & Symptoms

Fatigue



Pain



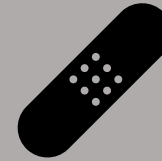
Neuro/Cog



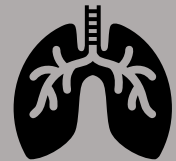
GI



Skin



Respiratory



Fatigue

Sleep

Body

Muscle

Joint

Memory

Mood

Headache

Diarrhea

Nausea

Rashes

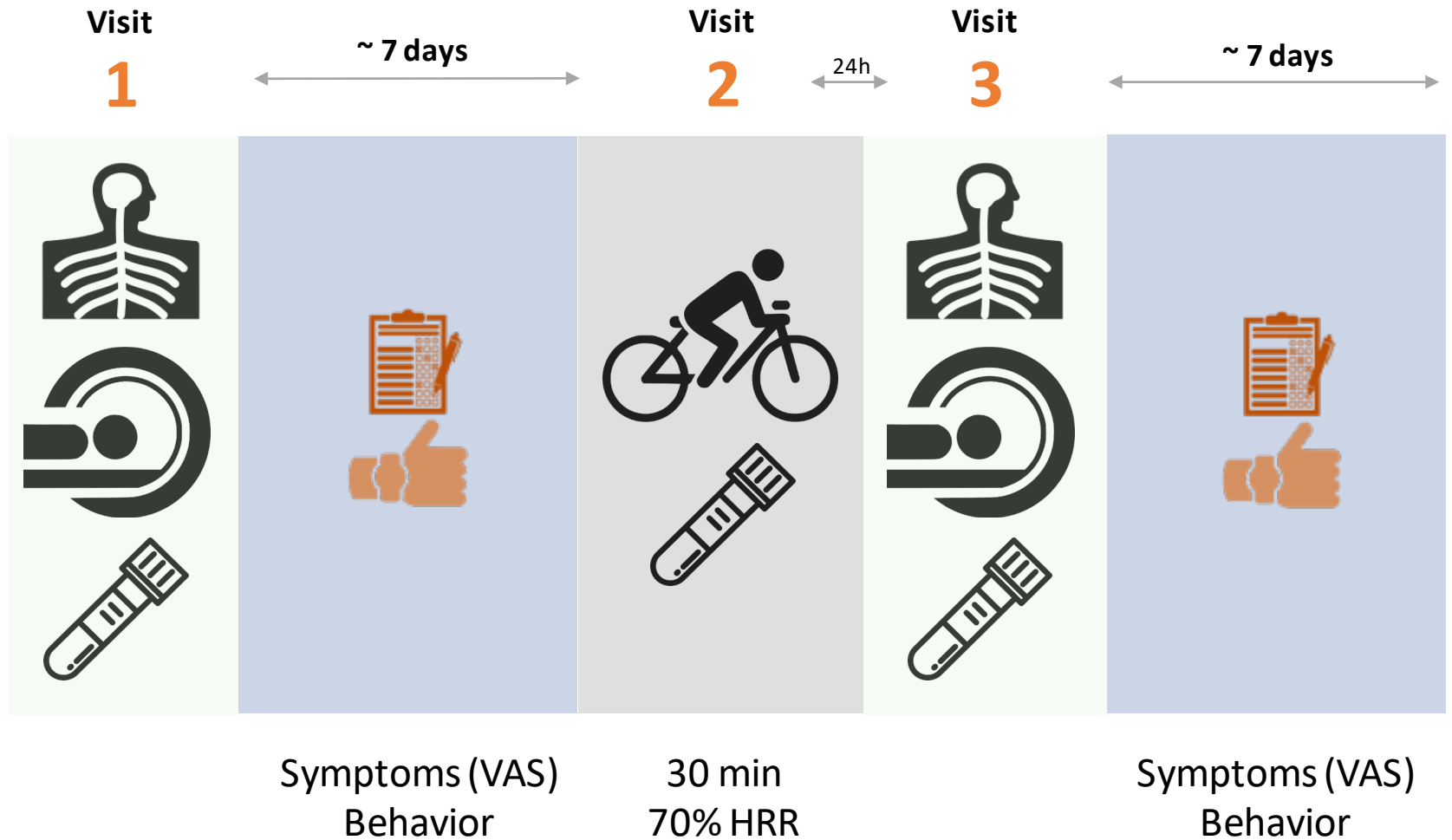
Dyspnea

Cough

GWJ = Score of 2+ (0-3 Scale) in at least 3/6 Kansas Domains

Minimum = 6 & Maximum = 87

Experimental Protocol



A row of wooden figures, with one red figure standing out in the center. The figures are arranged in a line, receding into the background. The red figure is the focal point, standing out from the others which are a light cream color. The background is a soft, out-of-focus grey.

Characterizing PEM in GWI

Presence, symptom type, severity, and variability

Not all Veterans with GWI respond negatively to exercise

International Journal of Psychophysiology 147 (2020) 202–212

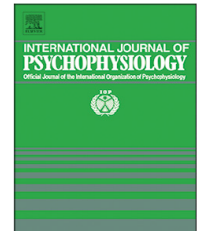


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International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho



Post-exertional malaise in veterans with gulf war illness

Jacob B. Lindheimer^{a,b}, Aaron J. Stegner^{a,b}, Glenn R. Wylie^{c,d,e}, Jacquelyn C. Klein-Adams^c, Neda E. Almassi^{a,b}, Jacob V. Ninneman^{a,b}, Stephanie M. Van Riper^{a,b}, Ryan J. Dougherty^b, Michael J. Falvo^{c,e,1}, Dane B. Cook^{a,b,*,1}



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Purpose

To determine
symptom severity
changes 24hrs post-
exercise in GWI



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Modeling PEM (Doubly-multivariate repeated measures MANOVA)



Mood: (6 subscales of POMS)

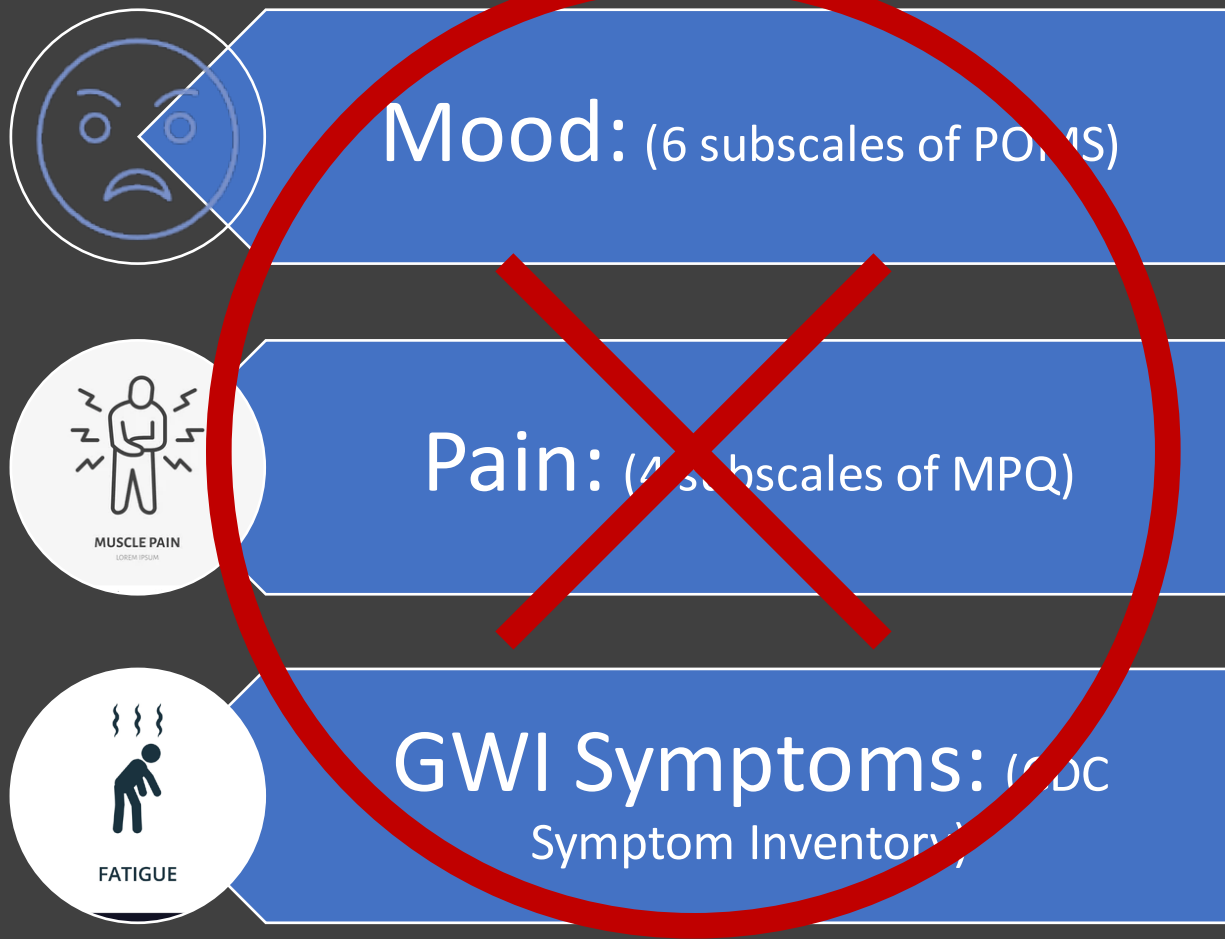


Pain: (4 subscales of MPQ)



GWI Symptoms: (CDC
Symptom Inventory)

No symptom exacerbation for full sample



GW+PEM experienced symptom exacerbation



Pain: (4 subscales of MPQ)



GW+PEM Symptoms: (CDC Symptom Inventory)



Preliminary conclusions

- Not all Veterans with GWI experience PEM
 - Immediately or 24hrs post-exercise
- Endorsement of feeling “unwell” with exercise or exertion mattered
- Deeper exploration is warranted



Purpose

To examine symptom responses for one-week prior to and one-week following acute aerobic exercise in Gulf War Veterans



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Participants

- N = 77 Deployed Gulf War Veterans
 - n=44 GWI
 - n=18 GWI
 - n=26 GWI+PEM
 - n=33 GV CON

Navy photo by Photographer
Abrahamson. (RELEASED)

Post-Exertional Malaise

- How are you feeling right now?
- How are you feeling compared to how you felt prior to exercise?

Fatigue

None-----Worst Imaginable

Pain in your Muscles

None-----Worst Imaginable

Difficulty Concentrating

None-----Worst Imaginable



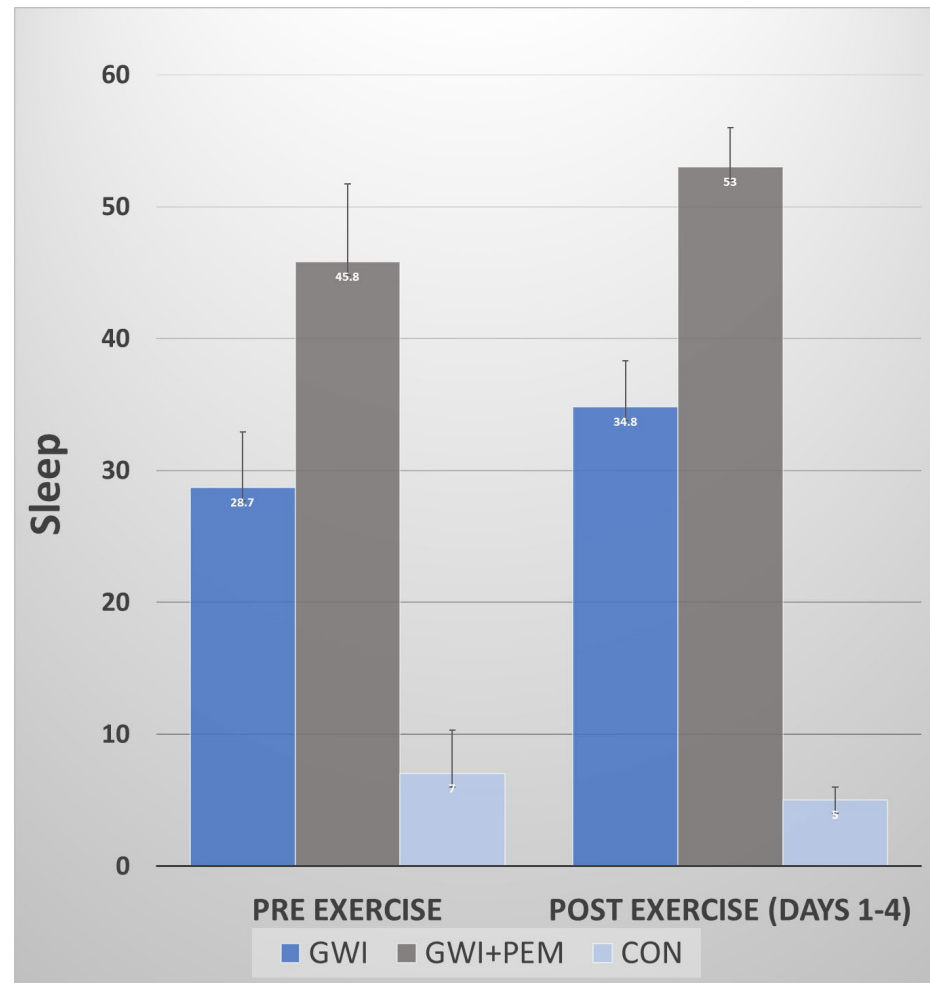
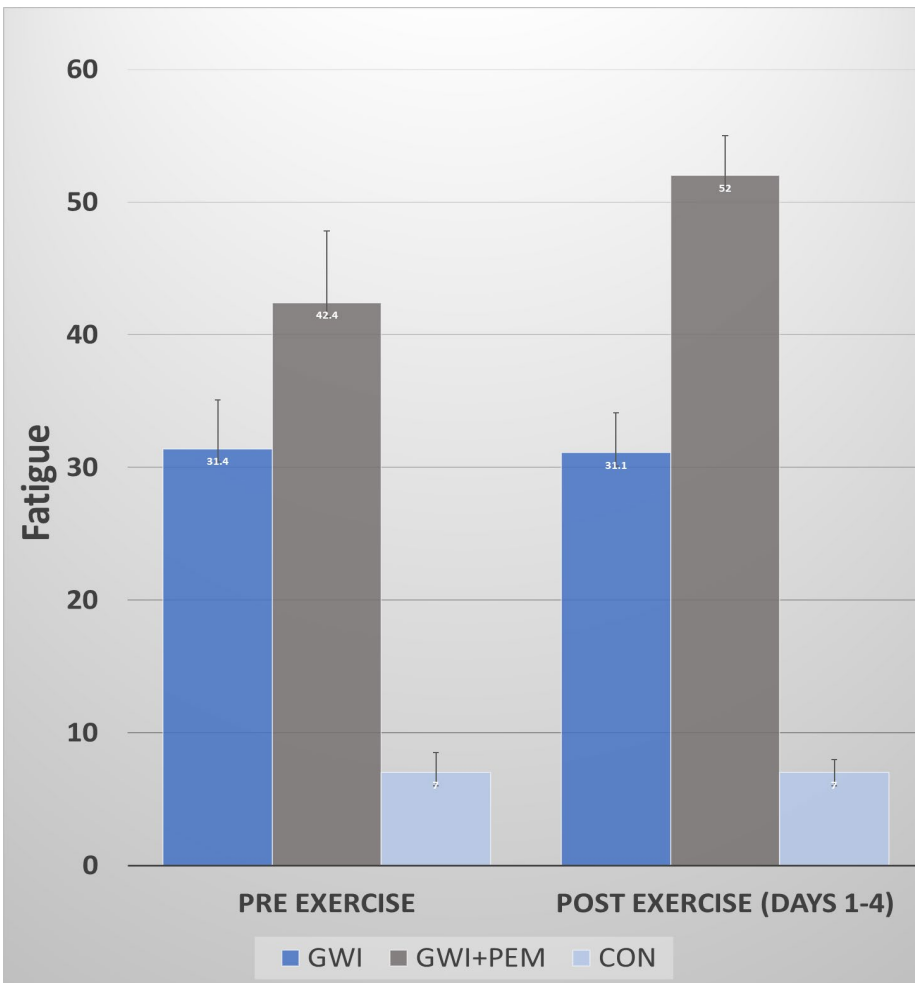
Statistical Analyses

- Linear Mixed Effects
 - Group, pre- post-exercise, Days of symptom measurement
 - Group stratification (GWI, GWI+PEM, CON)
 - Symptoms chosen a priori based on endorsement and category

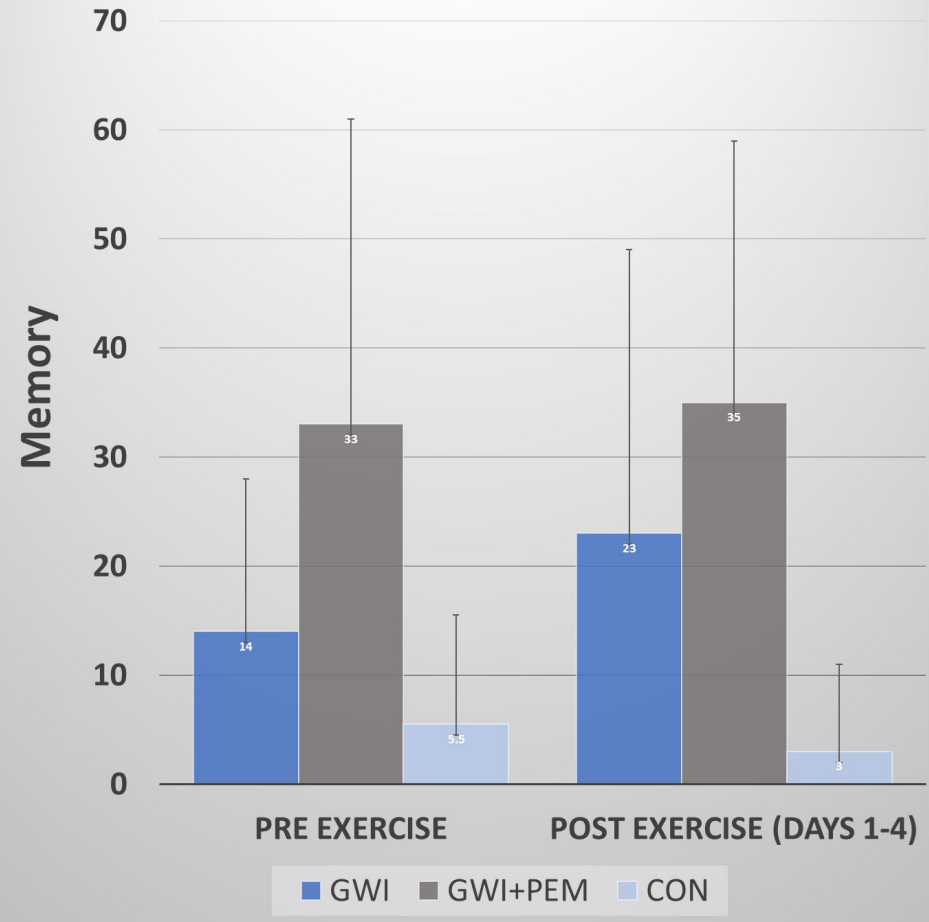
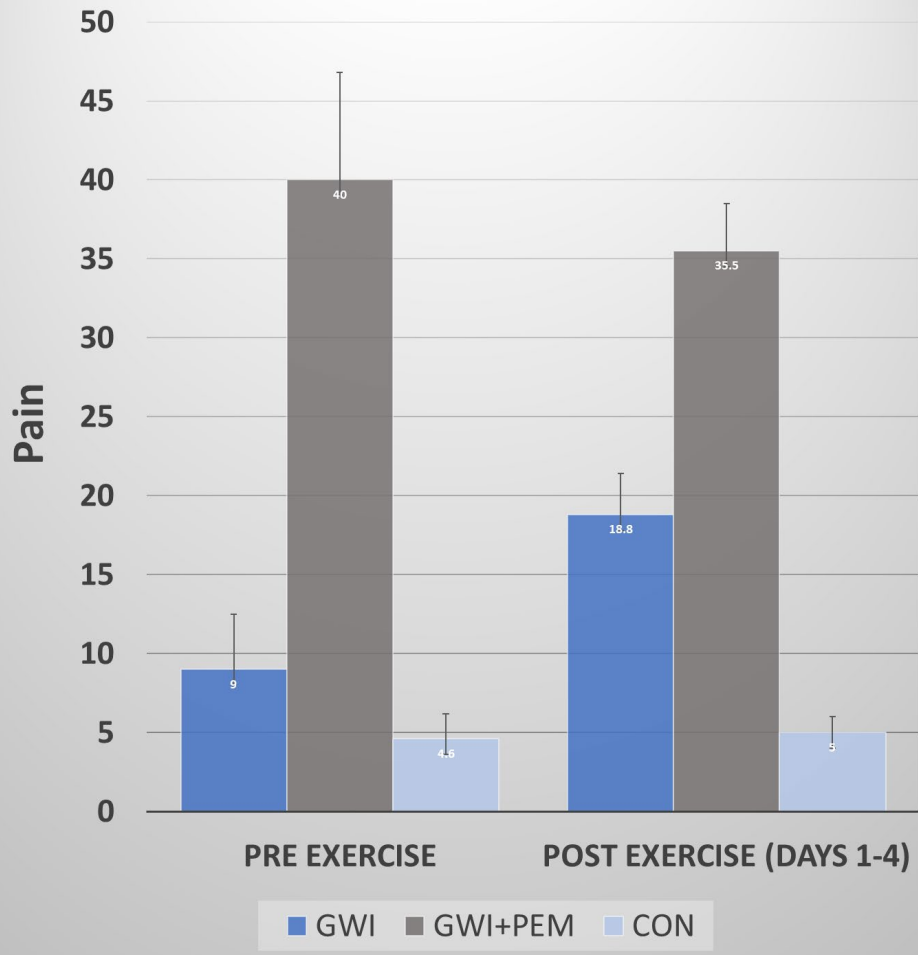
Demographic and Baseline Data

	GWJ (n=18)	GWJ+PEM (n=26)	CON (n=33)
Age (yrs)	52.3 (3.8)	52.2 (4.5)	52.3 (5.2)
Height (m)	1.8 (.08)	1.8 (.08)	1.8 (.10)
Weight (kg)	102.2 (19.2)	94.5 (18.6)	89.9 (15.7)
BMI	32.1 (5.8)	30.3 (5.7)	29.4 (4.8)
SF-36 PCS	64.1 (16.9)	55.8 (17.8)	89.5 (7.7)
SF-36 MCS	55.2 (21.9)	52.4 (16.8)	88.3 (8.0)
MFI Total	64.7 (13.0)	71.2 (11.0)	35.1 (11.8)
MPQ Total	1.9 (1.7)	2.9 (2.1)	0.3 (0.4)

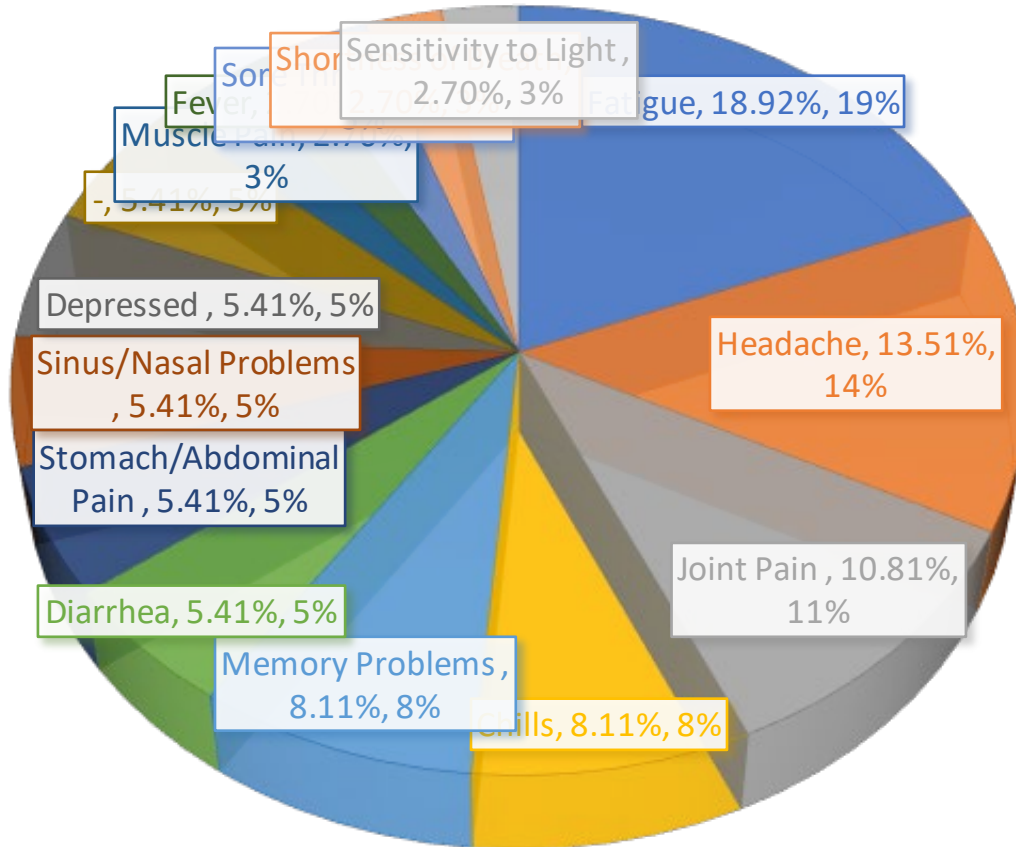
Group X Pre-Post Exercise Interaction ($p < 0.01$ *corrected)



Group X Pre-Post Exercise Interaction ($p < 0.01$ *corrected)



Peak Symptom responses





Conclusions & Future Directions

- GWI is associated with PEM
- No clear pattern of symptom worsening
- PEM endorsement important

- Biological predictors of PEM need to be explored

The background of the slide is a faded ECG (heart rate) tracing on a grid. The grid consists of small orange dots forming a fine grid and larger orange lines forming a coarser grid. The ECG line is black and shows several cardiac cycles with distinct P waves, QRS complexes, and T waves.

Predicting PEM in GWI

Cardiopulmonary, perceptual, and physical function



Contents lists available at [ScienceDirect](#)

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie



Predicting post-exertional malaise in Gulf War Illness based on acute exercise responses

Alexander E. Boruch^{a,b}, Jacob B. Lindheimer^{a,b,c}, Jacquelyn C. Klein-Adams^d,
Aaron J. Stegner^{a,b}, Glenn R. Wylie^{d,e,f,g}, Jacob V. Ninneman^{a,b}, Thomas Alexander^d,
Nicholas P. Gretzon^{a,b}, Bishoy Samy^d, Stephanie M. Van Riper^{a,b}, Michael J. Falvo^{d,f,g,1},
Dane B. Cook^{a,b,1,*}

Purpose

To test whether select
metabolic and perceptual
exercise responses predict
PEM in GWI

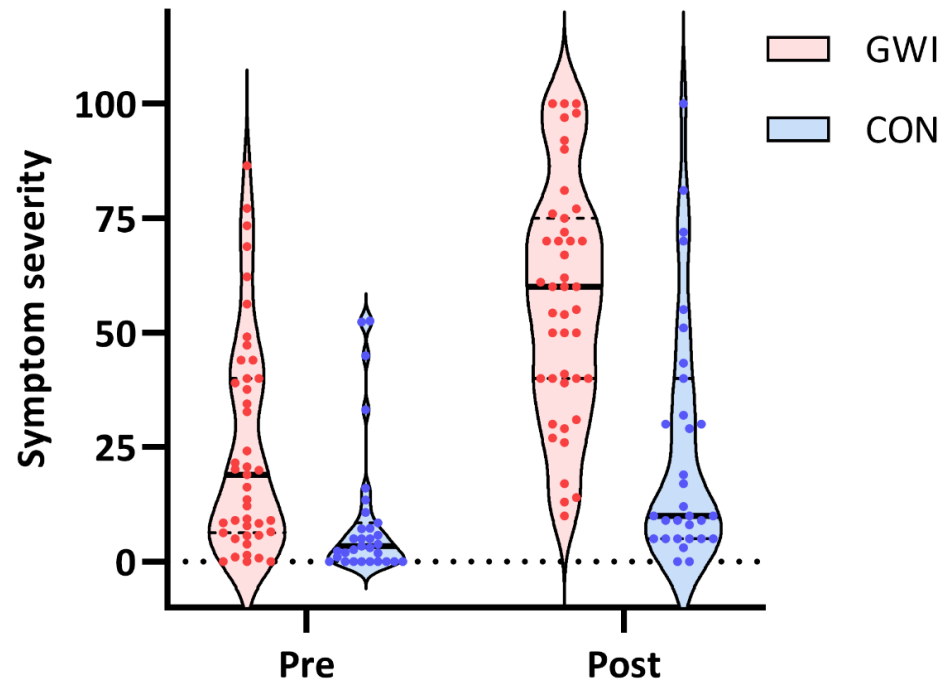
Statistical Analyses

- General Linear Regression Model
 - Independent Variables:
 - VR-36 Physical Component Score (PCS) – illness severity
 - VE/VCO₂ – ventilatory efficiency / clinical utility
 - Peak leg muscle pain – peripheral afferent signaling
 - Cumulative work – power*duration
 - Dependent Variable:
 - Peak PEM response – change from 1-week pre to 1-week post-exercise

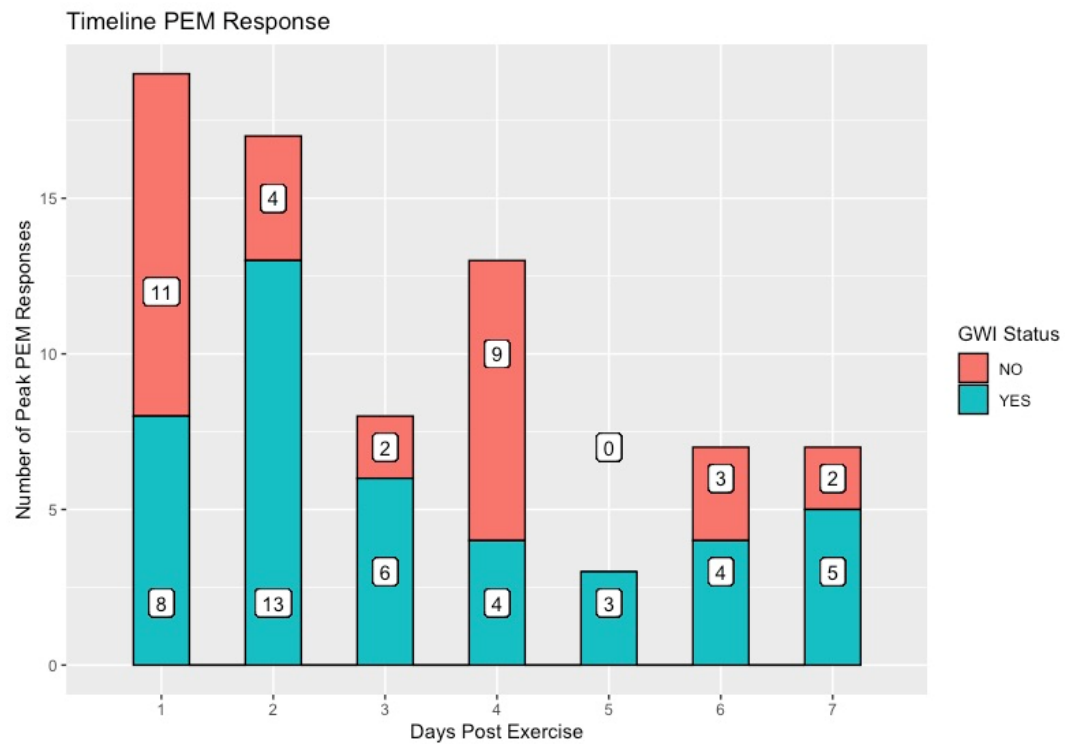
Steady state exercise response

	GWI	CON	Effect Size	
	(n = 43)	(n = 31)	GWI vs. CON	
	Mean (SD)	Mean (SD)	Hedges' g	95% CI
VO₂ (mL•kg•min⁻¹)*	16.15 (3.61)	19.42 (5.03)	-0.76	(-1.34, -0.28)
VCO₂ (mL)*	1402.14 (332.46)	1587.32 (342.85)	-0.54	(-1.02, -0.07)
VE (L•min⁻¹)	42.60 (12.46)	44.98 (9.84)	-0.21	(-0.67, 0.26)
VE/VO₂*	29.87 (4.49)	28.07 (4.04)	0.49	(0.02, 0.96)
VE/VCO₂	28.63 (4.45)	27.15 (4.00)	0.40	(-0.07, 0.86)
Heart rate (bpm)	134.75 (11.64)	135.46 (9.52)	0.07	(-0.44, 0.57)
Work (kJ)*	235.66 (35.27)	253.22 (23.27)	-0.50	(-0.98, -0.03)
Peak leg muscle pain*	4.45 (2.54)	2.66 (2.51)	0.70	(0.22, 1.18)

Median PEM response



Peak PEM timeline



$$F = G \frac{m_1 m_2}{d^2}$$

Model did not explain
PEM

Pooled $R^2 = 0.15$, Adjusted $R^2 = 0.03$, $p = 0.34$

$$\frac{\partial^2 u}{\partial t^2} = c \frac{\partial^2 u}{\partial x^2}$$

$$\frac{df}{dt} = \lim_{h \rightarrow 0} \frac{f(t+h) - f(t)}{h}$$

$$e^{\frac{(x-\mu)^2}{2\sigma^2}}$$
$$ds \geq 0$$

$$E = mc^2$$

$$E + V = 2$$

Conclusions & Future Directions

- Metabolic and perceptual responses during exercise alone do not predict PEM
- Other combinations of cardiopulmonary responses
- Alternative and complimentary measures of PEM
- Additional biological predictors of PEM need to be explored



Mediators of PEM

Exercise-induced changes in gene
expression

Purpose

- To determine gene expression responses to exercise challenge in GWI
- To determine whether gene expression changes mediate PEM in GWI



Gene Panel: n = 13 Genes of Interest

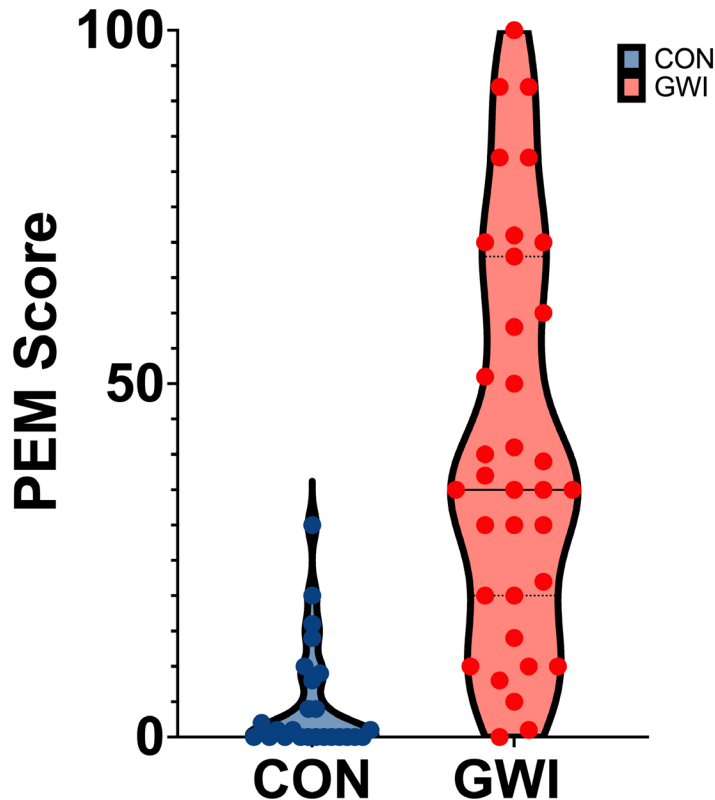
Gene (n = 13)	Domain	Assay ID
Beta -actin (ACTB)	Adrenergic	Hs01060665_g1
Beta-2 adrenergic receptor (ADRB2)	Adrenergic	Hs00240532_s1
Catechol-O-methyltransferase (COMT)	Adrenergic	Hs00241349_m1
Nuclear receptor subfamily 3, group C member 1 (NR3C1)	Cortisol	Hs00353740_m1
Cluster of differentiation 14 (CD14)	Immune	Hs02621496_s1
Interleukin 6 (IL6)	Immune	Hs00985639_m1
Lymphotoxin alpha (LTA)	Immune	Hs04188773_g1
Toll-like Receptor 4 (TLR4)	Immune	Hs00152939_m1
Interleukin 10 (IL10)	Immune	Hs00961662_m1
Acid Sensing Ion Channel 3 (ASIC3)	Metabolic	Hs00245097_m1
Purinoceptor 4 (P2RX4)	Metabolic	Hs00602442_m1
Purinoceptor 5 (P2RX5)	Metabolic	Hs01112471_m1
Transient receptor potential cation channel subfamily V member 1 (TRPV1)	Metabolic	Hs00218912_m1

Statistical Analyses

- Gene Expression processed and analyzed by Pharmacogenomics Analysis Lab (PAL) - Central Arkansas Veterans Health System
 - Quantitative Polymerase Chain Reaction (qPCR) analyses (white blood cells)
- Doubly-Multivariate Repeated Measures (RM) MANOVA
 - RM ANOVA for select genes

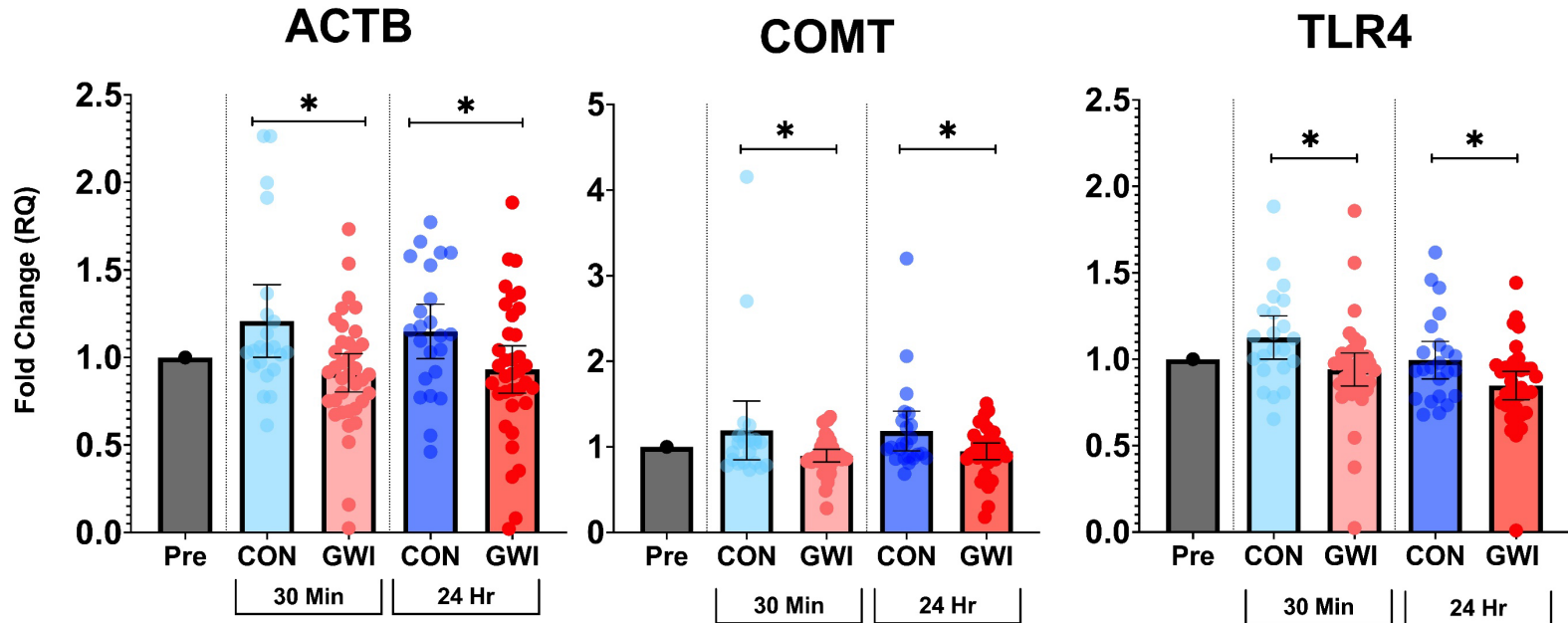
Peak symptom response

CDC VAS



CDC	PEM Score: Mean (SD)	Effect Size (<i>d</i>)
GWI N = 37	41.38 (27.87)	1.65 (1.06, 2.25)
CON N = 25	4.8 (7.80)	*

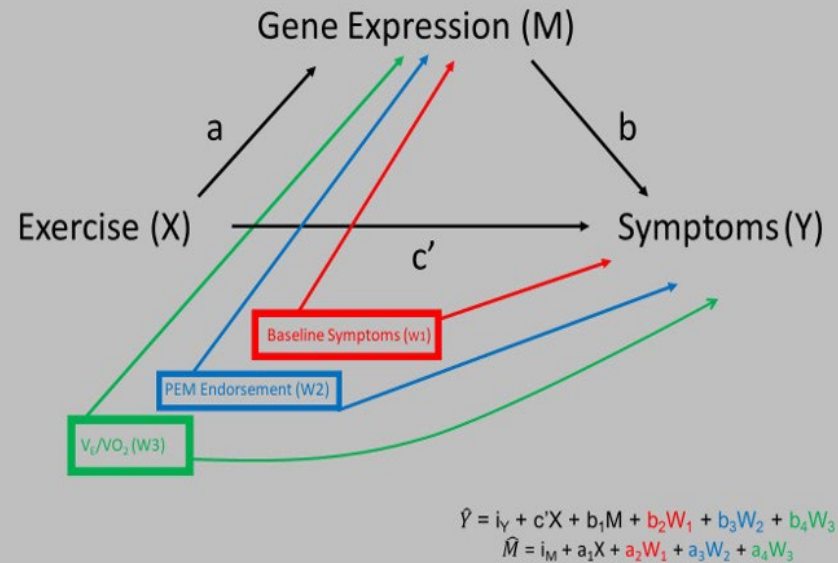
Differential changes in gene expression



RM-ANOVA				
Gene	Group:	partial η^2	30 Min Post	24 Hr Post
ACTB	<0.01	0.09	<0.05	<0.05
COMT	<0.01	0.10	<0.05	<0.05
TLR4	<0.001	0.10	<0.05	<0.05

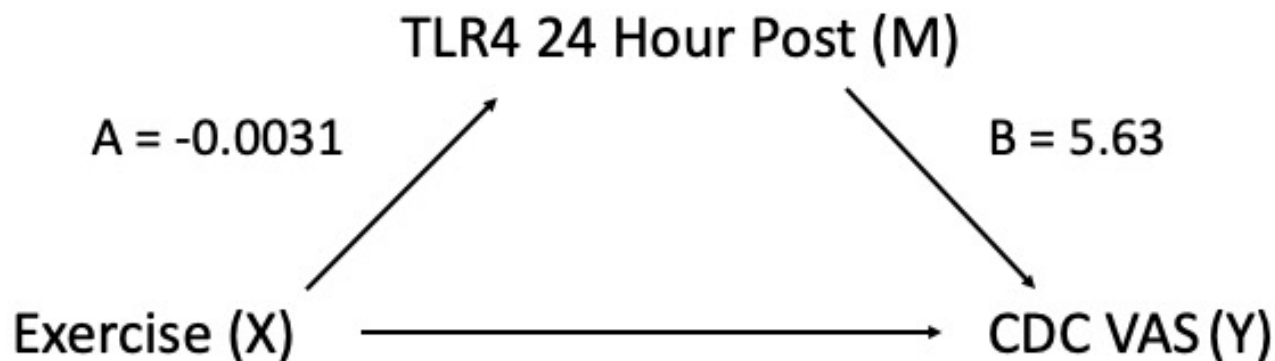
Mediation: CDC VAS

- C' = Direct Effect
- $A * B$ = Indirect Effect
- X = Independent Variable
- Y = Dependent Variable
- M = Mediator
- W = Moderator



No mediation

TLR4 24 Hour Post - CDC VAS



Direct Effect (C') = -0.12; 95% CI = (-0.35, 0.10)

Indirect Effect (A*B) = -0.02; 95% CI = (-0.07, 0.05)

Conclusions

- GWI had differential changes in gene expression to exercise challenge
 - Unexpected direction of change
- Gene expression alone did not explain PEM
 - Alternative and complimentary measures of PEM
 - Additional biological predictors of PEM need to be explored



Overall Discussion

- PEM occurs in GWI:
 - Not in all Veterans
 - Variable – intensity, type, timecourse
- Exercise responses differ
 - Model was not explanatory
- Gene expression responses differ
 - Not a mediator
- Multiple physiological systems within the same model (interactions) may be necessary to explain both GWI and PEM



Collaboration with Columbia

Center of Infection and Immunity (Dr. Ian Lipkin)



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Veterans Health Administration



WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON



RUTGERS
New Jersey Medical School



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- Nick Gretzon
- Jake Ninneman
- Neda Almassi
- Susan Schroeder
- Stephanie Van Riper, MS

- Mike Falvo, PhD (PI)
- Glenn Wylie, DPhil
- Jorge Serrador, PhD
- Jackie Klein-Adams, MS
- Duncan Ndirangu, BS
- Tom Alexander, MS
- Matt Watson, MS
- Bishoy Samy, MS
- Holly Klecha, BS
- Wei Qian, MS
- Nancy Eager, BS



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Thank you



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Discussion: Aim 1

ACTB

Beta-actin protein
Cell/receptor motility

Neuro/Cognitive/Mood
& GI Symptoms

COMT

Catecholamine
degradation

Impaired spatial
orientation,
depression, anxiety,
(Cognitive/Mood)

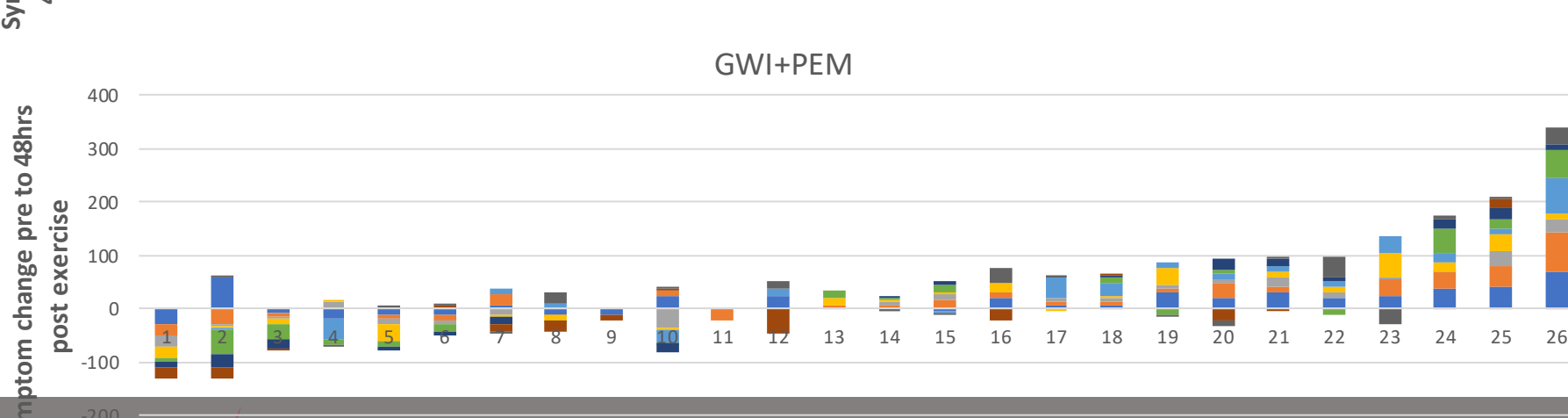
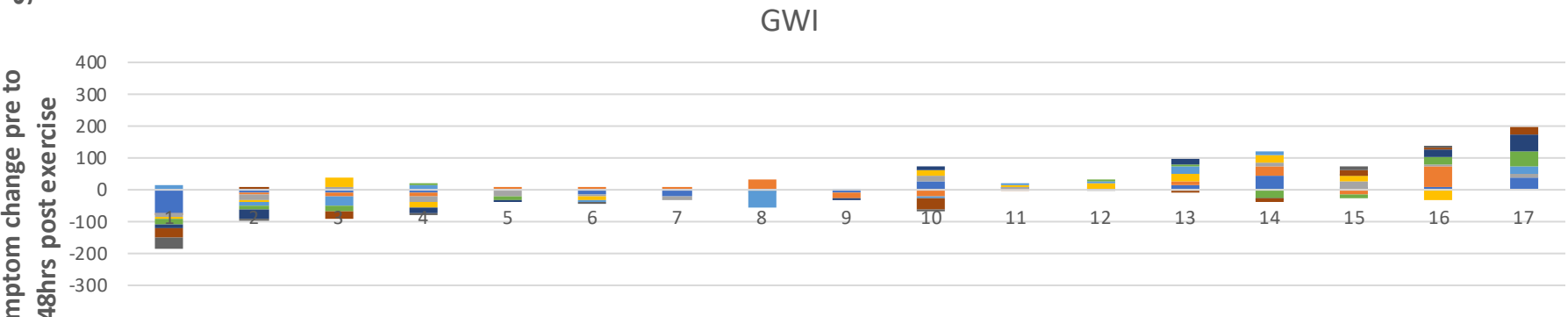
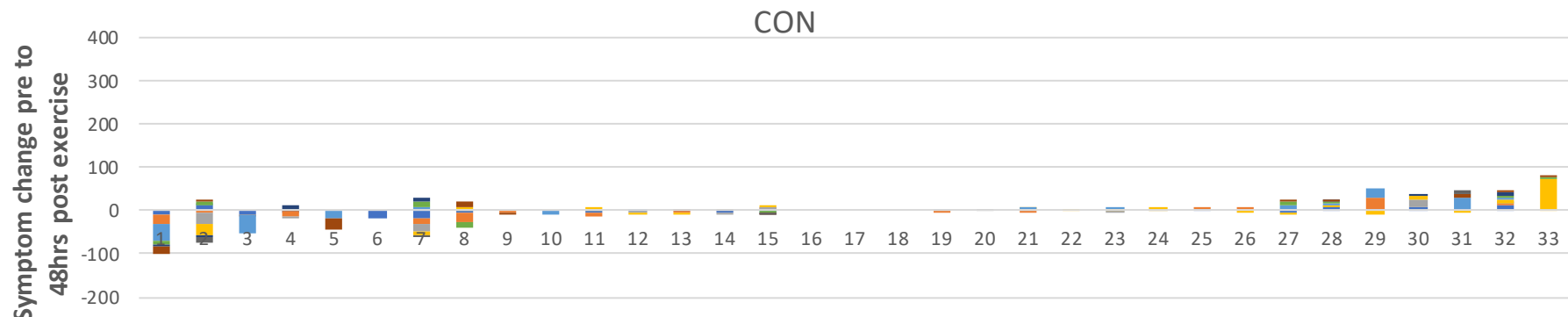
TLR4

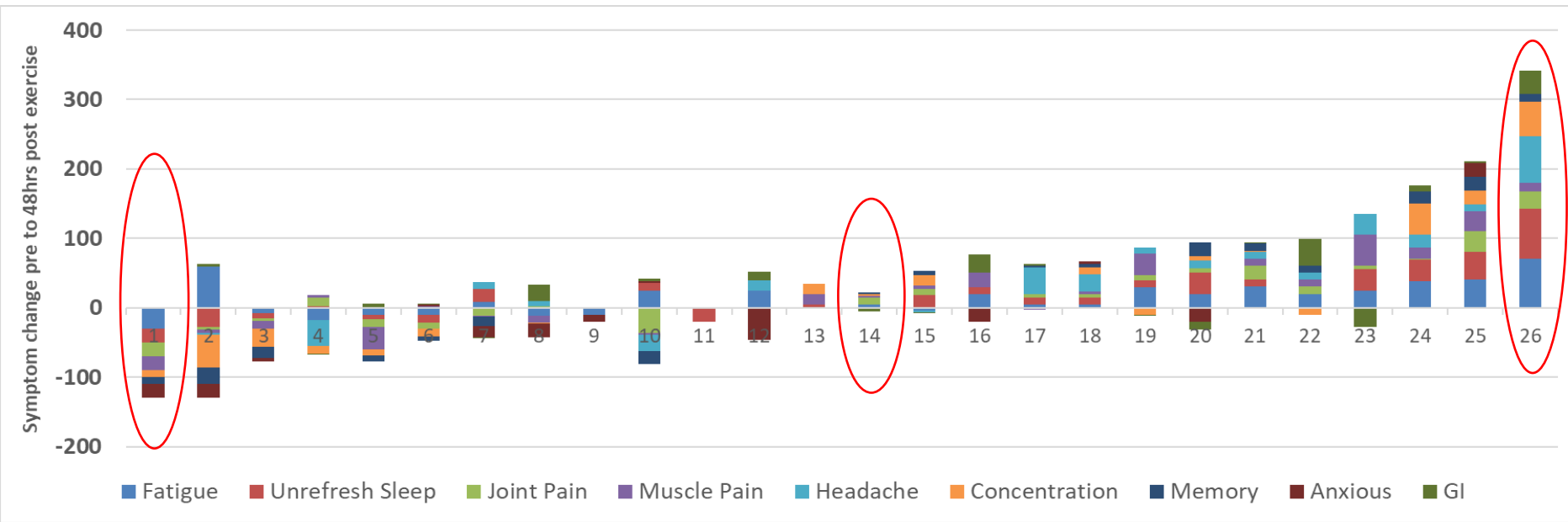
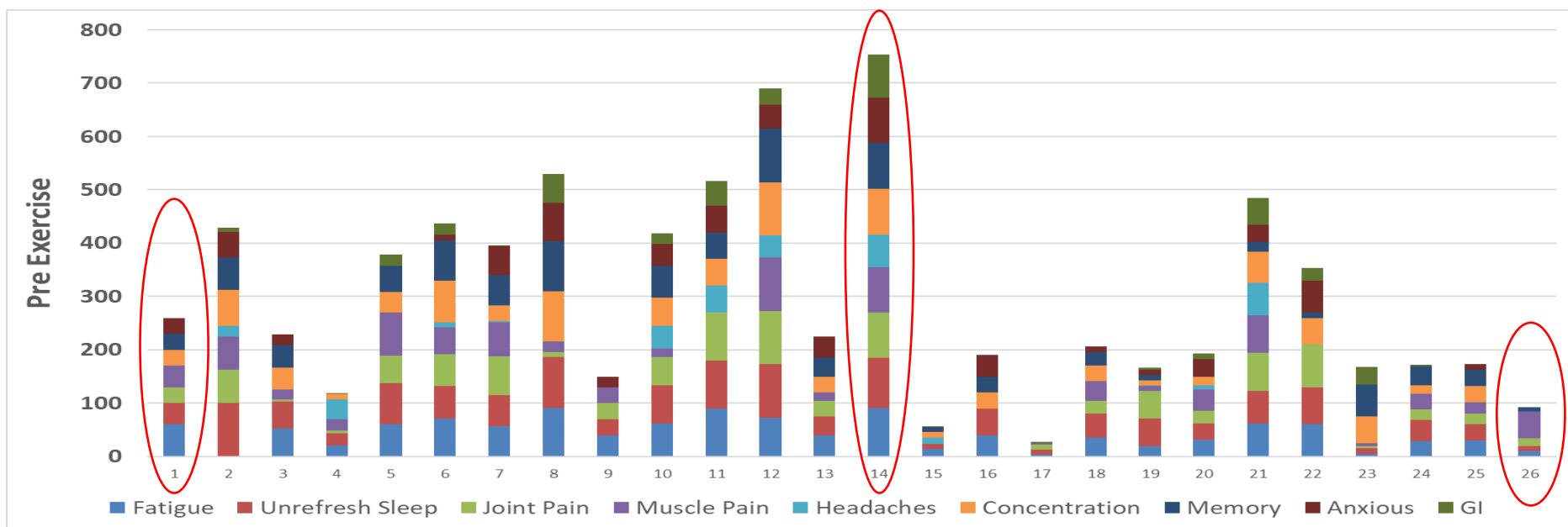
Pro-inflammatory
Mediator

Pain (different in males
vs. females) &
Cardiovascular
Function

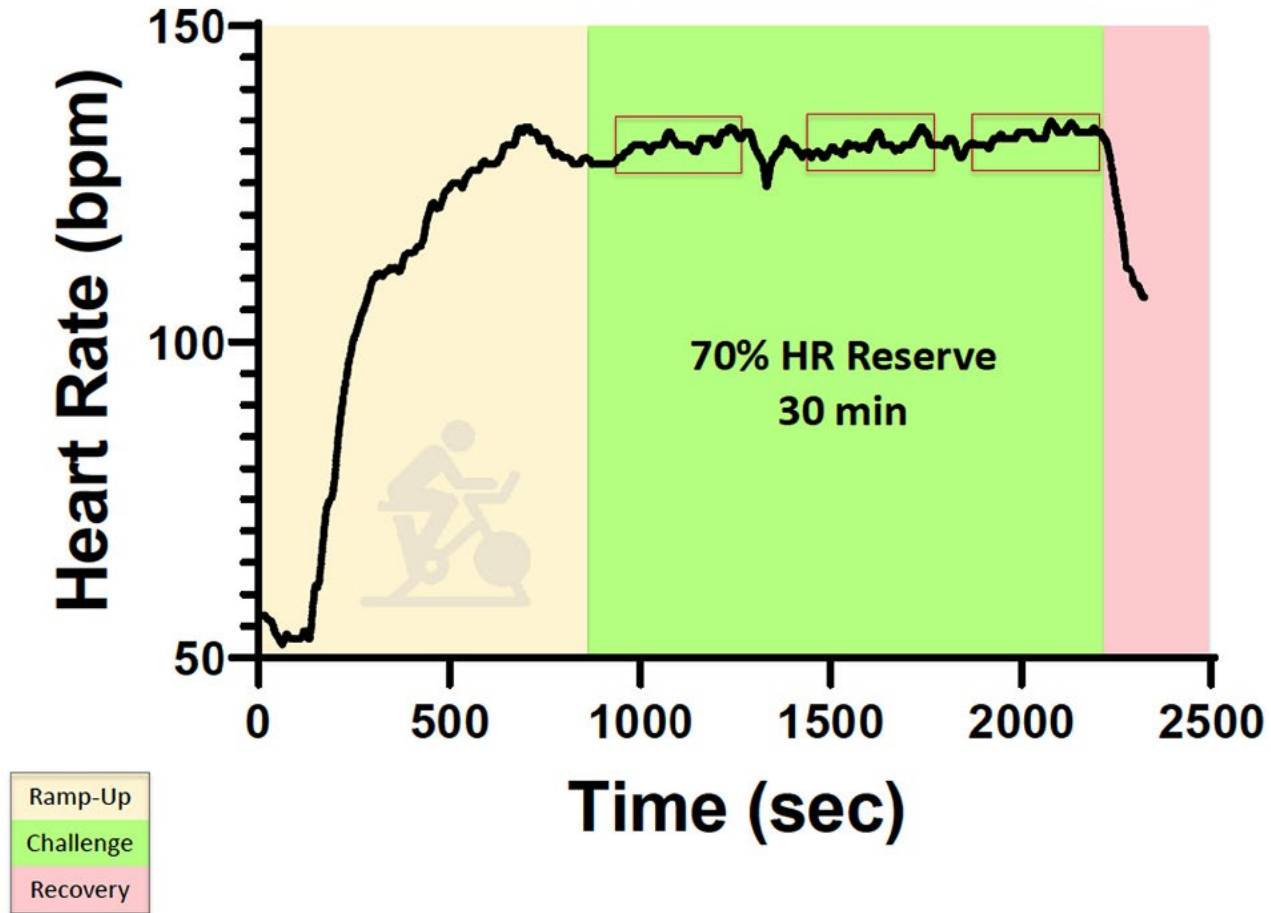


PEM: Group Differences & Variability





Metabolic data processing



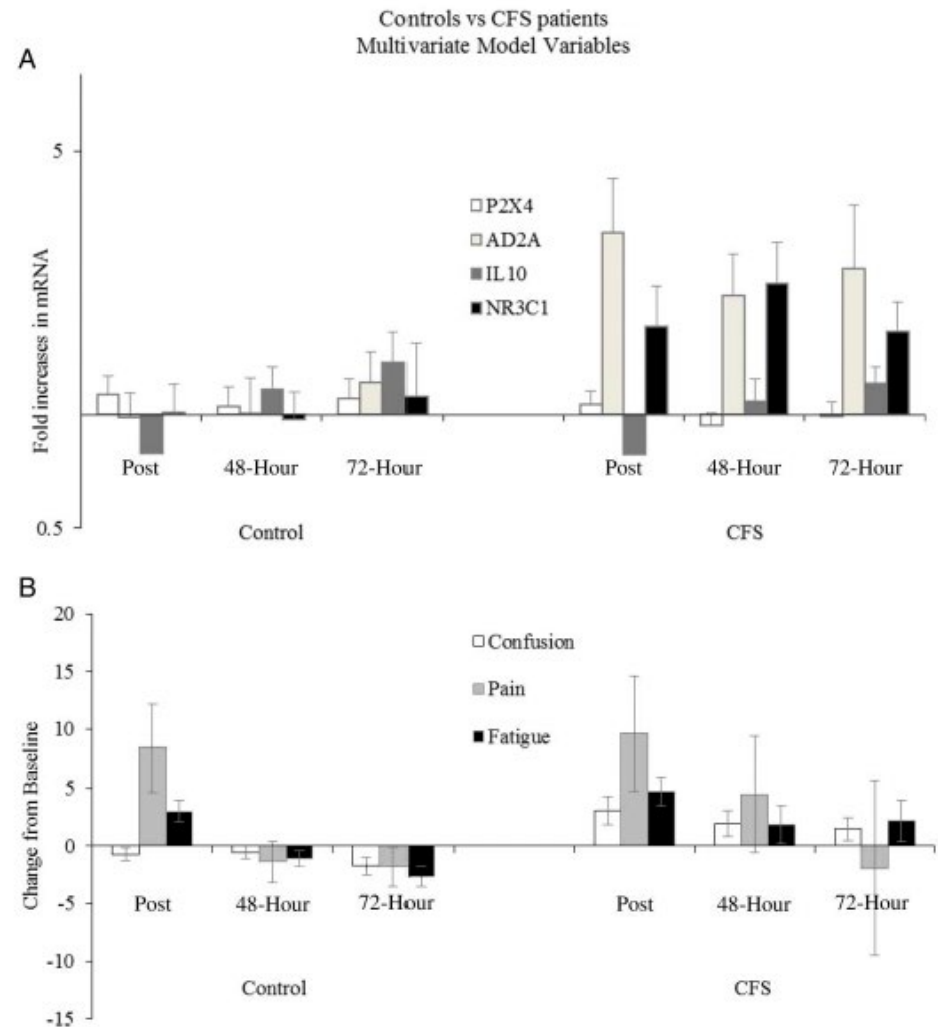
Exercise & Genetics - Considerations

Meyer et al., 2013

Maximal exercise test – no differences in VO_2

Associations between gene expression (mRNA) and fatigue, pain, and confusion symptoms

Fatigue, confusion, and pain all correlated with NR3C1 (glucocorticoid receptor)



Demographics & Baseline Symptoms:

	GWI (n = 37)	CON (n = 25)	GWI vs. CON		GWI vs. CON	
	Mean (SD)	Mean (SD)	t-statistic	p-value	Cohen <i>d</i>	95% CI
Age	51.75 (4.04)	52.56 (5.58)	-0.62	0.54	-0.17	(-0.69, 0.35)
Sex (Male/Female, %)	34/3 (91.8%)	24/1 (96%)	NA	0.64	NA	NA
Height (m)	1.76 (0.08)	1.76 (0.09)	0.06	0.95	0.02	(-0.50, 0.54)
Weight (kg)	95.97 (17.72)	88.63 (15.71)	1.69	0.09	0.43	(-0.10, 0.96)
BMI (kg/m ²)	30.87 (5.36)	28.53 (4.34)	1.88	0.06	0.47	(-0.06, 1.00)
Kansas*	27.35 (12.79)	2.78 (2.75)	11.31	<0.001	2.44	(1.76, 3.12)
VR-36 PCS*	59.72 (17.60)	90.46 (7.46)	-9.34	<0.001	-2.14	(-2.79, -1.49)
VR-36 MCS*	51.66 (17.76)	89.09 (7.92)	-11.15	<0.001	-2.57	(-3.27, -1.87)
FSS*	43.61 (13.89)	18.32 (7.99)	8.99	<0.001	2.13	(1.49, 2.78)
MFI Total*	70.25 (13.99)	33.36 (11.84)	11.1	<0.001	2.8	(2.08, 3.53)
SF-MPQ-2*	1.31 (1.08)	0.17 (0.56)	5.41	<0.001	1.25	(0.69, 1.81)
PSQI*	12.67 (4.22)	6.87 (3.58)	6.4	<0.002	1.46	(0.84, 2.09)
POMS TMD*	33.17 (19.45)	-0.68 (8.11)	9.34	<0.001	2.14	(1.49, 2.78)

NOTE. * = Significant difference between groups at p<0.05. **Kansas** = Kansas Symptom Inventory; **VR-36 PCS** = Veterans RAND 36-item Healthy Survey Physical Component Score; **VR-36 MCS** = Veterans RAND 36-item Healthy Survey Mental Component Score; **FSS** = Fatigue Severity Scale; **MFI Total** = Multiple Fatigue Inventory; **SF-MPQ-2** = Short Form McGill Pain Questionnaire; **PSQI** = Pittsburgh Sleep Quality Index; **POMS TMD** = Profile of Mood States Total Mood Disturbance.

Cardiopulmonary Exercise Testing (CPET) Response:

	GWI	CON	GWI vs. CON		GWI vs. CON	
	(n = 37)	(n = 25)	t-statistic	p-value	Effect Size (Cohen d)	95% CI
VO₂ (mL·kg·min⁻¹)*	14.94 (2.73)	20.11 (4.99)	-4.58	<0.01	1.28	(0.68, 1.88)
VCO₂ (mL)*	1335.74 (351.17)	1600.07 (360.83)	-2.72	<0.01	0.73	(0.18, 1.29)
VE (L·min⁻¹)	39.40 (10.41)	45.06 (11.37)	-1.9	0.06	0.51	(-0.03, 1.06)
V_T	1.94 (0.47)	2.17 (0.43)	-1.86	0.07	0.49	(-0.05, 1.05)
f_R	25.33 (4.70)	25.72 (4.68)	-0.3	0.76	0.08	(-0.46, 0.62)
VE/VO₂	29.61 (4.68)	27.85 (3.97)	1.46	0.15	-0.4	(-0.95, 0.16)
VE/VCO₂	28.20 (3.89)	26.85 (4.11)	1.22	0.23	-0.33	(-0.89, 0.22)
RER	0.92 (0.08)	0.91 (0.04)	0.4	0.69	-0.11	(-0.66, 0.44)
RPM	64.15 (7.42)	62.74 (14.71)	0.42	0.67	-0.12	(-0.68, 0.43)
Power*	72.11 (17.53)	100.59 (34.50)	-3.69	<0.01	1.03	(0.44, 1.62)
HR	134.94 (11.69)	136.20 (8.65)	-0.45	0.65	0.12	(-0.42, 0.66)
% of Test in HRR 70	95.10 (7.96)	97.95 (5.70)	-1.47	0.15	0.4	(-0.16, 0.97)
O₂ Pulse*	10.49 (2.42)	13.01 (3.08)	-3.19	<0.01	0.9	(0.31, 1.49)
Cumulative Work (kJ)*	237.43 (38.22)	254.28 (21.96)	-2.07	<0.05	0.52	(-0.02, 1.06)
Peak Leg Pain*	4.92 (2.49)	2.50 (2.11)	3.69	<0.01	-1.01	(-1.62, -0.41)
Peak RPE*	15.09 (2.18)	13.6 (1.72)	2.62	<0.05	-0.73	(-1.34, -0.13)
Peak Fatigue*	6.48 (2.03)	4.08 (1.96)	10.54	<0.01	-1.18	(-1.81, -0.55)

Note. VO₂ = oxygen consumption; VCO₂ = carbon dioxide consumption; VE = minute ventilation; V_T = Tidal Volume; f_R = Respiratory Frequency; VE/VO₂ = ventilatory equivalent for oxygen; VE/VCO₂ = ventilatory equivalent for carbon dioxide; RER = Respiratory Exchange Ratio; RPE = Rating of Perceived Exertion. Cardiopulmonary values are represented as averages across three steady-state periods that were identified during data processing. Perceptual ratings indicate the highest rating recorded during steady-state exercise. Positive and negative effect sizes indicate larger values in GWI and CON groups, respectively.

Aim 2: Primary & Secondary Analyses

GWI participants ONLY

Primary: Gene expression as mediator of symptom changes at 24h post-exercise

- **Exercise** (X; independent variable) – cumulative work (kJ)
- **Symptoms** (Y; dependent variable) – peak change CDC VAS at 24h **in lab**
- **Gene Expression** (M; mediator) – RQ
- Covariates: Age, BMI, baseline symptom scores
- Moderators (W_x) - PEM endorsement (W_2), and V_E/VO_2 (W_3) during exercise

Secondary: Gene expression as mediator of symptom changes days 1-5 post-exercise

- **Exercise** (X; independent variable) – cumulative work (kJ)
- **Symptoms** (Y; dependent variable) – peak change KSI VAS **at home**
- **Gene Expression** (M; mediator) – RQ
- Covariates: Age, BMI, baseline symptom scores
- Moderators (W_x) - PEM endorsement (W_2), and V_E/VO_2 (W_3) during exercise



Choose VA

Rockwood, 2023; Mediation analysis
version 4.0, 2021)

VA



U.S. Department
of Veterans Affairs

Primary Analysis: PEM & Gene Expression - Mediation

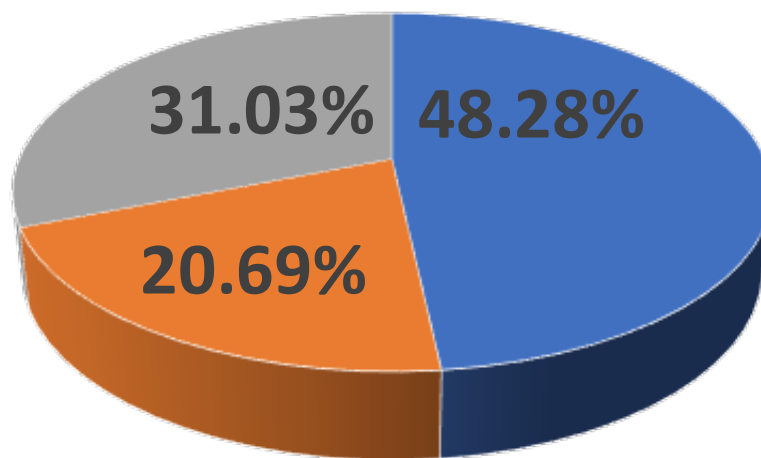
	CDC VAS Mediation Effect: 30 Min Post				CDC VAS Mediation Effect: 24 Hour			
Gene:	Direct:	95% CI	Indirect/Mediation:	Conclusion:	Direct:	95% CI	Indirect/Mediation:	Conclusion:
ACTB	0.06	(-0.38, 0.01)	(-0.14, 0.008)	No Mediation	<0.05	(-0.41, -0.009)	(-0.11, 0.03)	No Mediation
ADRB2	0.09	(-0.41, 0.03)	(-0.14, 0.02)	No Mediation	<0.05	(-0.45, -0.03)	(-0.03, 0.05)	No Mediation
ASIC3	0.07	(-0.40, 0.02)	(-0.16, 0.03)	No Mediation	0.054	(-0.42, 0.004)	(-0.10, 0.02)	No Mediation
CD14	0.07	(-0.39, 0.01)	(-0.14, 0.02)	No Mediation	0.051	(-0.41, 0.001)	(-0.11, 0.06)	No Mediation
COMT	0.054	(-0.37, 0.003)	(-0.16, 0.03)	No Mediation	<0.05	(-0.42, -0.003)	(-0.10, 0.02)	No Mediation
IL-6	<0.05	(-0.44, -0.01)	(-0.11, 0.04)	No Mediation	<0.05	(-0.45, -0.03)	(-0.03, 0.09)	No Mediation
IL-10	<0.05	(-0.42, -0.004)	(-0.11, 0.02)	No Mediation	0.055	(-0.43, 0.005)	(-0.11, 0.03)	No Mediation
LTA	0.07	(-0.40, 0.02)	(-0.11, 0.03)	No Mediation	<0.05	(-0.43, -0.02)	(-0.09, 0.05)	No Mediation
NR3C1	<0.05	(-0.44, -0.02)	(-0.05, 0.04)	No Mediation	<0.05	(-0.45, -0.03)	(-0.04, 0.05)	No Mediation
P2X4	<0.05	(-0.43, -0.008)	(-0.07, 0.03)	No Mediation	<0.05	(-0.44, -0.02)	(-0.03, 0.03)	No Mediation
P2X5	0.07	(-0.42, 0.02)	(-0.12, 0.02)	No Mediation	0.054	(-0.41, 0.004)	(-0.13, 0.02)	No Mediation
TLR4	<0.05	(-0.43, -0.01)	(-0.05, 0.04)	No Mediation	0.45	(-0.34, 0.16)	(-0.07, 0.02)	No Mediation
TRPV1	<0.05	(-0.44, -0.02)	(-0.05, 0.02)	No Mediation	0.4	(-0.36, 0.14)	(-0.04, 0.02)	No Mediation
GTF2B	NA	NA	NA	NA	NA	NA	NA	NA
PSMB6	<0.05	(-0.44, -0.03)	(-0.11, 0.02)	No Mediation	0.43	(-0.34, 0.15)	(-0.05, 0.05)	No Mediation
IPO8	<0.05	(-0.44, -0.02)	(-0.06, 0.05)	No Mediation	0.2	(-0.40, 0.09)	(-0.01, 0.16)	No Mediation

Note: Results lose Direct Effect with covariates Age, BMI, CDC 24 Pre-Scores

- Mediation was not observed at 30 min & 24-hour post-exercise
- Moderators (VE/VO₂ & PEM Endorsement) did not alter results (no mediation through moderation)

Secondary: Kansas VAS Breakdown

Kansas VAS PEM Responses



■ Fatigue ■ Pain ■ Neuro