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

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ABSTRACT

Aims: Post-exertional malaise (PEM) is poorly understood in Gulf War Illness (GWI). Exercise challenges have emerged as stimuli to study PEM; however, little attention has been paid to unique cardiorespiratory and perceptual responses during exercise. This study tested whether select exercise parameters explained variability in PEM responses.
Main methods: Visual analog scale (0–100) versions of the Kansas questionnaire were used for daily symptom measurements one week before and one week after 30-min of cycling at 70% heart rate reserve in 43 Veterans with GWI and 31 Veteran controls (CON). Cardiopulmonary exercise testing (CPET) methods were used to measure oxygen (VO_2), carbon dioxide (VCO_2), ventilation (VE), heart rate, work rate, and leg muscle pain. Symptom changes and CPET parameters were compared between groups with independent samples *t*-tests. Linear regression (GLM) with VE/VCO_2, cumulative work, leg muscle pain, and self-reported physical function treated as independent variables and peak symptom response as the dependent variable tested whether exercise responses predicted PEM.
Key findings: Compared to CON, Veterans with GWI had greater ventilatory equivalent for oxygen (VE/VO_2), peak leg muscle pain, fatigue, and lower VCO_2, VO_2, power, and cumulative work during exercise ($p < 0.05$), and greater peak symptom responses (GWI = 38.90 ± 29.06, CON = 17.84 ± 28.26, $g = 0.70$, $p < 0.01$). The final GLM did not explain significant variance in PEM (Pooled $R^2 = 0.15$, Adjusted $R^2 = 0.03$, $p = 0.34$).
Significance: The PEM response was not related to the selected combination of cardiorespiratory and perceptual responses to exercise.

1. Introduction

Following deployment to Operations Desert Storm and Desert Shield in 1990–91, approximately 35% of the ~767,000 returning U.S. Veterans began reporting multiple chronic and debilitating symptoms. These symptoms—primarily fatigue, pain, and problems with concentration and memory (i.e., cognitive fog) —are core components of the Gulf War Illness (GWI) case definition [1–3]. Another GWI symptom is feeling unwell after exercise or exertion [3]. We have previously interpreted this symptom as an endorsement of having experienced post-exertional malaise (PEM) [4], a phenomenon that is commonly conceptualized as a debilitating exacerbation fatigue, pain,

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or other symptoms lasting 24 h or more following exercise. Given the incomplete understanding of GWI pathophysiology, examining PEM in the laboratory setting via exercise challenge has gained currency among GWI researchers because of its potential to make pathophysiology more apparent, an approach also promoted in research involving people with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [5].

Despite observing that Gulf War Veterans (GWV) with GWI who endorse PEM (~47%) have poorer health-related functioning and cardiopulmonary responses to exercise, we also showed that PEM is not uniformly present for all GWV with GWI at 24 h post-exercise [4]. However, variability in the type, time-course, and severity of symptom exacerbation in people with ME/CFS [6–8] suggests that our prior approach to measuring PEM responses in GWI warrants consideration [4]. Conventionally, PEM studies have compared specific symptoms (e.g., fatigue) and/or post-exercise timepoints (e.g., 24 h post-exercise) between ill and healthy participants [7–9]. However, because of heterogeneity in GWI symptom profiles, PEM may vary from person to person in terms of the specific symptoms affected by physical exertion and the severity and time-course of the response. For instance, some participants whose primary GWI symptom is fatigue may show large changes in fatigue whereas others whose primary GWI symptom is musculoskeletal pain may show greater changes in muscle pain. Likewise, some participants may experience peak PEM at 24 h post-exercise whereas this response may occur at later timepoints for others (e.g., 48–72 h post-exercise). In the below methods section, we describe a novel approach for addressing these issues.

Exercise challenge is a useful research tool for studying PEM because it can be used to deliver a standardized physiological stressor [4,10,11]. However, relatively little attention has been paid to the unique respiratory, metabolic, and perceptual responses that occur during exercise and how these responses might contribute to symptom exacerbation. For instance, maximal CPET has revealed distinct respiratory patterns among GWV with GWI [12], and submaximal CPET shows that GWV with GWI have less efficient ventilation and rate exercise as more painful and effortful than non-ill GWV [4]. Given that these responses differ between GWV with and without GWI and occur during administration of the same stimulus being used to elicit symptom exacerbation, a logical assumption is that data collected *during* CPET may help better understand PEM.

The present study extends our previous findings by testing whether certain metabolic and perceptual exercise responses predict PEM [4]. We hypothesized that select cardiopulmonary and perceptual exercise responses would explain significant variability in the PEM response.

2. Methods

The present study is part of an ongoing multi-site investigation examining brain, autonomic, and immune function in GWV with GWI (Department of Veterans Affairs Merit Review Award #I01CX001329: Cook & Falvo: PIs) where participants complete three study visits over a 10-day span (Fig. 1). An in-depth description of inclusion/exclusion criteria, participant recruitment, and sample characteristics can be found in our prior work [4]. All study procedures were approved by the institutional review boards (IRB) and the Research and Development Committees of the University of Wisconsin – Madison (Protocol #2015-1226), Madison VA and the Department of Veterans Affairs, New Jersey Health Care System (#01332). All participants provided informed consent according to the Declaration of Helsinki prior to testing.

2.1. Participants and inclusion/exclusion criteria

Seventy-four GWVs, ages 45 to 65, who were deployed to the 1990–1991 Persian Gulf Operations Desert Storm/Desert Shield were recruited within the Veteran Integrated Service Networks '3' (NJ) and '12' (WI). GWV with GWI (n = 43) were required to meet the Kansas Case Definition criteria via the endorsement of moderately severe symptoms (2 on a scale of 0–3) in at least three of the following domains: pain, fatigue, neurological/cognitive/mood, skin, gastrointestinal, and respiratory – with symptoms first becoming a problem during or after the Gulf War [3]. GWVs who did not endorse having an illness associated with Gulf War deployment and were otherwise healthy as described previously [4] were considered healthy controls (CON, N = 31).

Potential participants were excluded if they met criteria for major psychotic/mood disorders or illicit substance abuse, based on the Mini-International Neuropsychiatric Interview. Exclusionary medications were beta & calcium channel blockers, anti-convulsants, non-steroidal anti-inflammatory drugs within 48-h of testing, unstable use of psychotropic medications (<3 months), or use of multiple sedatives. Per

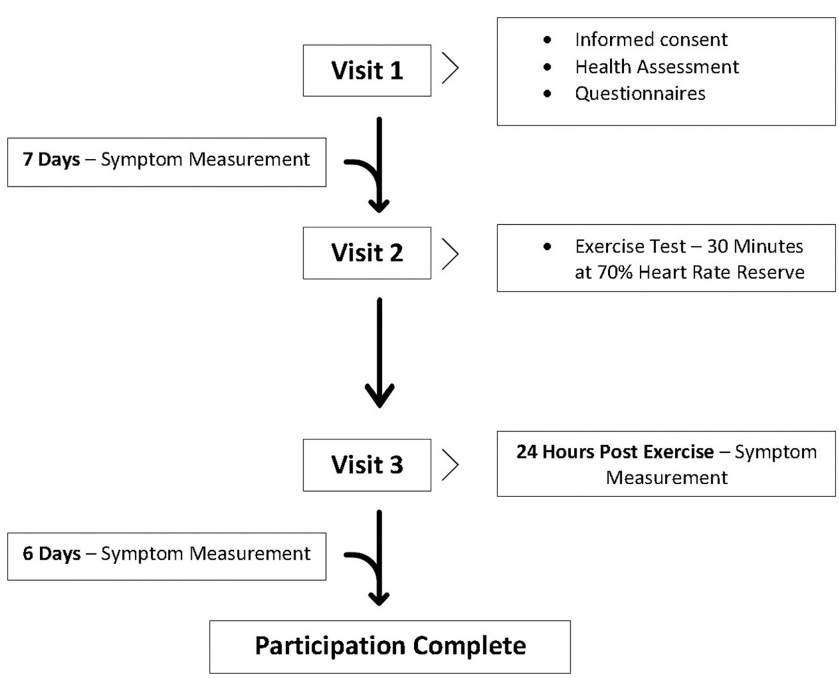

Fig. 1. Flow chart of study procedures.
Note. Visit 1 consists of consenting and screening, demographic and physical/mental health questionnaires, a blood draw, autonomic testing, and a magnetic resonance imaging (MRI) scan. Seven days later, participants return for Visit 2 and complete a second blood draw, a submaximal exercise test, and a post-exercise blood draw. Participants return to the lab on the following day for Visit 3 to complete an additional blood draw, autonomic test, and MRI scan. Symptom measurements are obtained on a daily basis via at-home questionnaires for seven days prior to and following exercise testing.

Kansas Case definition criteria, participants were also excluded if they presented with chronic conditions that might explain their symptoms (e.g., cancer, rheumatoid arthritis) or absolute contraindications to exercise testing according to American College of Cardiology/American Heart Association guidelines [13].

2.2. Participant characteristics

Baseline questionnaires were administered to characterize participant demographics and mental/physical health symptoms. Questionnaires included: 1) demographic and medical history; 2) the Veterans Rand 36-item Medical Health Survey (VR- 36) [14]; 3) the Pittsburgh Sleep Quality Index [15]; 4) the short-form McGill Pain Questionnaire-2 (SF-MPQ-2) [16]; 5) the Fatigue Severity Scale (FSS) [17]; 6) the Multidimensional Fatigue Inventory (MFI) [18].

2.3. Submaximal exercise challenge

Participants cycled at 70% (±5%) of age-predicted heart rate reserve (HRR) on an electronically braked cycle ergometer (Lode Corival, Lode B.V., Groningen, The Netherlands or Ergoselect 200, Ergoline GmbH, Bitz, Germany). Following a 2-min period of resting data collection, exercise began at 50 W and intensity was gradually increased until participants reached their target HR (~5 min). Next, participants completed 30 min of steady-state exercise at the target intensity followed by a 3-min active recovery period at 0 W (Fig. 2). Following standardized instructions, perceived exertion (RPE) [19], leg muscle pain [20], and overall fatigue [21] were measured every 5 min during exercise and every minute during recovery. See Lindheimer et al., 2020 for more detailed outline of CPET variable collection [4].

2.4. Characterization of the post-exertional malaise response

Home-based symptom assessments were obtained seven days prior to and following the exercise stressor (Fig. 1). At the end of the first laboratory visit, participants were given standard instructions to complete at-home symptom questionnaires online or via hard copy and were encouraged to complete these questionnaires daily around the same time of day, if possible. Briefly, Veterans were instructed to use the scales to indicate their symptom experience each day, were provided verbal anchor examples for using the range of the scale from 'none' to 'worst imaginable' and were informed that there were no right or wrong answers, but to answer as honestly and objectively as they could. Symptoms were measured using 0–100 visual analog scales representing the 29-items of the Kansas Symptom Questionnaire component of the Kansas Case Definition [3]. We have previously operationalized PEM as a greater increase in symptom severity from baseline to post-exercise for GWV with GWI relative to CON, with symptom assessment occurring in a laboratory setting immediately prior to-, immediately post- and 24-h post-exercise [4]. Here, this approach was modified to address concerns about heterogeneity in GWI (e.g., illness severity, symptom profile) and PEM (e.g., type, time-course, severity). First, we averaged each symptom across the seven days leading up to the exercise test to obtain a stable baseline measure of symptom severity. Next, we identified the greatest symptom change for each GWV with GWI from pre to post-exercise. For instance, for six Veterans, the Kansas VAS item corresponding to "muscle pain" showed the largest pre-to-post exercise change compared to the other 28 symptoms that were measured. On the other hand, responses from four Veterans indicated that difficulty remembering recent information (i.e., memory) was the symptom that was most affected by exercise. This strategy was used to identify each individual participant's "peak PEM response" which was treated as our primary dependent variable in the statistical analyses described below.

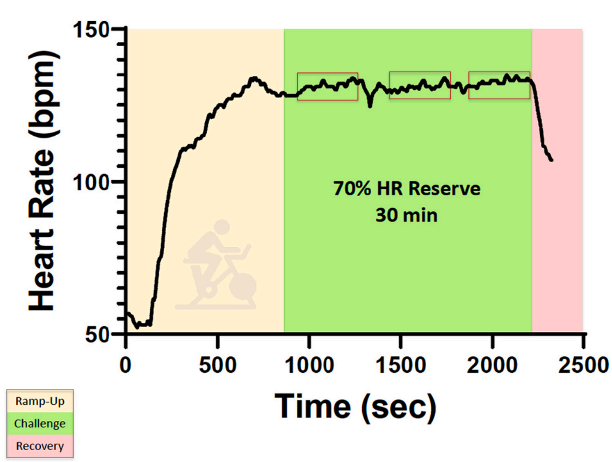

Fig. 2. Illustration of exercise challenge protocol.
Note. The exercise test began with a 2-min period of resting data collection, followed by a 5-min gradual ascension into the prescribed 70% HRR zone, starting at 50 W. Participants completed 30 min of steady-state exercise, ending with a 3-min active recovery period at 0 W. Cardiopulmonary exercise test variables were collected from three different 3-min steady state periods during the exercise test.

2.5. Exercise data processing

Raw data were exported from metabolic devices (.xlsx format) and imported into custom MATLAB (Mathworks, R2020b) scripts for post-processing. Breath-by-breath data were first interpolated (1 s intervals) and then primary variables (VO_2, VCO_2, VE, HR) were plotted for visual inspection and removal of errant points. Data were then smoothed (10-s) and re-plotted to confirm baseline, exercise, and recovery periods. Three steady-state time-points (17–20, 23–25, and 27–30 min) were automatically detected and second-by-second data were averaged over the three time-points. Peak RPE, fatigue, and leg muscle pain were defined as the highest value recorded during the exercise test. Cumulative work was calculated as the product of cycling power and duration (kJ = W * s). Processed data were then exported for statistical analysis. A satisfactory exercise test was determined by a participant's ability to perform within ±5% of their 70% HRR.

2.6. Identifying potential predictors of post-exertional malaise

Given the exploratory nature of this study, lack of prior work examining predictors of the PEM response, and the large number of potential predictors that could be derived from our data set (e.g., participant characteristics, physiological and perceptual responses to exercise), we used a combination of physiological and statistical considerations to guide our selection of potential predictors. This entailed (i) examining the empirical literature concerning cardiopulmonary responses to overlapping chronic diseases such as ME/CFS and fibromyalgia, (ii) selecting CPET parameters that could plausibly be related to changes in symptoms, (iii) plotting the distributions and variances of participant characteristics and responses to exercise, and (iv) determining which variables differed between GWV with GWI and CON GWV, and the degree to which they were correlated with symptoms pre- and post-exercise in the GWI group. To minimize concerns about model overfitting that can occur when the ratio of predictors to sample size is low [22], we decided a priori that the statistical model should contain no more than five predictors, thus resulting in a ~1:8 predictor-to-sample size ratio.

2.7. Statistical analyses

2.7.1. Data exclusion and missingness

Participant data were excluded because of inability to sustain required exercise test effort (n = 8) or missing because of technical difficulty ($n = 2$). Imputations for excluded or missing data were generated in R-studio (Version 1.2.5042) via the multivariate imputation by chained equations (MICE) package (Version 3.12.0).

2.7.2. Primary analyses

Data normality was confirmed via the Wilk-Shapiro Test, and data homoscedasticity was confirmed via the Levene Test. Non-normally distributed data were normalized using the Two-Step Normalization Procedure [23]. Differences between GWV with GWI and CON Veterans for participant characteristics, cardiopulmonary/perceptual responses, and pre-to-post exercise changes in symptoms were examined with descriptive statistics, independent samples t-tests ($\alpha = 0.05$) or Fisher's exact test. Hedges' g effect sizes of 0.25, 0.5, and 0.8 were interpreted as small, medium, and large differences, respectively. Medians and Interquartile range (IQR) were used to compare group compliance of the 70% HRR zone during exercise. Correlations between potential predictors and pre- and post-exercise Kansas symptoms were conducted with Pearson's r.

A general linear regression (GLM) model was used to test our primary aim (R-studio, Version 1.2.5042). Independent variables that were included in the model were: (i) VR-36 Physical Component Score (PCS), (ii) VE/VCO_2, (iii) peak exercise leg muscle pain, and (iv) cumulative work. The VR-36 PCS was selected as a predictor to account for baseline illness severity. Because the majority of peak PEM responses were fatigue related, VE/VCO_2 was included as a predictor. From a muscle energetics perspective, less efficient ventilation during exercise could translate to greater fatigue both during and following exercise. Naturally occurring leg muscle pain during exercise is a direct result of skeletal muscle contractions, and previous work by our lab confirmed that GWV with GWI have higher leg muscle pain ratings during exercise [4]. Significant group differences warranted adding cumulative work as a covariate to account for variability in the standardization of the exercise stimulus. An interaction term between VE/VCO_2 and VR-36 PCS was included to test whether individuals with worse physical health and ventilatory efficiency showed the greatest symptom responses. The PEM response (Section 2.4) was treated as our primary dependent variable.

3. Results

3.1. Participant characteristics

Data from 74 GWV were used in the final analysis (GWI = 43; CON = 31). Participant demographics and self-reported mental and physical illness severity are reported in Table 1. GWV with GWI had significantly worse physical health, mental health, and overall fatigue, with large between group differences (Effect size range: 1.83–2.25).

Table 1
Baseline group characteristics for Veterans with Gulf War Illness (GWI; $n = 43$) and healthy control Veterans (CON; $n = 31$).

	GWI ($n = 43$)	CON ($n = 31$)	GWI vs. CON	
	Mean (SD)	Mean (SD)	t-Statistic	p-value
Age	52.21 (4.17)	52.19 (5.12)	−0.05	0.99
Sex (Male/Female, %)	91%/9%	90%/10%	–	0.99
Height (m)	1.77 (0.08)	1.75 (0.10)	0.97	0.37
Weight (kg)	97.07 (18.51)	91.18 (16.13)	1.46	0.15
BMI (kg/m^2)	31.02 (5.63)	29.79 (4.65)	1.03	0.31
Kansas*	28.45 (12.79)	3.92 (4.32)	8.42	<0.001
VR-36 PCS*	59.82 (17.77)	90.81 (6.32)	−9.2	<0.001
VR-36 MCS*	54.24 (18.70)	89.41 (6.10)	−9.87	<0.001
FSS*	43.51 (13.58)	18.93 (9.67)	7.41	<0.001
MFI Total*	68.12 (12.24)	33.79 (10.37)	8.39	<0.001
SF-MPQ-2*	1.19 (1.26)	0.15 (0.51)	4.79	<0.001
PSQI*	11.73 (4.21)	6.79 (3.50)	5.34	<0.001

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.

* Significant difference between groups at $p < 0.05$.

3.2. Cardiopulmonary and perceptual responses to exercise

Group differences for exercise responses are detailed in Table 2. Briefly, Veterans with GWI had greater VE/VO_2, leg muscle pain, and fatigue, and lower VO_2, VCO_2, power, and cumulative work. Neither average heart rate nor the amount of time spent exercising at 70 ± 5% HRR differed between groups. Overall compliance was satisfactory for both the GWI and CON groups, indicating a high-integrity exercise stimulus (CON: median = 97.72, IQR = 5.42; GWI: median = 99.37, IQR = 5.67).

3.3. Post-exertional malaise response

GWV with GWI had a significantly greater PEM response compared to CON (GWI = 38.90 ± 29.06, CON = 17.84 ± 28.26, $g = 0.70$, $p < 0.01$) (Fig. 3, Supplemental Figs. 1–2). The distribution of PEM responses by symptom for GWI Veterans is displayed in Table 3. Of the nine different symptoms examined, difficulty "Getting to Sleep" was the most commonly (~30%) reported PEM response by GWV with GWI. The majority of GWV with GWI experienced their peak PEM response within 72 h of exercise (GWI = 27/43), but the overall distribution was variable (Fig. 4).

3.4. General linear model results

Three of four independent variables differed significantly between

Table 2
Average steady-state cardiorespiratory responses to exercise challenge at 70% HRR in Veterans with Gulf War Illness (GWI; $n = 43$) and healthy control Veterans (CON; $n = 31$).

	GWI (n = 43)	CON (n = 31)	Effect Size GWI vs. CON	
	Mean (SD)	Mean (SD)	*Hedges' g*	95% CI
VO_2 (mL)	1526.14 (332.69)	1716.77 (371.9)	−0.54	(−1.01, −0.07)
VO_2 ($mL \cdot kg \cdot min^{-1}$)	16.15 (3.61)	19.42 (5.03)	−0.76	(−1.34, −0.28)
VCO_2 (mL)	1402.14 (332.46)	1587.32 (342.85)	−0.54	(−1.02, −0.07)
VE ($L \cdot min^{-1}$)	42.60 (12.46)	44.98 (9.84)	−0.21	(−0.67, 0.26)
VE/VO_2	29.87 (4.49)	28.07 (4.04)	0.49	(0.02, 0.96)
VE/VCO_2	28.63 (4.45)	27.15 (4.00)	0.40	(−0.07, 0.86)
Heart rate (beats per min)	134.75 (11.64)	135.46 (9.52)	0.07	(−0.44, 0.57)
Power (watts)	80.26 (22.14)	99.37 (27.03)	−0.78	(−1.26, −0.30)
Work (kJ)	235.66 (35.27)	253.22 (23.27)	−0.50	(−0.98, −0.03)
Peak RPE	14.85 (2.39)	13.9 (2.02)	0.42	(−0.05, 0.89)
Peak fatigue	6.37 (2.42)	4.34 (2.14)	0.87	(0.39, 1.36)
Peak leg muscle pain	4.45 (2.54)	2.66 (2.51)	0.70	(0.22, 1.18)

Note. RPE = rating of perceived exertion; $\mathbf{VO_2}$ = oxygen consumption; $\mathbf{VCO_2}$ = carbon dioxide consumption; **VE** = minute ventilation; $\mathbf{VE/VO_2}$ = ventilatory equivalent for oxygen; $\mathbf{VE/VCO_2}$ = ventilatory equivalent for carbon dioxide. 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.

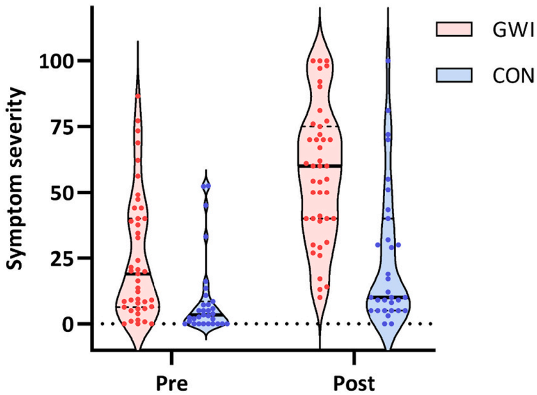

Fig. 3. Median (IQR) symptom severity for Veterans with Gulf War Illness (GWI; $n = 43$) and healthy control Veterans (CON; $n = 31$) pre and post-exercise.
Note. Symptom severity for GWI and CON groups pre-exercise and post-exercise. The GWI group experienced larger symptom exacerbation from pre-exercise (median = 19, IQR = 6.25, 40) to post-exercise (median = 60, IQR = 40, 75) compared to the CON group (pre-exercise: median = 3.33, IQR = 0, 8.5); post-exercise: (median = 10, IQR = 5, 40), indicating a post-exertional malaise response in the GWI group.

Table 3
Type and frequency of peak symptoms in Veterans with Gulf War Illness (GWI; $n = 43$).

Symptom	GWI Endorsing as Peak PEM Response ($n = 43$)	Total %
Fatigue	3	6.98%
Difficulty getting to Sleep	13	30.23%
Unrefreshing Sleep	5	11.63%
Joint Pain	0	N/A
Muscle Pain	6	13.95%
Body Pain	3	6.98%
Headache	8	18.60%
Difficulty with memory	4	9.30%
Nausea	1	2.33%

Note. Values in the Total % column represent the percentage of Veterans with GWI (n = 43) for whom a given symptom changed the most from pre to post-exercise.

GWI and CON groups, with effect sizes ranging from small to large (*g*: 0.4–2.25). Bivariate associations between PEM responses and predictors are shown in Fig. 5. The final GLM did not explain significant variance in the PEM response (Pooled $R^2 = 0.15$, Adjusted $R^2 = 0.03$, $p = 0.34$).

4. Discussion

Post-exertional malaise is an understudied aspect of GWI. Although prior exercise challenge investigations of PEM have revealed physiological responses suggestive of central nervous [24] or immune dysregulation [25,26], relatively little attention has been paid to the (i) cardiopulmonary and perceptual responses that occur *during* exercise challenge, (ii) heterogeneous symptom responses that follow, and (iii) strength of the association between these data. Here, we addressed these knowledge gaps by quantifying differences in CPET parameters and symptom responses between GWV with GWI and CON Veterans and exploring whether CPET data could explain variability in PEM among Veterans with GWI, as defined by their peak symptom responses following exercise. We observed clear differences between GWV with GWI and CON Veterans across several CPET parameters and peak symptom responses. However, contrary to our primary hypothesis, select CPET parameters did not explain significant variability in the PEM response.

4.1. CPET revealed several distinct cardiopulmonary and perceptual differences between GWI and CO Veterans

We observed small-to-moderate between-group differences characterized by lower consumption of oxygen (VO_2), lower carbon dioxide production (VCO_2), a higher ventilatory equivalent for oxygen (VE/VO_2), and lower power in Veterans with GWI. Further, Veterans with GWI experienced significantly greater leg muscle pain and overall fatigue, despite showing non-significant differences in RPE. The reason for these findings is unclear, but differences in aerobic fitness and illness pathophysiology are plausible explanations. Concerning aerobic fitness, our prior work in GWI [12] and ME/CFS with comorbid fibromyalgia [27] has shown that submaximal responses to exercise for select CPET parameters become smaller when ill and healthy participants are matched for peak VO_2, suggesting that fitness may play a role in the differences observed here. However, aerobic fitness does not always account for cardiopulmonary differences between groups, as shown by increased effect sizes for respiratory rate after matching GWI and CON Veterans for peak VO_2 in our prior study [12]. Notably, determining why groups differed on select CPET parameters was beyond the scope of the present study.

4.2. Peak symptom responses were greater in GWI than CON

A preliminary step to addressing the primary aim of this study involved determining whether Veterans with GWI presented with PEM, as measured by increases in symptom severity following exercise challenge. To address concerns with heterogeneity in the type, time-course, and severity of symptom responses to exercise, we focused on each Veteran's peak symptom response. In the GWI group, visual analog scores reflected high variability in symptom responses spanning the entire symptom severity range (Fig. 3). Comparing these peak responses between groups, we found a significant ($p < 0.01$) and moderate ($g = -0.70$) increase in symptom severity in Veterans with GWI relative to CON (Fig. 3).

Although the specific symptom showing the greatest response varied from person to person, the most frequent peak symptom was related to sleep (e.g., getting to sleep). Interestingly, this symptom is among the most prevalent complaints reported in population studies of GWV [28]. These findings are reminiscent of a prior ME/CFS-based study which observed that sleep disturbance was a consequence of physical/cognitive exertion in 67% of their study sample and recommended that future studies expand their outcome measures beyond pain and fatigue when characterizing PEM [29]. Here, this goal was accomplished with a visual analog scale adaptation of the Kansas questionnaire, which is a validated measure of GWI status, but had not previously been used to study the effect of physical exercise on GWI symptomology. Given its clear applicability to symptoms that GWV typically experience and its sensitivity to change following exercise, future work characterizing PEM in GWI may consider using the Kansas questionnaire in a similar fashion.

4.3. Select CPET parameters did not explain significant variability in the PEM response

Counter to our primary hypothesis, data collected during CPET did not predict PEM, as indicated by the non-significant regression model. Given the paucity of prior studies that have examined variability in the PEM response, our rationale for selecting each CPET parameter is worth revisiting. First, VE/VCO_2 was based on plausible physiological rationale, clinical applicability, and other statistical considerations. This variable has prognostic value for several cardiorespiratory illnesses [30,31], and people with ME/CFS [32,33] and GWI [4,12] have displayed less efficient ventilation patterns (among other CPET generated distinctions). Although significant group differences were not observed here, VE/VCO_2 was significantly correlated with peak PEM responses in our sample of GWV with GWI.

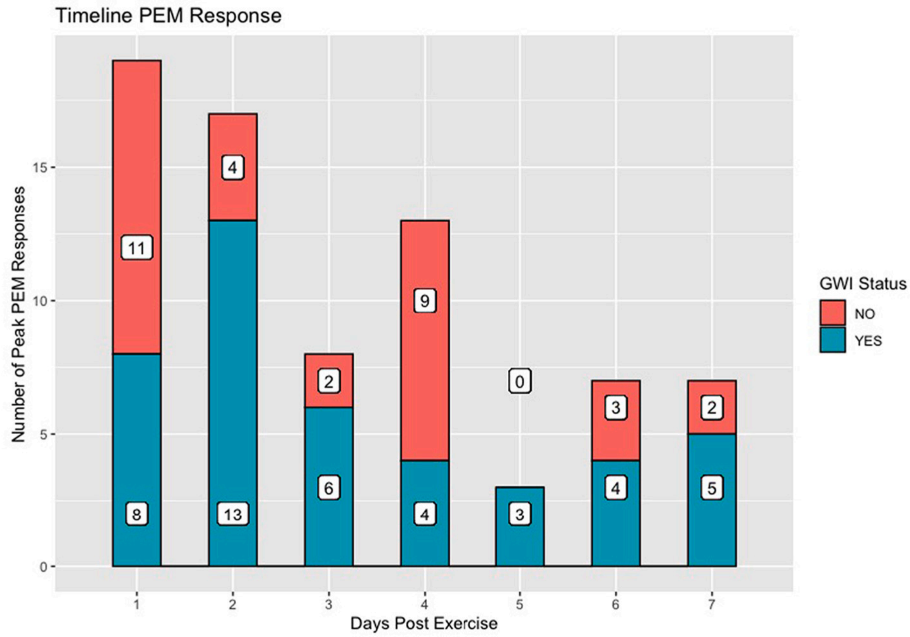

Fig. 4. Days post-exercise that PEM occurred in Veterans with Gulf War Illness (GWI; $n = 43$) and healthy control Veterans (CON; $n = 31$).
Note. Each column corresponds to the frequency of participants whose peak symptom response to exercise occurred that day. About 60% (44/74) of peak symptom responses were observed within 72 h post-exercise.

Another predictor in our model was leg muscle pain. Similar to VO_2 or heart rate, leg muscle pain increases in response to active skeletal muscle contraction during exercise [20]. Our previous work demonstrated that ME/CFS and GWI populations experience greater leg muscle pain during exercise [4,9], and we have also reported that GWV with chronic pain experience augmented leg muscle pain during exercise and exaggerated pain sensitivity following exercise, suggesting that repetitive peripheral stimulation of muscle nociceptors during physical exertion may contribute to central dysregulation of pain processing [34]. This response is opposite of that observed in healthy women [35] and suggests that pain experienced during exercise stimulates other physiological systems and can lead to post-exercise consequences such as changes in pain regulation and perhaps symptoms. Chronic musculoskeletal pain occurs in 22.8–33.3% of GWV [28]; however, pain-related symptoms represented only 9 out of 43 GWI peak PEM responses. Leg muscle pain during exercise was significantly correlated with GWI PEM responses, but as stated our model was not significant. It is possible that other physiological and perceptual factors may be playing a stronger role in stimulating symptoms.

The third CPET parameter selected for our model was cumulative work (kJ). Cumulative work is the product of cycling power and duration, thus it was included to more precisely estimate the standardization of our exercise stimulus beyond time spent exercising at 70% HRR and to help account for fitness-related effects (i.e., more fit individuals can perform more work at the same relative intensity as less fit individuals). Further, it is plausible that direct stimulus metrics of exercise intensity such as cumulative work would predict symptom worsening – i.e., greater exercise stress would trigger PEM. For these reasons and to avoid model overfitting, cumulative work was prioritized over other potential CPET parameters in the regression model, despite a non-significant bivariate association with the PEM response.

4.4. Limitations and future directions

Despite the exploratory nature of this study, we were somewhat conservative in our analytical approach to testing our primary hypothesis. Thus, it is possible that the PEM response can be predicted by CPET parameters, just not by the specific combination of variables included in our model (type-2 error). There are trade-offs between type-1 and -2 error risk in every study, and we prioritized minimizing type-1 error risk by (i) selecting variables with biologically plausible relationships with symptom worsening, (ii) limiting the overall number of predictors to avoid model overfitting, and (iii) avoiding predictors that were strongly correlated with one another to minimize multicollinearity concerns (e.g., VE/VCO_2 and VE/VO_2). Despite rejecting our primary hypothesis, our hope is that these methods and findings lay the groundwork for future efforts to examine the strength of association between CPET data (or other potential predictors) and symptom responses. A second point worth discussing is our novel choice to operationalize PEM as a peak symptom response rather than adopting a traditional approach of examining specific symptoms (e.g., fatigue) and timepoints (e.g., 24 h post-exercise). Our approach provided a simple solution for navigating the heterogeneity that is commonplace in PEM studies; however, it is possible that focusing on a single symptom may have resulted in a different outcome. Nevertheless, this method of analyzing PEM may serve as a useful framework for maximizing the signal-noise ratio in the PEM response which may be applicable in other situations such as establishing the prevalence and severity of PEM in GWI or exploring whether the PEM response is associated with biological alterations following exercise. Importantly, prior approaches and the approach used here are not mutually exclusive and instead may be viewed as providing complimentary perspectives on studying PEM.

4.5. Conclusion

The PEM response is variable in GWI and does not appear to be related to a model that includes ventilatory efficiency, exercise-induced muscle pain, cumulative work, and physical health related quality of life. This study may aid several future research directions, including exploring associations between PEM and dysregulation within other physiological systems of relevance to GWI. Upcoming projects will evaluate if other select variables representative of central nervous, autonomic, and immune system function improve our ability to explain variance in the GWI PEM response.

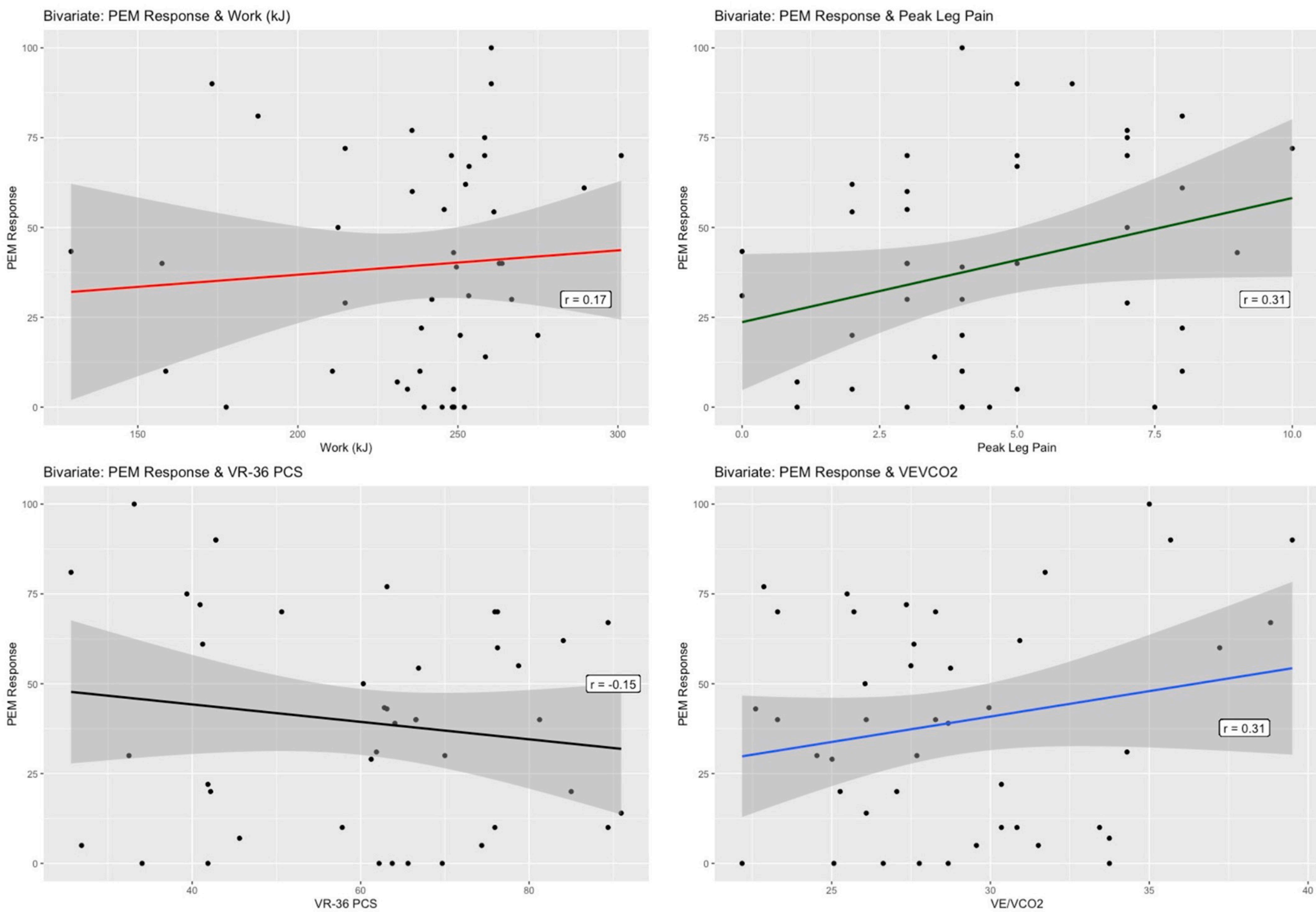

Fig. 5. Scatter plots and bivariate correlations between symptom responses and predictors.
Note. Cumulative Work (kJ), Peak Leg Pain, VR-36 Physical Component Score (PCS), and VE/VCO_2 were included as independent variables in the final general linear model. Both VE/VCO_2 (*Pearson* $r = 0.31$, 95% CI = 0.01, 0.56, $p = 0.05$) and leg muscle pain (*Pearson* $r = 0.31$, 95% CI = 0.01, 0.55, $p < 0.05$) were significantly correlated with peak symptom responses. Cumulative work (*Pearson* $r = 0.17$, 95% CI = −0.14, 0.45, $p = 0.27$) and VR-36 PCS (*Pearson* $r = -0.15$, 95% CI = −0.43, 0.16, $p = 0.49$) were not significantly correlated with peak symptom responses.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

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Appendix A. Supplementary data

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References

[1] Committee on Gulf War and Health, in: D. Cory-Slechta, R. Wedge (Eds.), Volume 10: Update of Health Effects of Serving in the Gulf War, Board on the Health of Select Populations, Institute of Medicine, National Academies of Sciences, Engineering, and Medicine. Gulf War and Health: Volume 10: Update of Health Effects of Serving in the Gulf War, 2016 [Internet], National Academies Press, Washington, D.C., 2016 [cited 2016 May 12]. Available from: http://www.nap.edu/catalog/21840.

[2] Research Advisory Committee on Gulf War Veterans'' Illnesses, Gulf War Illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations, U. S. Government Printing Office, Washington, D.C., 2008.

[3] L. Steele, Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service, Am. J. Epidemiol. 152 (10) (2000) 992–1002.

[4] J.B. Lindheimer, A.J. Stegner, G.R. Wylie, J.C. Klein-Adams, N.E. Almassi, J. V. Ninneman, et al., Post-exertional malaise in veterans with gulf war illness, Int. J. Psychophysiol. 147 (2020) 202–212. Jan 1.

[5] A.L. Komaroff, Advances in understanding the pathophysiology of chronic fatigue syndrome, JAMA 322 (6) (2019) 499. Aug 13.

[6] K. Yoshiuchi, D.B. Cook, K. Ohashi, H. Kumano, T. Kuboki, Y. Yamamoto, et al., A real-time assessment of the effect of exercise in chronic fatigue syndrome, Physiol. Behav. 92 (5) (2007) 963–968. Dec.

[7] J.D. Meyer, A.R. Light, S.K. Shukla, D. Clevidence, S. Yale, A.J. Stegner, et al., Post-exertion malaise in chronic fatigue syndrome: symptoms and gene expression, Fatigue Biomed. Health Behav. 1 (4) (2013) 190–209.

[8] A.R. Light, L. Bateman, D. Jo, R.W. Hughen, VanHaitsma TA, A.T. White, et al., Gene expression alterations at baseline and following moderate exercise in patients with chronic fatigue syndrome and fibromyalgia syndrome, J. Intern. Med. 271 (1) (2012 Jan) 64–81.

[9] D.B. Cook, A.R. Light, K.C. Light, G. Broderick, M.R. Shields, R.J. Dougherty, et al., Neural consequences of post-exertion malaise in myalgic encephalomyelitis/chronic fatigue syndrome, Brain Behav. Immun. 62 (2017) 87–99.

[10] G. Broderick, R. Ben-Hamo, S. Vashishtha, S. Efroni, L. Nathanson, Z. Barnes, et al., Altered immune pathway activity under exercise challenge in Gulf War Illness: an exploratory analysis, Brain Behav. Immun. 28 (2013) 159–169. Feb.

[11] J.N. Baraniuk, N. Shivapurkar, Exercise - induced changes in cerebrospinal fluid miRNAs in Gulf War Illness, chronic fatigue syndrome and sedentary control subjects, Sci. Rep. 7 (1) (2017) 15338. Nov 10.

[12] J.B. Lindheimer, D.B. Cook, J.C. Klein-Adams, W. Qian, H.Z. Hill, G. Lange, et al., Veterans with Gulf War Illness exhibit distinct respiratory patterns during maximal cardiopulmonary exercise. Weston KL, PLoS One 14 (11) (2019) e0224833. Nov 12.

[13] Committee Members, R.J. Gibbons, G.J. Balady, J. Timothy Bricker, B.R. Chaitman, G.F. Fletcher, et al., ACC/AHA 2002 guideline update for exercise testing: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (committee to update the 1997 exercise testing guidelines), Circulation 106 (14) (2002 Oct) 1883–1892.

[14] L.E. Kazis, The veterans SF-36 health status questionnaire: development and application in the Veterans Health Administration 5 (1) (2000) 18.

[15] D.J. Buysse, C.F. Reynolds, T.H. Monk, S.R. Berman, D.J. Kupfer, The Pittsburgh Sleep Quality Index (PSQI): a new instrument for psychiatric research and practice, Psychiatry Res. 28 (1989) 193–213.

[16] R.H. Dworkin, D.C. Turk, D.A. Revicki, G. Harding, K.S. Coyne, S. Peirce-Sandner, et al., Development and initial validation of an expanded and revised version of the Short-form McGill Pain Questionnaire (SF-MPQ-2), Pain 144 (1) (2009) 35–42. Jul.

[17] L.B. Krupp, N.G. LaRocca, J. Muir-Nash, A.D. Steinberg, The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus, Arch. Neurol. 46 (1989) 1121–1123.

[18] Smets EMA, B. Garssen, B. Bonke, De Haes JCJM, The multidimensional fatigue inventory (MFI): psychometric qualities of an instrument to assess fatigue, J. Psychosom. Res. 39 (1995) 315–325.

[19] Borg GAV, Psychophysical bases of perceived exertion, Med. Sci. Sports Exerc. 14 (1982) 377–381.

[20] D.B. Cook, P.J. O'Connor, S.A. Eubanks, J.C. Smith, M. Lee, Naturally occurring muscle pain during exercise: assessment and experimental evidence, Med. Sci. Sports Exerc. 29 (8) (1997) 999–1012.

[21] A.G. Gift, Visual analogue scales: measurement of subjective phenomena, Nurs. Res. 38 (1989), 286–8.

[22] M.A. Babyak, What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models, Psychosom. Med. 11 (2004).

[23] G. Templeton, A two-step approach for transforming continuous variables to Normal: implications and recommendations for IS research, Commun. Assoc. Inf. Syst. 28 (2011).

[24] R.U. Rayhan, M.P. Raksit, C.R. Timbol, O. Adewuyi, J.W. Vanmeter, J.N. Baraniuk, Prefrontal lactate predicts exercise-induced cognitive dysfunction in Gulf War Illness, Am. J. Transl. Res. 5 (2) (2013) 212–223.

[25] T. Whistler, M.A. Fletcher, W. Lonergan, X.R. Zeng, J.M. Lin, A. Laperriere, et al., Impaired immune function in Gulf War Illness, BMC Med. Genet. 2 (2009) 12. Mar 5.

[26] G. Broderick, A. Kreitz, J. Fuite, M.A. Fletcher, S.D. Vernon, N. Klimas, A pilot study of immune network remodeling under challenge in Gulf War Illness, Brain Behav. Immun. 25 (2) (2011) 302–313. Feb.

[27] D.B. Cook, P.R. Nagelkirk, A. Poluri, J. Mores, B.H. Natelson, The influence of aerobic fitness and fibromyalgia on cardiorespiratory and perceptual responses to exercise in patients with chronic fatigue syndrome, Arthritis Rheum. 54 (10) (2006) 3351–3362. Oct.

[28] A.L. Maule, P.A. Janulewicz, K.A. Sullivan, M.H. Krengel, M.K. Yee, M. McClean, et al., Meta-analysis of self-reported health symptoms in 1990–1991 Gulf War and Gulf War-era veterans, BMJ Open 8 (2) (2018) e016086. Feb.

[29] L. Chu, I.J. Valencia, D.W. Garvert, J.G. Montoya, Deconstructing post-exertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: a patient-centered, cross-sectional survey, PLoS One 13 (6) (2018), e0197811.

[30] R. Arena, J. Myers, S.S. Aslam, E.B. Varughese, M.A. Peberdy, Peak VO2 and VE/VCO2 slope in patients with heart failure: a prognostic comparison, Am. Heart J. 147 (2) (2004) 354–360. Feb.

[31] M. Guazzi, R. Arena, M. Halle, M.F. Piepoli, J. Myers, C.J. Lavie, 2016 focused update: clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations, Circulation 133 (24) (2016) e694–e711.

[32] O. Inbar, R. Dlin, A. Rotstein, B.J. Whipp, Physiological responses to incremental exercise in patients with chronic fatigue syndrome, Med. Sci. Sports Exerc. 33 (9) (2001) 1463–1470. Sep.

[33] D.B. Cook, A.J. Stegner, P.R. Nagelkirk, J.D. Meyer, F. Togo, B.H. Natelson, Responses to exercise differ for chronic fatigue syndrome patients with fibromyalgia, Med. Sci. Sports Exerc. 44 (6) (2012 Jun) 1186–1193.

[34] D.B. Cook, A.J. Stegner, L.D. Ellingson, Exercise alters pain sensitivity in gulf war veterans with chronic musculoskeletal pain, J. Pain 11 (8) (2010) 764–772. Aug.

[35] K.F. Koltyn, A.G. Brellenthin, D.B. Cook, N. Sehgal, C. Hillard, Mechanisms of exercise-induced hypoalgesia, J. Pain 15 (12) (2014) 1294–1304. Dec.