

Original Article

Prefrontal lactate predicts exercise-induced cognitive dysfunction in Gulf War Illness

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Abstract: Background: 25% to 30% of Veterans deployed to the 1990 to 1991 Persian Gulf War exhibit an idiopathic syndrome of chronic fatigue, exertional exhaustion, pain, hyperalgesia, cognitive and affective dysfunction known as Gulf War Illness (GWI). Methods: Gulf War veterans (n=15) and sedentary veteran and civilian controls (n=11) completed a 2-back working memory test in an fMRI before and after two bicycle exercise stress test. We performed single voxel ¹H MRS to evaluate brain metabolic differences in the left anterior cingulate cortex and the changes associated with exercise. Results: Eight GWI subjects increased their 2-back scores after exercise (labelled increasers) and seven GWI subjects decreased their 2-back scores after exercise (labelled decreasers). These phenotypic responses were absent for controls. Decreasers had significantly elevated prefrontal lactate levels compared to Increasers prior to completion of the exercise stress tests. Evaluation of prefrontal lactate levels prior to exercise demonstrated predictability (ROC analysis) of the two diametrically opposed subgroups. Conclusion: Prefrontal lactate levels may be a potential biomarker for exercise-induced subgroups in GWI. The alterations in brain energetics may be in part responsible for a subgroup of GWI and underlie some of the symptoms present in the patient population.

Keywords: Gulf War Illness (GWI), pre-frontal lactate, exercise-induced cognitive dysfunction, biomarker

Introduction

Gulf War Illness (GWI), also known as Chronic Multi-symptom Illness (CMI), affects approximately a quarter of the one million military personnel who served in the 1990 - 1991 Persian Gulf War [1, 2]. Veterans with GWI present with symptoms of cognitive dysfunction, chronic fatigue, and widespread pain that overlap with a larger group of idiopathic illnesses that include Chronic Fatigue Syndrome (CFS) [3], Myalgic encephalomyelitis [4] and fibromyalgia [5-8].

A common complaint is exertional exhaustion [1]. Physiological stressors such as exercise at greater than usual levels leads to an exacerbation of symptoms that may develop immediately or be characteristically delayed as long as 24

hours [2, 4]. Experimental exercise models demonstrate acute symptom exacerbations in these disorders [9-11]. In animal models, exercise-induced pain, hyperalgesia and fatigue were not attributed to lactate build-up in peripheral muscles [12]. Instead, up-regulation of expression for the immediate early gene c-fos in brainstem nuclei and dysfunctional brain energetics suggested that central mechanisms may be responsible for exercise-induced changes in muscular performance, decreased activity ("fatigue"), and hyperalgesia (pain and tenderness) [13]. These studies suggest the hypothesis that differences in brain energetics may be associated to the heterogeneity of exercise-induced symptom exacerbations in GWI.

Glucose has traditionally been considered the principal energy source for the brain [14].

However, recent findings in brain energetics suggest that lactate is an alternative fuel under conditions of high neural demand for energy metabolism during cognition and memory acquisition [15, 16]. Cerebral lactate metabolism is maintained by an interaction between astrocytes and neurons known as the astrocyte - neuron lactate shuttle [14]. Astrocytes generate and release lactate into the brain interstitial fluid. Neurons import the lactate and convert it to pyruvate for use in mitochondrial oxidative phosphorylation [14]. Lactate is a more efficient starting point for oxidative respiration than glucose since the neuron has a net gain of one ATP molecule, and the glucose can be reserved for skeletal muscle ATP production [17, 18].

Lactate derived from exercising muscles is another energy source for neurons [17]. Proton - coupled monocarboxylate transporters (MCT) shuttle lactate across the blood - brain barrier and through astrocytes to neurons and other cells. Under normal conditions, astrocytes supply lactate by glycolysis followed by lactate export, and during muscular exercise by absorption of lactate from across the blood - brain barrier into the neuropil [14, 17]. The flux is efficient so importation matches neural energy needs and brain lactate levels do not rise [19]. In contrast, elevated brain lactate has been associated with disrupted metabolite homeostasis, mitochondrial failure, and neuronal dysfunction in conditions such as aging [20].

Eighty percent of the energy consumed by the brain is for neuronal activity, with glutamate as the predominant neurotransmitter which is known to play a vital role in memory function [19, 21]. Astrocytes play a critical role in glutamatergic neuronal activity. They ensheath synapses and rapidly absorb the glutamate that is released [14]. The glutamate is converted to glutamine that is then exported to neurons for conversion back into glutamate. This pathway is the glutamate - glutamine cycle. The molecular spectroscopy ratio of glutamate to glutamine is a measure of astrocyte function [22]. High glutamate turnover in astrocytes triggers glycolysis and the production of lactate that is shuttled to neurons. These processes link the glutamate - glutamine cycle and lactate shuttle in astrocytes. Astrocyte dysfunction leads to impaired synaptic glutamate clearance with

inappropriately high synaptic glutamate concentrations and dysregulated neuronal activity [23]. There is no information on either of these indices in GWI, or their responses to exercise.

Lactate in the cerebral ventricles of CFS subjects is significantly higher than anxiety, major depression, and healthy volunteers [24-26]. Our own receiver operator analysis of the published data suggested an upper threshold of 0.5 institutional units for lactate for a combined anxiety and healthy volunteer group with sensitivity of 0.65 and specificity of 0.88 to discriminate from CFS values [26]. Frequency analysis of the CFS lactate distribution data demonstrated a wide range of levels suggesting a continuum or potential bimodal distribution. These authors did not find differences in lactate for brain parenchyma at rest between CFS and controls. There is no information on lactate after exercise.

Based on these data and the subjective overlap of GWI and CFS subjects, we predicted that GWI subjects would also show heterogeneous lactate levels. Molecular spectroscopy evidence of heterogeneous astrocyte - neuron lactate shuttle and glutamate - glutamine cycle dynamics were also anticipated to possibly show distinctions before and after exercise. These studies are of importance since the relationships between exercise - induced changes in brain metabolites, energetics and cognitive function have not been studied in GWI. Furthermore, biomarkers defined by molecular spectroscopy may be valuable for defining subsets, pathophysiological mechanisms, and responses to treatments. In order to address these issues, functional magnetic resonance imaging (fMRI) scans were performed at baseline prior to, and immediately following, two bicycle exercise stress tests. The second fMRI scan demonstrated post - exertional changes in the brain.

Cognition was tested during each fMRI scan using the letter variant 2-back working memory paradigm [27]. Working memory is a system that actively retains multiple fragments of information during goal directed behavior that is used at later moments in cognitive operations [28]. The cognitive testing revealed 2 GWI subsets that had diametrically opposite working memory responses to exercise and may represent functional phenotypes.

Metabolite concentrations were measured by single voxel ^1H magnetic resonance spectroscopy (MRS) in the left anterior cingulate cortex. This region is dysfunctional in prolonged pain, fatigue, and affective disorders [29, 30]. The level of lactate in this region before and after exercise was the primary outcome planned for this study.

Materials and methods

Subjects and recruitment

The protocol was approved by Georgetown University Institutional Review Board and USAMRMC Human Research Protection Office (HRPO #A-15547.0) (clinicaltrials.gov identification number NCT01291758). The participant pool composed of 11 age and gender matched controls and 15 CMI subjects who also met CFS criteria (Georgetown University IRB #2009-229). All subjects signed informed consent, completed questionnaires, and physical examinations to validate CMI and CFS diagnosis. On-line questionnaires assessed an extensive set of psychometric qualities to investigate the distinctions between CMI, CFS and control subjects [31]. The current analysis is focused on pre- and post-exercise working memory accuracy scores and metabolite concentrations for subjects who met both CMI and CFS criteria.

Pre-arrival instructions and questionnaires

Fatigue was assessed with the ordinal fatigue rating and verified with the Chalder fatigue scale [32, 33]. The ordinal fatigue assessment was anchored with 0 = no complaint, 1 = trivial, 2 = mild, 3 = moderate or 4 = severe intensity [32, 34]. Inclusion of "trivial" allowed participants to verify complaints that were present but not bothersome enough to warrant treatment and/or other lifestyle changes [35]. Subjective pain perceptions were quantified with the McGill short form with its sensory, affective and total scores [36]. Relative disability and quality of life were compared using the Medical Outcomes Survey Short Form 36 (SF-36) [37].

Protocol

Upon arrival to the Georgetown University Clinical Research Unit, subjects reviewed and signed their informed consent forms to partici-

pate in this four day protocol. On the 1st day, all subjects had history and physical examinations to assess CMI [2] and CFS [3] criteria, blood tests, and had a tour of the facilities. On the 2nd day subjects completed a pre-exercise (baseline) fMRI scan prior to the 1st bicycle exercise stress test. On the 3rd day, subjects completed their second, identical bicycle exercise stress test followed by a post-exercise fMRI and MRS scan, which was on average within 1 hour of the stress test. The 4th day was their discharge day. The neuroimaging analysis included ^1H MRS, functional MRI (fMRI) during the working memory paradigm, structural/anatomic acquisition, and white matter diffusion tensor imaging. This study focuses on ^1H MRS spectroscopy data and other fMRI data will be reported separately.

To assess changes in cognition, we used the working memory N-back paradigm during both fMRI scans. Prior to both fMRI scans, subjects completed N-back practice sessions on a computer. Blocks of 9 randomized letters (A, B, C, D) were presented. Subjects were given instructions to respond by pressing a button for the same letter ("O-Back") or the one seen 2 letters previously ("2-Back"). Blocks for O-back then 2-back tasks were presented for 5 cycles. A total score of 35 letters correct were possible during the 2-back working memory cognitive paradigm.

Bicycle stress tests

Two protocols were evaluated. First, standard VO₂MAX cardiopulmonary stress tests were performed using Vmax equipment and software (SensorMedix) [38, 39]. Second, subjects had submaximal stress tests at 70% predicted heart rate for 25 minutes which was followed by a brief climb to 85% heart rate to standardize exertion on a Schwinn AirDyne bicycle using the Vmax software and equipment (SensorMedix) [40]. Cardiac stress tests were negative and ruled out arrhythmias, ischemias, or other alterations. Outcomes related to the stress tests will be described in greater detail elsewhere.

Functional magnetic resonance imaging (fMRI) and MRI spectroscopy (MRS)

Data was acquired on a Siemens 3T Tim Trio scanner equipped using a transmit-receive body coil and commercial twelve-element head

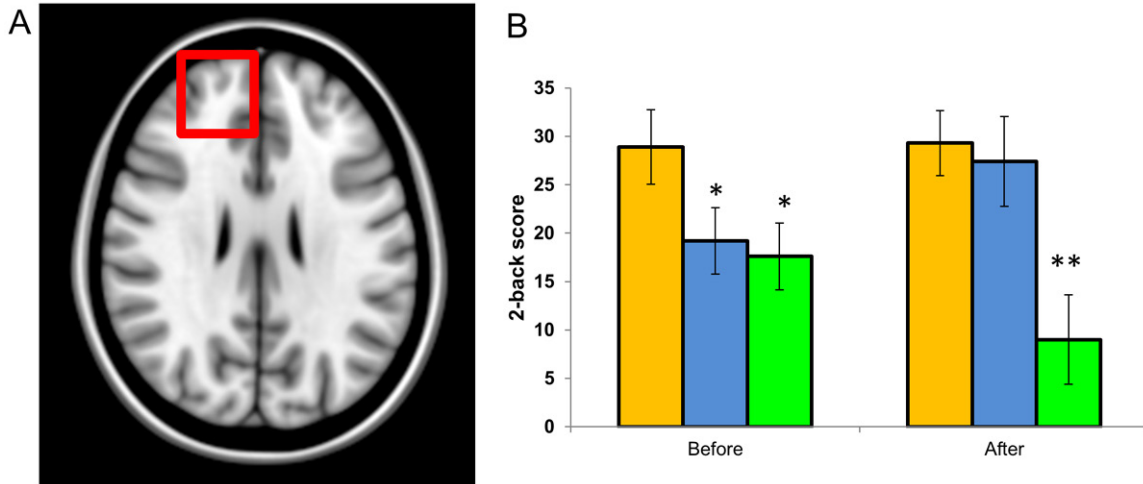


Figure 1. Voxel placement and 2-back working memory scores. A. A single voxel was placed in the left anterior cingulate cortex to obtain metabolite concentrations. B. Before exercise, controls had significantly higher accuracies compared to CMI subjects ($*P < 0.001$, 2-tailed unpaired t-test vs. controls). After exercise, scores for the 8 Increasers (blue) were comparable to controls (orange), while the 7 Decreasers (green) had significantly lower scores ($**F_{2,23} = 27.8$, $P = 0.000007$; ANOVA, Tukey's post hoc test compared to controls and increasers).

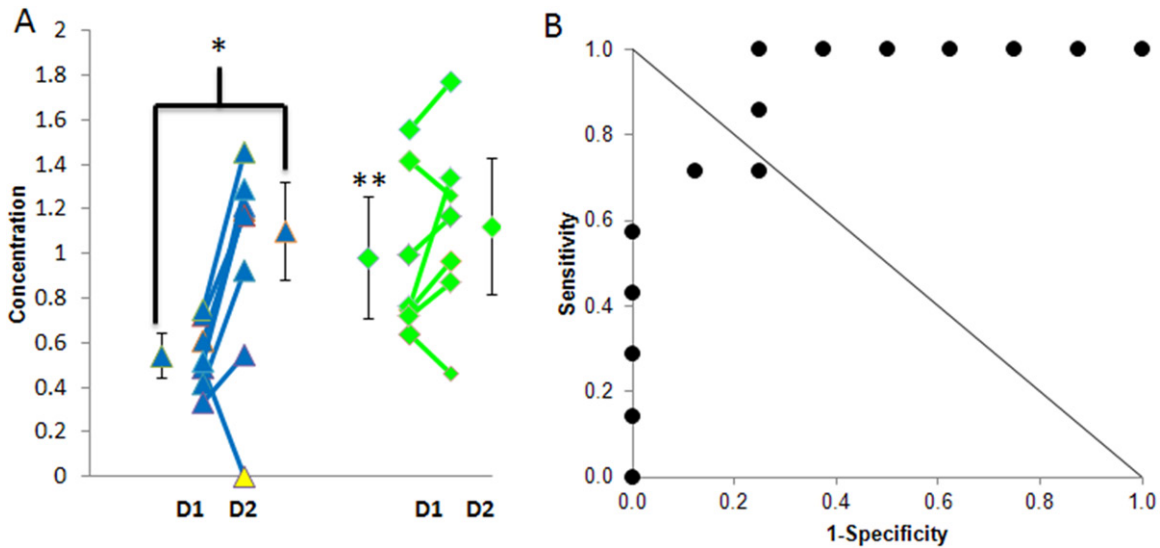


Figure 2. Exercise - induced changes in prefrontal lactate for GWI subgroups. A. Absolute values for lactate levels are shown for pre-exercise Day 1 (D1) and post-exercise Day 2 (D2) for Increasers (blue triangles) and Decreasers (green diamonds). Baseline levels were significantly different between Increasers and Decreasers ($**P = 0.008$, 2-tailed unpaired t-test). Exercise - induced lactate levels rose significantly in Increasers ($*P = 0.02$, 2-tailed paired t-test). The value of 0 for lactate on D2 was highlighted (yellow triangle) as a potential erroneous spectroscopy reading. B. ROC curve separating increasers and decreasers based upon Pre-exercise lactate levels. AUC=0.920, Sensitivity= 71.4%, Specificity=87.5%; $P=0.007$, Asymptotic Significance. (Errors bars are mean[\pm 95% confidence intervals]).

coil array. Structural 3D T1-weighted MPRAGE images parameters were: TE= 2.52 ms, TR= 1900 ms, TI= 900 ms, FOV= 250 mm, 176 slices, slice resolution= 1.0 mm, voxel size 1x1x1 mm. All MPRAGE images were processed using

the Statistical Parametric Mapping software package (SPM8, Wellcome Department of Imaging Neuroscience, London, UK), which was run on MATLAB 10b (The MathWorks Inc., Natick, MA, USA).

Pre-frontal lactate in GWI

Table 1. Demographic and behavioral parameters

Parameter	Controls (n=11)	CMI (n=15)	Increasesers (n=8)	Decreasers (n=7)
Age	47.4 [41.3 to 53.6]	44.6 [41.5 to 46.8]	45.8 [41.7 to 49.9]	43.2 [38.1 to 48.4]
Males, n (%)	9 (81.8%)	11 (73.3%)	7 (87.5%)	4 (57.1%)
Pain and Fatigue ratings				
Ordinal Fatigue	1.63 [0.74 to 2.5]	3.73 [3.50 to 3.97]**	3.63 [3.27 to 3.98]	3.86 [3.58 to 4.14]
Chalder Fatigue	12.1 [8.8 to 15.3]	26.9 [24.8 to 29.0]**	26.0 [23.2 to 28.7]	27.8 [24.5 to 31.2]
McGill Total	5.45 [0.95 to 9.9]	28.2 [24.2 to 32.1]**	26.0 [20.0 to 31.9]	30.4 [25.4 to 35.4]
MOS-SF-36 Quality of Life Domains				
Physical Func.	79.5 [63.7 to 95.3]	40.3 [26.5 to 54.1]**	49.2 [32.8 to 65.7]	31.4 [10.1 to 52.6]
Role Physical	72.9 [50.7 to 95.0]	16.1 [-1.43 to 33.5]**	32.1 [0.57 to 63.7]	0.0 [0.00 to 0.00]
Bodily Pain	70.0 [55.6 to 84.3]	20.2 [9.88 to 30.5]**	19.4 [2.35 to 36.5]	21.0 [7.9 to 34.1]
General Health	70.5 [58.2 to 82.8]	26.1 [14.4 to 37.7]**	26.7 [15.8 to 37.5]	25.6 [3.9 to 47.3]
Vitality	56.6 [43.3 to 69.9]	11.4 [4.13 to 18.7]**	8.5 [-2.1 to 19.2]	14.3 [3.9 to 24.6]
Social Func.	76.0 [58.8 to 93.2]	20.5 [10.7 to 30.3]**	19.6 [7.86 to 31.4]	21.4 [4.8 to 38.1]
Role Emotional	88.8 [74.2 to 103.5]	33.3 [9.61 to 57.0]**	52.3 [17.8 to 86.8]	14.3 [-13.7 to 42.3]
Mental Health	74.0 [66.8 to 81.1]	52.8 [42.2 to 67.4]**	50.8 [35.3 to 66.3]	58.9 [38.1 to 79.7]
2-Back accuracy				
	Controls (n=11)	CMI (n=15)	Increasesers (n=8)	Decreasers (n=7)
Pre-exercise	28.9 [24.9 to 32.9]	18.4 [14.8 to 22.1]*	19.2 [15.3 to 23.1]	17.6 [10.8 to 24.3]
Post-exercise	29.4 [25.6 to 33.2]	18.8 [13.3 to 24.3]*	27.4 [24.0 to 30.7]	9.00 [4.37 to 13.6]

CMI subjects had significantly higher fatigue and pain ratings, and lower SF-36 quality of life scores than controls. After stressors the subjects were classified as Increasesers or Decreasers based upon the exercise - induced change in their 2-back working memory accuracy (maximum accuracy = 35). There were no significant differences between these CMI subgroups except for post-exercise accuracy. Mean [95% confidence intervals]. * $P < 0.001$, ** $P < 0.00001$ vs. controls; 2-tailed unpaired Student's t-tests.

Single voxel spectroscopy was used to assess metabolite concentrations. A voxel of 2 x 2 x 2 cm³ on edge (8.0 ml) was placed over the left anterior cingulate cortex (ACC) (**Figure 1**). Single voxel spectroscopy of ¹H MRS uses the volume localized STEAM sequence with echo times (TE) of 20ms–144ms, repetition time (TR) 2000ms, modulation time TM 10ms, 300 transients, spectral width of 3 kHz, 1k complex data points. Automatic outer volume suppression (7 min scan) eliminated lipid and water signals.

Metabolite concentrations of the in vivo spectrum were acquired using “LCModel” (Provencher Inc., Oakville, Canada) [41]. The method is fully automated excluding subjective inputs, referencing, and initial estimates. The in vivo spectrum is modelled as a linear combination of lined broadened individual metabolite spectra. A high-resolution anatomic scan was used for graphical positioning of the MRS voxels (**Figure 1**), which was also used for partial volume correction by determining the tissue composition and as previously described [42].

The voxels from the different locations were transformed onto the VBM (GM, WM, and CSF) maps to obtain the voxel tissue content. Reliability of the metabolite concentrations were based on Cramer-Rao lower bounds (CRB) from the Cramer-Rao inequality given as percent standard deviation of the brain parenchyma which was implemented for voxel CSF content correction [43]. The Cramer-Rao lower bounds for glutamate and glutamine were from 7% to 44% standard deviation for CMI subjects. The Cramer-Rao lower bounds for lactate were from 19% to 50% for all CMI subjects. One CMI subject had a lactate concentration level of 0 with a standard deviation of 999% and highlighted as a yellow triangle (**Figure 2A**). Even though this is undoubtedly a measurement error, this data point was included to minimize attrition given the small N for these analyses.

Statistical analysis

SPSS for Windows version 20 (Armonk, New York) and Microsoft Excel 2007 (Redmond, Washington) were used for database and sta-

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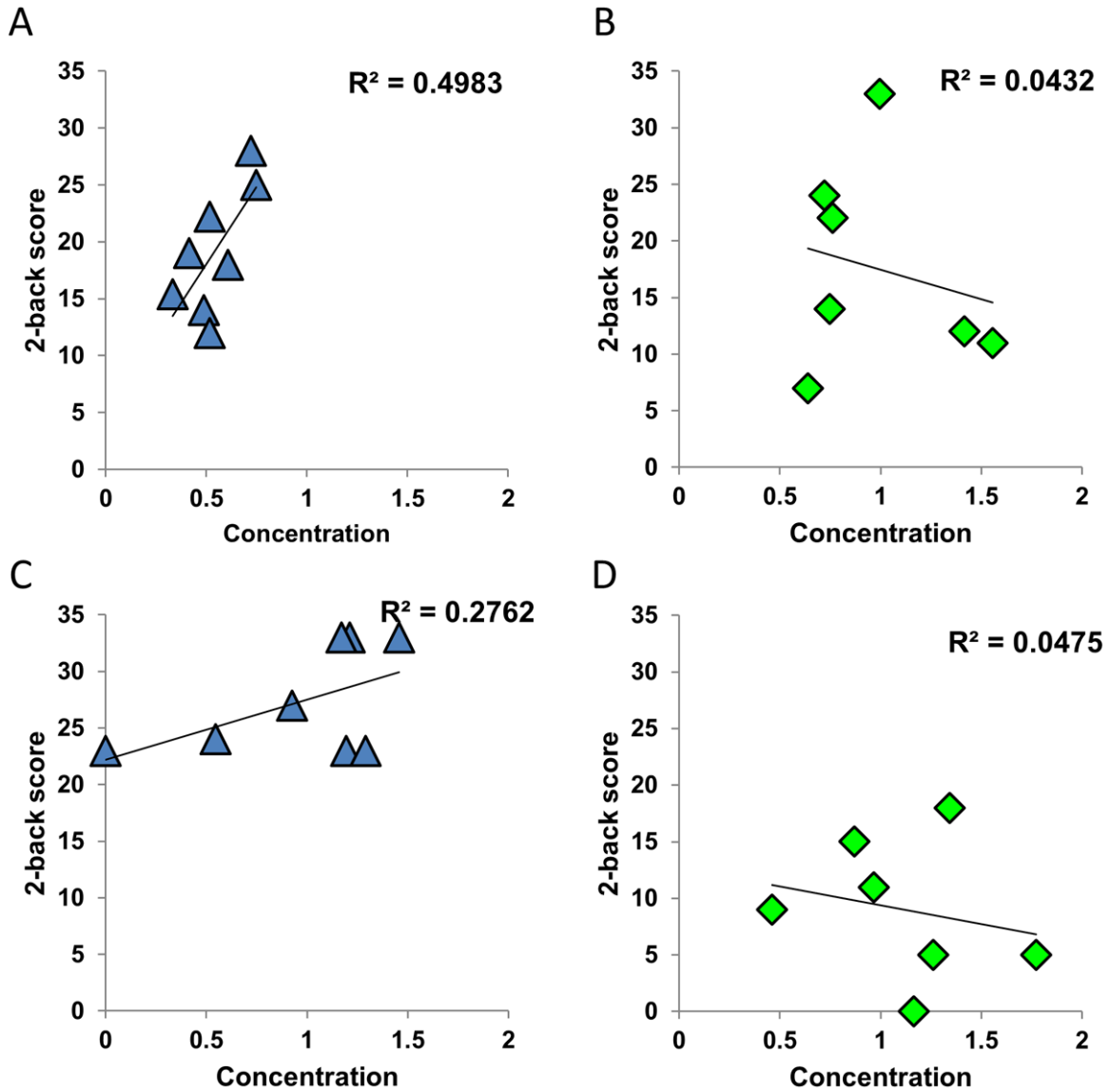


Figure 3. Pre-frontal lactate levels and correlation with working memory scores. A. Prior to exercise, prefrontal lactate concentration correlated with increasers but not (B) decreasers 2-back WM scores. C. After exercise, prefrontal lactate levels correlated with increasers but not (D) decreasers 2-back WM scores.

tistical analysis. 2-back working memory scores, metabolite concentrations, pain and fatigue ratings were compiled and reported as means with [95% confidence intervals]. Significant differences between groups ($P \leq 0.05$) were identified by two-tailed unpaired Student's t-tests or Fisher's exact tests. P values were not corrected for multiple comparisons due to the exploratory nature of this analysis. Metabolite concentrations were evaluated by receiver - operator curve (ROC) analysis as predictive measures with sensitivity, specificity,

area under the curve (AUC), and asymptotic significance.

The differences in 2-back accuracy between pre - and post - exercise fMRI scans were determined for each subject. Visual inspection followed by receiver operator analysis identified two groups. (i) "Increasers" had higher accuracies with Day 2 minus Day 1 $\Delta \geq 4$ out of 35 letter responses after exercise. (ii) "Decreasers" had lower or essentially no change ("floor effect") in accuracy with $\Delta \leq 2$ out of 35

Pre-frontal lactate in GWI

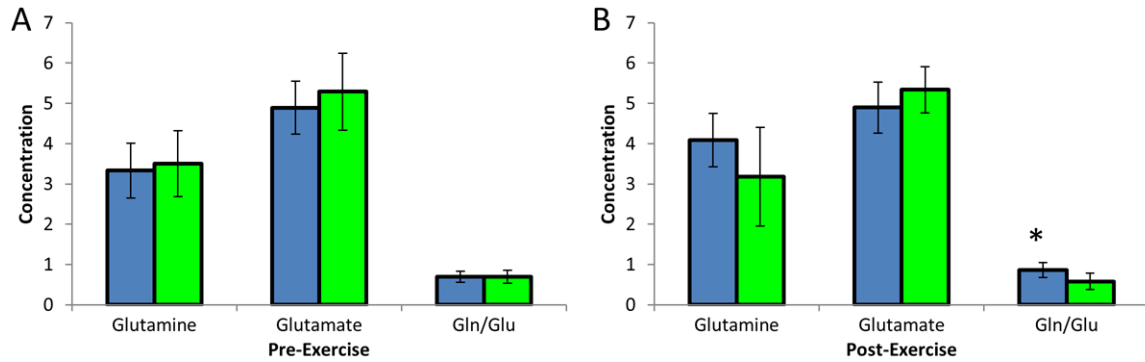


Figure 4. Glutamine, Glutamate, Gln/Glu, and Receiver Operator Curve for Prefrontal Lactate Levels at baseline. A. Prior to exercise there was no significant difference between increasers (blue bars) and decreasees (green bars) for Glutamine (Gln), Glutamate (Glu) and Gln/Glu ratio. B. Following exercise, there was an elevated trend for increasers compared to decreasees in the Gln/Glu ratio. (* $P=0.062$, 2-tailed unpaired t-test).

responses. Hence, Increasers significantly improved their cognitive function following exercise, while Decreasees did significantly worse after exercise. These findings and correlations with blood oxygen dependent level (BOLD) findings are being reported in detail elsewhere.

Lactate, glutamine, and glutamate concentrations were correlated with 2-back working memory scores in univariate fashion using two-tailed Pearson's function.

Results

Demographics and behavioral data

Fifteen veterans who met the 1998 CDC criteria for CMI [2] and eleven controls (age and gender matched) were recruited. There were no significant differences in demographics between groups (Table 1). Similar to previous reports of increased prevalence, all veterans (CMI subjects) also met CFS criteria [4, 7]. CMI subjects had significantly higher pain and fatigue ratings and lower SF-36 quality of life scores than controls ($P<0.0001$; Table 1).

Working memory accuracies before and after exercise

Before exercise, CMI subjects had significantly lower accuracy than controls ($P<0.001$, 2-tailed unpaired t-test) (Table 1, Figure 1B). Exercise-induced changes in accuracy defined the CMI subgroups. Increasers raised their accuracy on average by 29.6% ($n=8$, $P=0.000018$, 2-tailed paired t-test) whereas Decreasees on average had a drop of 24.5% ($n=8$, $P=0.087$) (Figure 1B).

Lactate concentrations and accuracies

At baseline, prefrontal lactate was significantly higher in Decreasees (0.98 [0.71 to 1.25]) than Increasers (0.54 [0.44 to 0.643]; $P<0.008$, 2-tailed unpaired t-test) (Figure 2A). Receiver operator analysis demonstrated that pre-exercise lactate levels could predict Increaser and Decreaser status prior to the completion of the exercise stress tests (AUC=0.920, sensitivity=71.4%, specificity=87.5%; $P=0.007$, asymptotic significance; Figure 2B).

The presence of distinct CMI subgroups was confirmed by the exercise-induced increase in prefrontal lactate in the Increaser subgroup ($P=0.021$, paired t-test; Figure 2A). In contrast, Decreasees had no significant change ($P=0.19$, paired t-test; Figure 2A). Prior to exercise, lactate levels were significantly correlated with working memory scores in Increasers ($R^2=0.50$; Figure 3A) but not Decreasees ($R^2=0.04$; Figure 3B). The same relationship was found after exercise for Increasers ($R^2=0.28$; Figure 3C) but not Decreasees ($R^2=0.05$; Figure 3D).

Prefrontal glutamine and glutamate

Prefrontal glutamine, glutamate, and glutamine/glutamate ratio were not significantly different between Increasers and Decreasees prior to exercise (Figure 4A), and did not correlate with working memory scores, pain, or fatigue indices. The glutamine/glutamate ratio was marginally higher in Increasers compared to Decreasees after exercise ($P=0.062$, unpaired t-test; Figure 4B) driven primarily by changes in glutamine concentration post-exer-

cise relative to pre-exercise such that there was an rise in the Increasers and a slight drop in the Decreasers.

Discussion

The primary observation of this study was that baseline prefrontal lactate levels predicted future exercise - induced cognitive responses in CMI subjects. These concentration differences were observed before the two exercise stress tests when behavioral measures and 2-back task accuracies were equivalent between CMI subgroups. Prefrontal lactate levels and accuracies were significantly correlated for the Increaser subgroup both before and after exercise. No such relationship between brain lactate and cognition was found for Decreasers.

This represents an important advance by using objective findings to delineate functional subtypes. Exercise proved to be a critical stressor that perturbed the baseline state of CMI compensatory adaptations to more clearly reveal distinctions from controls as well as by Increaser and Decreaser cognitive - prefrontal lactate status. These CMI subjects also met CFS criteria, so future studies will be needed to confirm and expand our conclusions for the entire population of Persian Gulf War veterans, and to identify pathophysiological similarities with CFS.

The comparison of pre - exercise to post - exercise scans provided insights into brain energetics in CMI subgroups. At baseline, normal physiological activity in the adult brain oxidizes glucose [13, 44]. However, working memory constitutes a cognitively demanding state where glutamatergic afferents to the prefrontal cortex underlie efficient activity [45-48]. During increased neural synaptic activity, lactate is the preferred energy source even in the presence of glucose [49]. Mono-carboxylate transporters (MCT) are responsible for shuttling lactate in the brain. MCT-2 is the predominate neuronal lactate transporter. MCT-1 and MCT-4 are the primary astrocytic lactate transporters [50]. The relationship between lactate and working memory scores in Increasers was consistent with preferential lactate metabolism by neurons during highly demanding cognitive situations [14, 15].

In contrast, the lack of correlation between working memory scores and the persistently elevated prefrontal lactate levels of Decreasers implied an inability to import or utilize lactate by neurons. MCT-2 transporter dysfunction is one potential explanation. Murine models show that blocking neuronal MCT-2 leads to an increase in glucose metabolism and dysfunction of the proposed astrocyte - neuron lactate shuttle [51]. Extrapolation of our data would suggest that cerebral glucose may have played a compensatory metabolic role in Decreasers so that their baseline 2-back accuracies were comparable to Increasers.

Mitochondrial dysfunction provides an alternative hypothesis to explain the significantly elevated prefrontal lactate levels before and after exercise in Decreasers. Loss of oxidative capabilities in neurons and astrocytes may cause a shift to glycolysis for ATP generation [20]. Lactate dehydrogenase (LDH) is responsible for the catalytic conversions between pyruvate and lactate. The isoform LDH-A is responsible for converting pyruvate to lactate whereas isoform LDH-B is responsible for converting lactate to pyruvate [13]. Mitochondrial dysfunction leads to increased transcriptional activity with higher relative levels of LDH-A [20]. This facilitates the regeneration of NAD⁺ substrates that can be reused in glycolysis to maintain ATP generation. This compensatory dependence on glycolysis may explain the increased lactate levels in Decreasers.

Exercise causes a multitude of changes in brain, muscle, liver and other organs [52]. During vigorous exercise, skeletal muscles dramatically increase glucose uptake in order to generate ATP for sustained contractions [52]. This depletes plasma glucose availability leading to a 32% decrease in brain glucose uptake [53]. In the normal brain, the lower glucose availability as a fuel is met by compensatory replacement with lactate [54].

Our results suggest Increasers were able to metabolically shift to the use of lactate as an energy source leading to an increase in the availability of glutamate, a key transmitter for memory function[19] leading to an increase in 2-back accuracy after exercise. However, Decreasers were not able to utilize lactate in the post-exercise setting when glucose would be preferentially sequestered to muscle.

Inability to use lactate may have required decreaseers to rely more heavily on glycolytic metabolism. This compensatory reliance on glucose combined with the fact that exercise is known to cause depleted plasma glucose levels [53] may have accounted for their neuronal dysfunction, inefficient working memory, and observed drop in their 2-back scores after exercise.

Vigorous exercise can increase brain lactate, glutamate and glutamine in healthy humans [55]. Glutamate is converted to glutamine in astrocytes. The glutamine/glutamate ratio may indirectly measure astrocyte function as the astrocyte is the only cell capable of transforming glutamate to glutamine [22]. Increases in glutamine and/or glutamate concentration are associated with active memory formation and subsequent consolidation to long term memory [56]. Although speculative, the trend towards elevated glutamine/glutamate ratios in Increaseers but not Decreaseers after exercise is suggestive of astrocyte dysfunction and inefficient glutamate-glutamine cycling in Decreaseers [57]. An inability of astrocytes to uptake and recycle synaptically - released glutamate may have hindered synaptic activity and contributed to the lower 2-back scores of Decreaseers after exercise.

Myo-inositol, choline, γ -aminobutyric acid, N-acetylaspartate, creatine and other analytes were not different between Increaseers, Decreaseers and controls either before or after exercise. This could indicate that their metabolism is not altered in CMI, or that molecular spectroscopy is not satisfactory to detect dynamic changes in their turnover during cognition.

The main limitation of this study was the small sample sizes in CMI and controls. Only 3 control subjects had post - exercise molecular spectroscopy over the prefrontal region because of a change in voxel placement (results to be reported elsewhere). Prefrontal metabolite concentrations should be cautiously interpreted as the spectroscopic voxels contains varying amounts of gray matter, white matter and cerebrospinal fluid. Statistics were not corrected for multiple comparisons due to the pilot nature of the study. No comparisons were made to civilians with CFS alone, or to anxiety, major affective disorder, or other conditions. Future

studies should incorporate larger CMI sample sizes and multiple voxels in regions associated with pain and fatigue to assess the global changes in metabolite concentrations.

Conclusion

In this pilot study, we compared working memory scores and prefrontal metabolite concentrations via ^1H MRS spectroscopy to assess underlying pathophysiological processes of CMI criteria and exertional exhaustion. To our knowledge, this is the first controlled study to compare the causal relationship between exercise induced changes in prefrontal metabolite concentrations in subgroups with Gulf War Illness.

Our primary finding is a proportion of subjects had elevated prefrontal lactate that was predictive of exercise induced cognitive dysfunction. Lactate levels significantly correlated with scores in the Increaseer but not Decreaseer subgroup both before and after exercise suggesting dysfunctional neuronal-astrocyte relationships in decreaseers. We suggest that alterations in brain energetics may be in part responsible for a subgroup of GWI and underlie some of the symptoms present in the patient population.

Results may also provide broader support for pathophysiological processes underlying overlapping syndromes such as CFS and fibromyalgia. Future studies should validate our protocol with larger samples to confirm the exploratory data. Our findings may indicate ways to identify subgroups and metabolites that could serve as targets for therapeutic strategies for the treatment of GWI and other idiopathic "functional" syndromes.

Acknowledgments

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Competing interests

The authors declare they have no competing interests.

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