Multimodal Validation of Oxidative Stress as a Pathophysiological Model of Chronic Fatigue Syndrome

Dikoma C Shungu[1], Nora Weiduscat[1], James W Murrough[2], Xiangling Mao[1], Sarah Pillemer[2], Marvin S Meddow[3], Jonathan P Dyke[1], Benjamin H Natelson[4], Julian M Stewart[3], and Sanjay J Mathew[5]

[1]Radiology, Weill Cornell Medical College, New York, New York, United States, [2]Psychiatry, Mount Sinai School of Medicine, [3]New York Medical College, [4]Beth Israel Medical Center, [5]Baylor College of Medicine

INTRODUCTION

Chronic fatigue syndrome (CFS) is a complex illness, which is often misdiagnosed as a psychiatric illness. In two previous studies, we used ^{1}H MRSI to compare neurometabolites in CFS with generalized anxiety disorder (GAD) [1] and major depressive disorder (MDD) [2], common neuropsychiatric disorders with extensive symptom overlap with CFS. In those reports, CFS patients showed significantly elevated ventricular cerebrospinal fluid (CSF) lactate compared to healthy control subjects [1,2] and to patients with GAD [1], while no differences were found between CFS and MDD [2]. Importantly, our replicated finding of significant elevations of ventricular lactate in CFS suggested a potential illness-associated biomarker, whose understanding could shed new light onto the pathophysiology of the illness. In the present third independent cross-sectional study, we aimed to investigate a pathophysiological model of CFS, which postulates that sustained oxidative stress [3] and associated oxidant damage lead to cerebral hypoperfusion and/or to secondary mitochondrial dysfunction that could potentially explain our observed cross-sectional elevations of ventricular lactate. Specifically, this study had two primary objectives: (a) to use ^{1}H MRSI to replicate in a new cohort our finding of cross-sectional elevations of ventricular lactate in CFS, and (b) to determine whether the postulated [3] and experimentally documented [4,5] oxidative stress increases in the disorder are associated with antioxidant capacity deficit by using ^{1}H MRS to measure *in vivo* brain levels of glutathione (GSH), the most abundant antioxidant in CNS. In addition, we used arterial spin-labeling (ASL) MRI to replicate prior observations of decreased regional cerebral blood flow (rCBF) in CFS [6,7] that may explain the observed lactate elevations, and ^{31}P MRSI to measure regional brain levels of high-energy phosphates (HEPs) as indices of a possible secondary mitochondrial dysfunction in CFS [3], whose presence might also be associated with elevations in lactate.

METHODS

Subjects: Participants included 15 unmedicated patients with CFS diagnosed according to the CDC criteria [8], 15 unmedicated patients with major depressive disorder (MDD), as established by DSM-IV-TR criteria who served as "disease controls", and 13 age- and sex-matched healthy volunteer (HV) subjects.

In vivo Neuroimaging Measurements: A GE 3.0T MR system was used to conduct the following neuroimaging studies in a single 60-90 min session: (a) *In vivo brain GSH data* were acquired from a 3x3x2-cm^3 occipital cortex voxel using the standard J-editing sequence (**Fig. 1**); (b) *In vivo ventricular CSF lactate* levels were obtained by multislice ^{1}H MRSI [1,2]; (c) *Regional and global cerebral blood flow* was acquired using fast spin echo-based continuous ASL-MRI method; and (d) high-energy phosphates were obtained by ^{31}P MRSI using the DRESS sequence. *In vivo* levels of all compounds derived by MRS were corrected for brain matter content using segmented volumetric MRI.

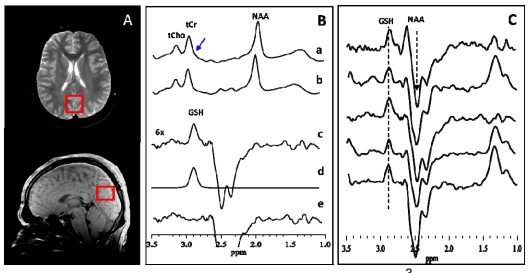

Fig. 1: [A] Location of a 2.0 x 3.0 x 3.0-cm^3 occipital lobe voxel targeted for GSH measurement. [B] J-editing. [C] Consistent detection of GSH in 5 subjects.

RESULTS AND DISCUSSION

(a) Ventricular CSF Lactate: Mean ventricular CSF lactate, measured by ^{1}H MRSI and expressed in institutional units (i.u.), differed significantly between the CFS, MDD and HV groups ($F_{2,33}$ = 16.78; p < .001) (**Fig. 2**). Post-hoc analyses found that CFS patients had significantly higher ventricular lactate levels than HV (p < .001). Elevated ventricular lactate levels were also found in MDD compared to HV (p = .009). There was a weak trend toward higher ventricular lactate in CFS compared to MDD (p = .114). *This finding represents a third independent replication of our previous observation of increased CSF lactate in CFS, suggesting this to be a feature of the disorder.*

(b) Cortical GSH: Comparisons of occipital GSH levels measured by J-editing and normalized to the peak area of the unsuppressed voxel tissue water (W) revealed a main effect of diagnostic group ($F_{2,40}$ = 15.93; p < .001), which post-hoc testing attributed to **reductions of GSH/W** (**Fig. 2, bottom**) in both CFS (p < .001) and MDD (p = .004) compared to HV. There was a non-significant trend towards lower GSH/W in CFS compared to MDD (p = .086). *To our knowledge, this is the first study to document in vivo cortical GSH deficits in CFS (a **36%** decrease) and in MDD (a **21%** decrease), which supports a role for increased oxidative stress in both disorders, and provides a compelling rationale for investigating treatment strategies, such as supplementation with N-acetylcysteine (NAC) or other synthetic precursors, that can restore cortical GSH reserves to potentially lower oxidative stress.*

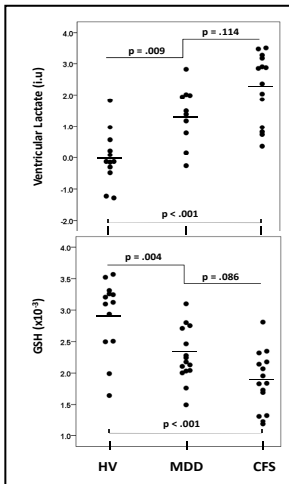

Fig. 2: LAC and GSH scatter plots

(c) Regional Cerebral Blood Flow (rCBF): Following intensity and morphological normalization and statistical analysis using the Statistical Parametric Mapping (SPM) software, Version 5, we found significantly different ASL-derived CBF values at the uncorrected significance level of .001 in two brain regions. The CFS group had lower rCBF values in the left anterior cingulate cortex (p = .039) and in the right lingual (p = .016) regions compared to the HV group. In addition, there was a trend toward lower rCBF in the left anterior cingulate cortex in MDD subjects compared to HV (p = .08). There were no significant differences in rCBF values between CFS and MDD in any brain region. *These results are consistent with prior reports of decreased rCBF in CFS* [6,7].

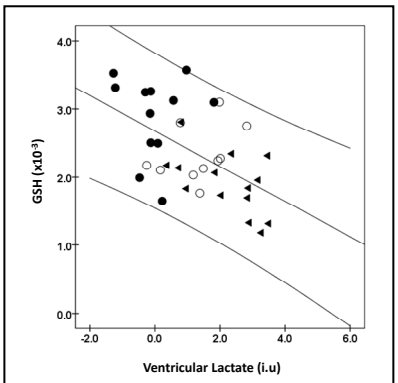

Fig. 3: Correlation of occipital GSH and ventricular lactate levels across all CFS (◄), MDD (○) and HV (●) participants (r = -.545, p < .001)

(d) High-Energy Phosphates: We found no differences between the groups in any phosphate metabolites, *suggesting that mitochondrial dysfunction may not be a key factor in our reported lactate elevations in CFS.*

(e) *Correlations among Lactate, GSH and Clinical Variables*: In exploratory correlational analyses, we found ventricular lactate and cortical GSH to correlate inversely (**Fig. 3**), not only with each other (r=-.545; p <.001), but also with several key indices of physical health and disability across all participants, further supporting a role for oxidative stress the pathophysiology of CFS and MDD.

CONCLUSION

Our finding of a significant 36% cortical GSH deficit in CFS has provided both mechanistic and face validity for an emerging oxidative stress model of this poorly understood illness, documenting for the first time a significant decrease in antioxidant capacity in living brain

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