

AWARD NUMBER: **W81XWH-17-1-0457**

TITLE: **Glutamate Neuroexcitotoxicity in GWI**

PRINCIPAL INVESTIGATOR: **Kathleen Holton, PhD, MPH**

CONTRACTING ORGANIZATION: **American University**  
4400 Massachusetts Ave NW  
Washington DC 20016

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# REPORT DOCUMENTATION PAGE

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		<b>5c. PROGRAM ELEMENT NUMBER</b>
<b>6. AUTHOR(S)</b>  Kathleen Holton, PhD, MPH  E-Mail: holton@american.edu		<b>5d. PROJECT NUMBER</b>
		<b>5e. TASK NUMBER</b>
		<b>5f. WORK UNIT NUMBER</b>
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<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited		
<b>13. SUPPLEMENTARY NOTES</b>		
<b>14. ABSTRACT</b>  The objective of the proposed research is to examine whether dietary exposure to food additives containing glutamate may be contributing to symptoms in Gulf War Illness (GWI). The rationale for proposed study comes from data in the fibromyalgia field which suggests that reducing the consumption of dietary glutamate can reduce over-excitation in the nervous system, leading to symptom improvement. Since there is almost complete symptom overlap between fibromyalgia and GWI, it is of utmost importance to test this diet as a low-cost treatment option in GWI patients. Herein, we report on our preliminary findings of this research which include highly significant and clinically meaningful improvements in overall symptom load, pain, fatigue, quality of life, cognitive function, and mood symptoms.		

<b>15. SUBJECT TERMS</b> Gulf War Illness, GWI, glutamate, diet, nutrition, intervention					
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**INTRODUCTION:** This is a phase II clinical trial being completed to examine the effect of using a low glutamate diet as a low-cost treatment option for Gulf War veterans who are suffering from Gulf War Illness (GWI). We recruited 40 veterans of the Gulf War from across the US who are suffering from symptoms according to the CDC and Kansas City criteria for GWI. We have completed the third year of the 3-year grant and are currently in a no-cost extension period.

**KEYWORDS:** Gulf War Illness, GWI, multi-symptom illness, neurological symptoms, glutamate, diet, intervention, nutrients, nutrition

**ACCOMPLISHMENTS:** We successfully recruited all 40 subjects for the study and have begun analyzing and publishing/presenting data from the study.

### **What were the major goals of the project?**

The major goals of the project for year 3, as stated in the approved SOW, included:

1. Recruiting, scheduling, and collecting data, diet training, challenge period data collection, data entry/cleaning, data analysis, publication, and presentation of findings.

### **What was accomplished under these goals?**

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. Major Activities – finished recruiting subjects, all data has been double entered and cleaned, some analyses have been completed, first publications have been submitted, and the primary data was presented at the Gulf War Illness State of the Science conference last month.
2. Specific Objectives – our objective was to complete recruitment, finalize data, run analyses, and begin publishing the data. These objectives were all met.
3. Significant Results -

The demographic breakdown for those in our recently completed GWI study is presented below. Most subjects tended to be overweight or obese. Because of this, many subjects can additionally benefit from a healthy diet in many ways outside of just treating their GWI symptoms. We have been successful at recruiting a good number of women, as well as identifying a more racially diverse sample.

Table 1. Demographic characteristics of all subjects consented and enrolled

<b>Demographic (N=40)</b>	<b>Mean (SD)</b>
Age (yrs)	54.35 (6.02)
BMI (kg/m <sup>2</sup> )	32.10 (5.34)
Systolic blood pressure (mm/Hg)	130.38 (14.11)
Diastolic blood pressure (mm/Hg)	76.00 (8.79)

	N (%)
Sex	
Female	11 (27.5%)
Male	29 (72.5%)
Race	
Caucasian	37 (92.5%)
African American	3 (7.5%)
BMI	
Healthy weight	4 (10.0%)
Overweight	11 (27.5%)
Obese	25 (62.5%)
Military Branch	
Army	20 (52.6%)
Air Force	6 (15.8%)
Navy	5 (13.2%)
Marine Corps	7 (18.4%)
Highest Education	
High School Graduate	3 (7.7%)
Some college	15 (38.5%)
Graduate school	11 (28.2%)
Postgraduate school or degree	10 (25.6%)
Marital Status	
Single (never married)	1 (2.6%)
Married	28 (71.8%)
Divorced/Separated	7 (17.9%)
Widowed	3 (7.7%)
Employment Status	
Currently working	17 (43.6%)
Homemaker	1 (2.6%)
Retired (not due to illness)	4 (10.3%)
Disabled/Retired due to illness	15 (38.5%)
Unemployment	2 (5.1%)
Cigarette Smoking	
Never smoked	26 (65.0%)
Quit <6 months	13 (32.5%)
Quit >6 months	1 (2.5%)

When comparing responses obtained from subjects randomized to the diet intervention group (one-month dietary intervention) as compared to the waitlisted controls after one month, there was a significant difference between groups,  $p=0.0009$ , and the effect size was calculated as Cohen's  $d = 1.16$ , which is considered a 'very large' effect size for the low glutamate dietary intervention. As demonstrated below, after everyone completed one month on the low glutamate diet, highly significant improvements were noted in every single outcome assessed. There was a highly significant reduction in the overall number of symptoms (with an average of 9 symptoms going away after one month on the diet), with highly significant reductions in pain (e.g.

dolorimetry, number of tender points, myalgic score), fatigue, depression, anxiety, and PTSD; and highly significant improvements in cognitive function (neurocognition index) and quality of life. To our knowledge, no other treatment for GWI has come close to this magnitude of improvement, nor has a treatment demonstrated improvements across such a wide range of symptoms experienced.

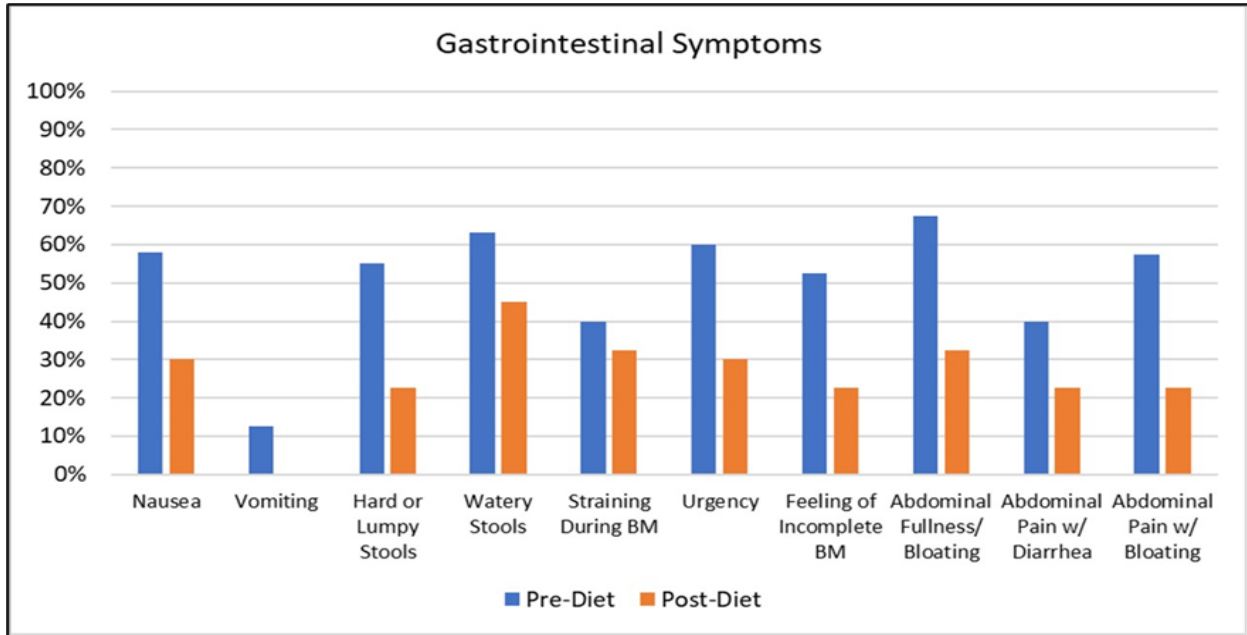
**Table 2. Changes in Outcome Measures after 1-Month on the Low Glutamate Diet**

<b>N=40</b>	<b>Pre-Diet</b>	<b>Post-Diet</b>	<b>P-value*</b>
	<b>Median (IQ Range)</b>		
Dolorimetry - Avg Amount of Pressure (kgs) Before Pain Onset in 18 Tenderpoints - higher better	15 (11)	18 (10)	0.0006
	<b>Mean (SD)</b>		<b>P-value**</b>
Total Number of Symptoms	21 (5)	12 (5)	<0.0001
Number of Tender Points (0-18)	12 (5)	10 (5)	<0.0001
Myalgic (Pain) Score (0-54)	22 (12)	14 (9)	<0.0001
Chalder Fatigue Scale (0-42)	29 (8)	16 (9)	<0.0001
Neurocognition Index (NCI) - higher is better	91.4 (14.6)	95.6 (17.7)	0.002
Depression (CES-D) Measure (0-60)	27.8 (13.5)	18.7 (11.2)	<0.0001
Anxiety (GAD-7) Measure (0-21)	9.5 (7.0)	6.6 (6.5)	0.0008
PTSD (PCL-C) Measure (0-85)	52.4 (18.4)	41.8 (17.5)	<0.0001
Quality of Life (SF-36) - higher is better			
Physical	46.5 (37.2)	60.9 (37.8)	<0.0001
Emotional	52.6 (30.6)	68.8 (27.9)	<0.0001
Energy	20.8 (23.2)	53.2 (33.2)	<0.0001
General Health	30.0 (27.4)	39.4 (30.9)	<0.0001

\*Wilcoxon Signed Rank Test    \*\*Paired t-test

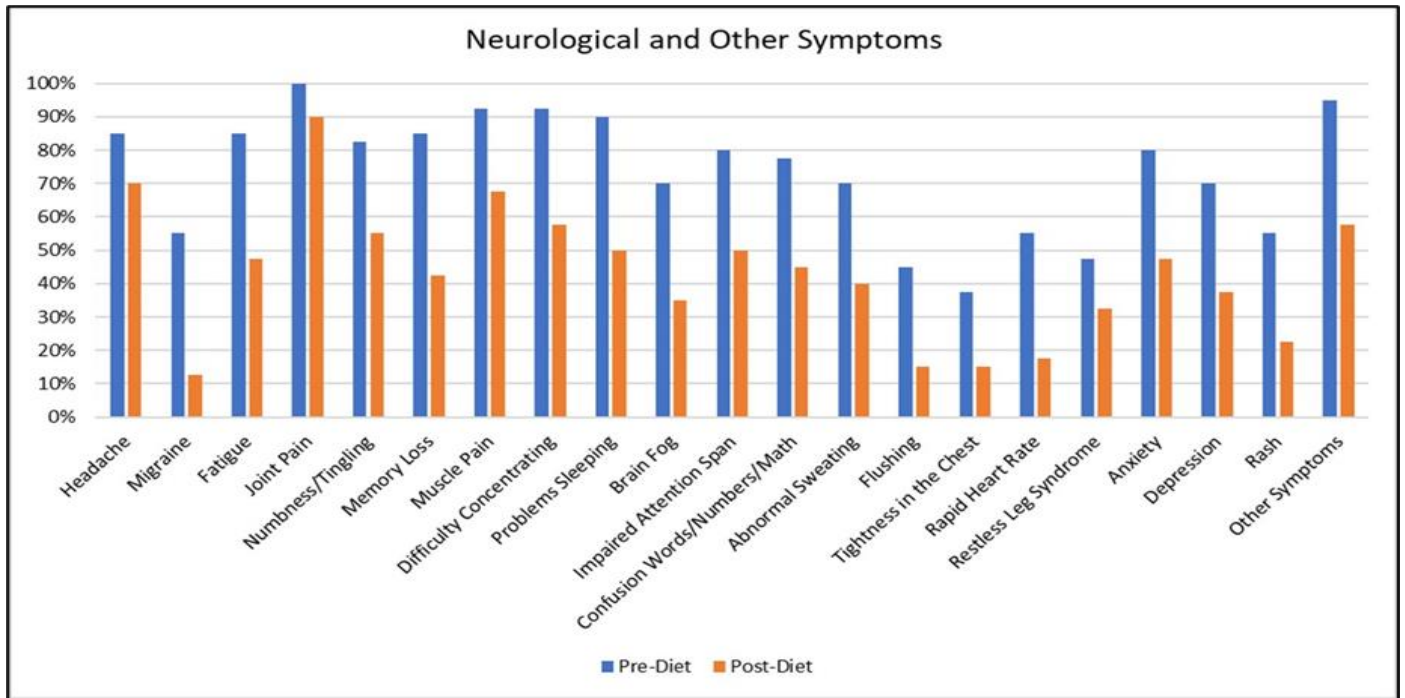
To help visualize the change in the report of specific symptoms (from the total symptom score measure), the two figures below illustrate the percentage of subjects reporting gastrointestinal, neurological, and other symptoms pre-diet, as compared to after one month on the low glutamate diet. As demonstrated, there were reductions in the report of every single symptom.

**Figure 1. Comparison of the percentage of GW veterans reporting each gastrointestinal symptom pre- versus post-diet.**



All gastrointestinal symptoms responded very well to the low glutamate diet. Similarly, the report of most neurological symptoms also decreased dramatically after one month on the diet. The exception to this would be joint pain, which the majority of subjects still reported post-diet. However, the quality of the pain was found to differ, with widespread joint pain becoming more localized, and the remaining joint pain commonly being located in joints which were previously injured. For example, paratroopers tended to have joint pain remaining in their knees and ankles. Reductions in joint pain *severity* were also reported. In contrast, migraines were very responsive to the diet, with almost all subjects reporting remission of this symptom after one month on the diet. This is consistent with our prior research,<sup>34</sup> and with the literature, suggesting a strong influence of glutamate on migraine.<sup>35</sup> The most common “other” symptoms which were reported include tinnitus, peripheral neuropathy, visual disturbances, PTSD, sleep apnea, and reports of specific locations and types of pain (like shooting or burning pain in a specific location of the body). Many of these symptoms also remitted on the diet.

**Figure 2. Percentage of GW veterans reporting neurological and other symptoms pre-versus post-diet**



Upon challenge with MSG versus placebo in the randomized double-blind, placebo-controlled crossover challenge portion of the previous GWI study, we observed significant overall models for the pain measures, overall cognitive function, depression, anxiety, PTSD, and all four domains of the SF-36. Interestingly, these significant models were driven by inter-individual variation within sequence (MSG/placebo, or placebo/MSG), with some participants significantly worsening upon challenge, while others significantly improved. (Table 3) This differs from our previous research in fibromyalgia and deserves further study to understand why there is consistent improvement on the low glutamate diet, but differential response to challenge.

**Table 3. Results of Challenge with MSG versus placebo**

Measure	Placebo Mean (SD)	MSG Mean (SD)	F-statistic	Model P-value*
Total Symptom Score	12.00 (6.65)	11.33 (5.88)	1.21	0.28
Number Tender Points	9.62 (5.49)	9.48 (5.49)	5.10	<0.0001
Total Myalgic Score	16.13 (11.78)	15.36 (11.34)	5.08	<0.0001
Average Dolorimetry -higher is better	17.35 (6.16)	11.27 (5.85)	10.31	<0.0001
Chalder Fatigue Scale	16.28 (9.44)	16.55 (8.57)	0.79	0.77
Neurocognition Index (NCI) - higher better	98.70 (13.45)	99.20 (13.60)	5.81	<0.0001

Depression (CES-D) Measure (0-60)	15.48 (11.05)	15.33 (10.55)	5.51	<0.0001
Anxiety (GAD-7) Measure (0-21)	4.80 (5.05)	4.40 (5.72)	6.59	<0.0001
PTSD (PCL-C) Measure (0-85)	35.50 (16.60)	34.15 (15.09)	22.19	<0.0001
Quality of Life (SF-36) - higher is better				
Physical	57.88 (37.72)	63.00 (38.37)	2.45	0.003
Emotional	76.40 (27.03)	75.90 (27.15)	5.76	<0.001
Energy	55.63 (30.81)	56.25 (32.13)	2.56	0.002
General Health	40.00 (29.97)	42.25 (30.43)	13.32	<0.0001

\*General Linear Model accounting for sequence, period, challenge material, and subject nested within sequence. Bonferroni corrected p-value of p=0.004.

4. Other achievements – On Sept 10<sup>th</sup> we submitted a GWI grant proposal for a phase III clinical trial to confirm these findings in a larger group of veterans while also working to identify biomarkers of improvement and attempting to understand the differential effects observed during the challenge period of this study. If successful, this will allow for important next steps in this research.

### **What opportunities for training and professional development has the project provided?**

The project has provided many learning opportunities for students in the lab. One PhD student was able to identify a hypothesis she could test during the study. She successfully completed her PhD and has a manuscript currently under review at *Brain and Cognition*. Multiple undergraduate students helped with the study, gaining valuable experience in clinical trial research, and this has led to a published review article in *Neurotoxicology*, successful poster presentations at local and international conferences, and a post-bac student is currently working on analyses of fMRI data which we hope to submit as a manuscript this winter.

### **How were the results disseminated to communities of interest?**

The main outcomes of the study were presented last month at the GWI State of the Science conference, and preliminary data was presented last Fall at the International Society for Nutritional Psychiatry Research in London and the American Society for Nutrition 2019 conference in Boston. Unfortunately, the abstracts which were accepted for the 2020 conferences of the American Society for Nutrition and the International Association for the Study of Pain were never presented due to COVID-related cancellations. The primary study results were recently published in *Nutrients*, the cognitive results are currently under review, and a third manuscript on effects on mood outcomes should be completed this Fall, and will be submitted to *Biological Psychiatry* for review.

**What do you plan to do during the next reporting period to accomplish the goals?**

Our goals for the no-cost extension period is to complete all other statistical analyses and submit the remaining manuscripts from this work.

**IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

**What was the impact on the development of the principal discipline(s) of the project?**

The low glutamate appears to substantially reduce the overall number of symptoms of GWI, and significantly improves pain, fatigue, quality of life, cognitive, and mood outcomes. To our knowledge, there is no current treatment for GWI which comes close to this widespread clinically meaningful improvement observed in this research. This data supports the next step of pursuing funding for a phase III clinical trial. If these results hold in this larger study, this suggests that the low glutamate diet should offered as a low-cost treatment for GWI via VA dietitians, which could positively impact the quality of life of veterans with GWI.

**What was the impact on other disciplines?**

This research also impacts the fields of nutrition, neuroscience, psychiatry, and pain research. The low glutamate diet may have use in treating non-GW veterans with similar multi-symptom profiles, as well as other neurological/psychiatric disorders where abnormal glutamatergic neurotransmission has been implicated, such as epilepsy, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS).

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**

Nothing to report yet.

**CHANGES/PROBLEMS:**

**Changes in approach and reasons for change**

Nothing to Report.

**Actual or anticipated problems or delays and actions or plans to resolve them**

No expected or actual delays to report.

**Changes that had a significant impact on expenditures**

Nothing to Report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report for any of the below items:

**Significant changes in use or care of human subjects**

**Significant changes in use or care of vertebrate animals.**

**Significant changes in use of biohazards and/or select agents**

**PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

**Publications, conference papers, and presentations:**

**Journal publications.**

Holton KF, Kirkland AE, Baron M, Ramachandra SS, Langan MT, Brandley ET, Baraniuk JN. The low glutamate diet effectively improves pain and other symptoms of Gulf War Illness. *Nutrients*. 2020.12: 2593. DOI: [10.3390/nu12092593](https://doi.org/10.3390/nu12092593)

**Submitted Manuscripts**

Kirkland AE, Baron ML, VanMeter JW, Baraniuk JN, **Holton KF**. The Low Glutamate Diet Improves Cognitive Functioning in Veterans with Gulf War Illness and Resting-State EEG Potentially Predicts Response. Submitted to *Brain and Cognition*. Sept. 10, 2020.

**Books or other non-periodical, one-time publications.** Nothing to Report.

**Other publications, conference papers, and presentations.**

**Presentations**

- Oral presentation at the GWI State of the Science Conference (online) Aug 18-19<sup>th</sup>, 2020, titled "The low glutamate diet effectively improves overall symptom load, pain, and fatigue in veterans with GWI"
- Oral presentation at the International Society for Nutritional Psychiatry Research in London, England Oct 20-22, 2019 titled, "A Low Glutamate Diet Reduces Anxiety and PTSD Symptoms in Veterans with Gulf War Illness"
- Poster presentation (student) at the International Society for Nutritional Psychiatry Research in London, England Oct 20-22, 2019 titled, "The Effects of a Low Glutamate Diet on Depression in Gulf War Veterans"
- Poster presentation (student) at the International Society for Nutritional Psychiatry Research in London, England Oct 20-22, 2019 titled, "A Low Glutamate Diet Affects Gamma Power in Veterans with Gulf War Illness: Preliminary Data"
- Poster presentation and oral presentation (student) at the American Society for Nutrition conference in Baltimore, MD June 8-11<sup>th</sup>, 2019 titled, "The Effects of a Low Glutamate Dietary Intervention on Anxiety and PTSD in Veterans with Gulf War Illness"

**Website(s) or other Internet site(s)**

Not applicable.

**Technologies or techniques**

Nothing to Report.

**Inventions, patent applications, and/or licenses**

Nothing to Report.

**Other Products**

None.

**PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS****What individuals have worked on the project?**

Name:	Kathleen Holton, PhD, MPH
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-2619-7983
Nearest person month worked:	5
Contribution to Project:	Oversight of all aspects of the study.
Funding Support:	

Name:	James Baraniuk, MD
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Medical oversight for safety of participants during challenges.
Funding Support:	

Name:	John VanMeter, PhD
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Oversight of collection of MRI and MRS data for the study.
Funding Support:	

Name:	Michael Baron, PhD
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Statistician
Funding Support:	

Name:	Elizabeth Brandley, MS (see explanation below)
Project Role:	Research Coordinator Position
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Marketing, recruitment, scheduling, data management
Funding Support:	

Name:	Meissa Jones, MS
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Marketing and Outreach Coordinator
Funding Support:	

Name:	Anna Kirkland
Project Role:	Grad Student Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Collecting data during subject visits, data entry, data analysis, manuscript writing
Funding Support:	

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

No

**What other organizations were involved as partners?**

**Organization Name:** Georgetown University

**Location of Organization:** Washington, DC

**Partner's contribution to the project** includes medical oversight for the challenges and collection of MRI data for the study.

**Financial support:** None.

**In-kind support** None

**Facilities** MRI facility is used.

**Collaboration** Two of the Co-Is work at GU.

**Personnel exchanges** None.

**Other.** None.

## **SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** Not applicable.

**QUAD CHARTS:** I was told that this does NOT need to be included.

**APPENDICES:** None.