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Effect of the Low Glutamate Diet on Inflammatory Cytokines in Veterans with Gulf War Illness (GWI): A Pilot Study

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Abstract

Aim: To examine the effects of the low glutamate diet on inflammatory cytokines in veterans with Gulf War Illness (GWI).

Main methods: Forty veterans with GWI were recruited from across the country. Anthropometric measurements and blood samples were collected at baseline and after one month on the low glutamate diet. Dietary adherence was measured with a glutamate food frequency questionnaire (FFQ). Inflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α) were measured in pre- and post-diet serum (N=34). Improvement was defined as being "much" or "very much" improved on the patient global impression of change scale (PGIC), or as having ≥30% of their symptoms remit. Correlations of the FFQ and the cytokines were calculated, followed by multivariable linear regression for significant findings. Mann Whitney *U* tests were used to compare cytokine levels according to improvement on the diet, and then logistic regression was used to estimate the association after adjustment for potential confounders. Classification trees were also produced to determine the ability of change in the inflammatory cytokines to predict improvement on the diet.

Key findings: Dietary adherence was significantly associated with reduction in TNF-α, and PGIC improvement was significantly associated with reduced IL-1β, after adjustment for potential confounders. Classification trees demonstrated that IL-1β, TNF-α, and IL-6 can predict improvement on the diet with 76.5% accuracy.

Significance: Finding suggest that the low glutamate diet may be able to reduce systemic inflammation in veterans with GWI.

Introduction

Gulf War Illness (GWI) is a chronic multi-symptom condition characterized by symptoms such as widespread chronic pain, headache/migraine, cognitive dysfunction, gastrointestinal illness, mood disorders, and sometimes lung problems or skin rashes [1, 2]. Inflammation has been implicated in the pathophysiology of GWI in humans [3-7], and animal models have also suggested that changes in systemic proinflammatory cytokines parallel brain dysfunction in GWI [8, 9]. These studies have led to the proposal of a neuroimmune model for GWI [10]. The inflammation in GWI is thought to be due to neurotoxic exposures during the Gulf War, including exposure to pesticides, pyridostigmine bromide pills (PB), and low-dose exposure to chemical warfare agents like sarin gas [11]. Interestingly, all three of these exposures impair function of acetylcholine esterase [12-14], which causes downstream release of glutamate that can lead to excitotoxicity in the central nervous system (CNS), as well as blood-brain barrier (BBB) permeability [15]. Excitotoxicity from excess glutamate is intimately connected with both neuroinflammation and oxidative stress, and these three conditions have been shown to reinforce one another in a self-sustaining manner [16]. Thus, effective treatments may need to be able to address neuroinflammation, excitotoxicity, and oxidative stress in order to stop this cycle.

Recent work has suggested evidence of neuroinflammation in veterans with GWI using PET imaging [7]. Systemic cytokines have also been associated with GWI. For example, a higher number of fatigue severity days was associated with elevated IL-1β and IL-15 in one study [5]. Animal models have demonstrated the strongest relation of elevated proinflammatory cytokines with GWI symptoms. One study demonstrated that male rats exposed to low doses of PB, permethrin, and DEET, combined with restraint stress, had mitochondrial dysfunction in the

hippocampus and associated elevations in proinflammatory cytokines (TNF-α and IL-1β) and chemokines [8]. Higher levels of the proinflammatory cytokines IL-6, IL-1β, and TNF-α have also been observed in the cortex of GWI rats, when compared to non-exposed rats [17]. Furthermore, neuroinflammatory signaling (via IL-1β and IL-6 in the spinal cord) has also been linked to allodynia (pain from typically non-painful stimuli) in male rats exposed to corticosterone and diisopropylfluorophosphate (DFP; as a sarin surrogate) [18].

We have previously reported the substantial benefits of treatment with the low glutamate diet on overall symptom number, pain, and fatigue in veterans with GWI, with highly significant improvements noted in all three areas [19]. This diet removes sources of free glutamate (i.e. glutamate not bound to a protein), while also increasing consumption of nutrients protective against excitotoxicity, as well as dietary antioxidants with protective action against oxidative stress. To our knowledge, there are no published reports on the effects of potential GWI treatments on cytokine levels in humans. Thus, the objective of this pilot study was to examine the effects of the low glutamate diet on systemic inflammatory cytokines in veterans with GWI.

Materials and Methods

The methods of this study have been reported in detail previously [19] but are also presented here in brief form. Forty veterans with GWI were recruited from across the US. Written informed consent was obtained from all participants prior to participation in the study. Subjects travelled to Washington DC for a baseline visit where questionnaires and blood samples were collected, and then returned to DC for a post-diet visit where baseline measures and blood samples were repeated, and then subjects were randomized into a double-blind, placebo-

controlled crossover challenge with MSG/placebo to test for a return of symptoms. Due to limited funding, blood samples were only obtained pre- and post-diet; thus, this paper focuses on these two time points.

A glutamate food frequency questionnaire (FFQ) (specially designed to capture the frequency of consumption of foods high in free glutamate) was used as a measure of dietary compliance and a quantification for how well each subject followed the diet. In addition to the FFQ, three-day food diaries were also collected for each study time period to aid in the accurate collection of dietary free glutamate exposures.

Improvement on the diet was defined in two ways. The first was the patients' global impression of change scale (PGIC), where reports of "much improved" or "very much improved" on the PGIC were considered 'improved,' and all other answers like ("slightly improved" or "no change") were considered 'not improved.' The PGIC is a self-rated 7-item measure (ranging from very much improved to very much worse) which is commonly used in clinical trial research, especially for studies evaluating changes in pain [20], where it has been recommended by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) [21]. Similar to fibromyalgia research which has used this measure [22], it is also helpful for assessing improvement in GWI, since subjects suffer from a very high symptom load and improvements could potentially vary among the symptoms (allowing the individual to give a ranking of their perceived overall improvement).

The second measure of improvement was based on the total symptom score. Remission of ≥30% of their symptoms on the total symptom score was considered 'improved,' and anything less than that was considered 'not improved.' The total symptom score measure asked about symptoms commonly experienced by veterans with GWI, including gastrointestinal symptoms, pain symptoms (headache, migraine, muscle pain, joint pain), cognitive symptoms (memory, attention), sleep issues, autonomic nervous system dysfunction (abnormal heart rate, flushing, sweating), mood symptoms (depression, anxiety), and skin rash. There was also an option to check 'other' with the ability to write in any additional symptoms. This measure was scored by adding together the number of checked symptoms, with one potential additional point given for 'other,' resulting in a score range from 0-33.

Following the baseline visit, subjects received intensive dietary training via Skype along with a binder of dietary materials including a list of food additives to avoid, common places these additives are found, a list of foods highest in each micronutrient, foods highest in antioxidants, and sample recipes. The low glutamate diet excludes all dietary sources of free glutamate (i.e. glutamate not bound to a protein like in meat). Free glutamate is found mostly in food additives (which are used to enhance the flavor of food by exciting neurons on the tongue); but can also occur naturally in foods like soy sauce, aged cheeses, seaweed, and tomato sauce. The diet also increases consumption of micronutrients which are protective against the excitotoxicity mediated by glutamate, as well as antioxidants which counteract the oxidative stress caused by this excitotoxicity. Participants were given the weekend to prepare, then followed the diet for one month before returning to the lab for post-diet assessment, where all measures were again collected.

Pre- and post-diet blood samples were successfully obtained for 34 participants with GWI. Venipuncture was completed in the morning of each visit (pre-diet and post-diet) after an overnight fast. Blood samples were centrifuged, and aliquots of serum were stored at -80 degrees C before being sent for analysis at the INIM EM Papper Clinical Immunology Laboratory. Measurements were obtained for the inflammatory cytokines IL-1β, IL-6, IFN-γ, and TNF-α. Cytokine analysis was performed using Quansys custom 18-multiplex chemiluminescent assay (Quansys). Briefly, plasma samples were thawed at 4°C overnight. Samples were plated in duplicate following manufactures protocol. The plates were read at 270 sec using the Q-view Imager LS (Quansys). Individual cytokine concentrations were obtained using image analysis software (Q-view v3.09, Quansys Biosciences). Sample concentrations were calculated from standard curves created by a five-parameter logistic regression (5PL) with √y weighting. The average value from each duplicate was then used for subsequent analyses. Samples were normalized between plates using the internal controls run on each plate [23].

Statistical Analyses

Statistical analyses were completed in SAS 9.4 and R (R Core Team 2013), and significance was considered an alpha of 0.05. Analyses were completed for the inflammatory cytokines IL-1β, IL-6, IFN-γ, and TNF-α. Data were checked for normality using the Shapiro-Wilk test and some cytokines were found to have a non-normal distribution. Spearman correlations were calculated to compare change in the glutamate FFQ (as an estimate of how well glutamate was removed from the diet) with change in each inflammatory cytokine (pre-diet values minus post-diet values). Significant correlations were followed by multivariable linear regression (for normally

distributed variables) adjusting for the effects of age, sex, and change in BMI. Changes in cytokine concentrations were compared for those who did and did not improve on the diet, as described earlier, using Wilcoxon Rank Sum (Mann Whitney *U*) tests for independent data. Outcomes were double checked for the normally distributed variables using independent t-tests and all findings remained consistent (as detailed in table footnotes). Logistic regression was then used to model those with significant results, adjusting for age, sex, and change in BMI, as well as models containing the other three inflammatory cytokines. Additionally, classification trees were used to examine the optimal thresholds of change in the inflammatory cytokines as a prediction model for improvement on the low glutamate diet. Classification trees were produced with the classification response being "improved" (defined with PGIC or remission of ≥30% of symptoms) and the four inflammatory cytokines as predictors. Decision classification trees [24, 25] are used for stratifying or segmenting the predictor space of explanatory variables (change in cytokines) into intuitively simple regions. Each node of a tree creates two branches, and consequently, the corresponding region of the predictor space splits into two regions. Each split occurs according to one of the explanatory variables, and patients are classified to one or the other branch depending on their measures of the chosen variable relative to the threshold. At its final or terminal nodes (leaves), the tree provides a classification of the entire population according to the most frequent class observed at the corresponding node. The tree selects variables and thresholds at each node in an optimal way, maximizing the accuracy of the obtained classification.

Results

The study sample is described in Table 1. Average age was 55 years old, 91% of the sample was Caucasian, and females were well represented, making up 32% of the sample. The veterans came

from all branches of the military except the Coast Guard, and on average, the BMI of the group was considered obese, which is defined as a BMI ≥30 kg/m^2. Average weight loss from the diet was minimal, with participants losing approximately 3.5 lbs after one month on the diet.

Table 1. Demographics of the study sample (N=34)

Quantitative Variable	**Mean (SD)**
Age (yrs)	54.65 (6)
BMI at baseline (kg/m^2)	31.92 (5.63)
Change in BMI after one month diet (kg/m^2)	-0.52 (0.86)
Change in weight (lbs)	-3.59 (5.45)
Categorical Variable	**N (%)**
Sex	
Female	11 (32%)
Male	23 (68%)
Race*	
Caucasian	30 (91%)
Black/ African American	3 (9%)
Military Branch*	
Army	17 (52%)
Air Force	6 (18%)
Navy	5 (15%)
Marine Corps	5 (15%)

*N = 33

Note: One participant was listed as serving for the Army, Navy, and Marine Corps. Their count was included under Marine Corps for this table.

Greater reduction in score on the GFFQ (showing highest compliance with the low glutamate diet) was positively correlated with change in TNF-α (r = 0.58, p=0.01) but not with the other inflammatory cytokines. These results held using multivariable linear regression adjusting for the effects of age, sex, and change in BMI (β (SE) = 0.11 (0.05); p=0.03). (Data not shown.) Cytokine concentrations were compared between those who did and did not improve on the diet based on (1) reporting that they were "much" or "very much" improved on the PGIC and (2) those reporting that ≥30% of their symptoms remitted after one month on the diet. (Table 2) Participants who reported improvements on the diet (using either measure) were observed to have reductions in three of the four inflammatory cytokines, IL-1β, TNF-α, and IL-6, as

compared to increased concentrations in those who did not improve on the diet. However, this difference was only significant for IL-1β in those reporting improvement based on the PGIC. Median comparisons are shown in Table 2, and the overall median reduction in IL-1β was 10% for those who improved on the diet based on the PGIC.

Table 2. Change in median cytokine concentrations according to improvement on the low glutamate diet.

Cytokine (pg/mL)	Time point	Improved on Diet Based on PGIC			Improved Based on ≥30% Symptom Remission		
		No N = 10	Yes N = 24		No N=13	Yes N=21	
		Median (IQR)	Median (IQR)	P-value*	Median (IQR)	Median (IQR)	P-value*
IL-1β	Pre-Diet**	11.42 (3.95)	11.42 (4.23)	0.60	10.25 (3.94)	12.11 (3.14)	0.22
	Post-Diet	11.13 (12.94)	9.74 (3.40)	0.03	9.86 (3.19)	10.04 (2.71)	0.93
	Change	-1.57 (10.72)	1.27 (4.92)	0.03	-0.68 (6.07)	0.88 (4.64)	0.40
IL-6	Pre-Diet	3.68 (2.68)	2.93 (1.68)	0.23	2.94 (1.44)	3.08 (1.75)	0.89
	Post-Diet	2.53 (0.96)	2.55 (1.18)	0.86	2.53 (0.66)	2.59 (1.44)	0.59
	Change	-0.41 (4.52)	0.09 (1.34)	0.84	-0.24 (1.27)	0.31 (2.29)	0.33
IFN-γ	Pre-Diet	10.35 (9.03)	13.24 (6.53)	0.58	10.97 (6.32)	13.14 (7.64)	0.57
	Post-Diet**	10.12 (6.86)	11.69 (8.44)	0.23	11.37 (5.82)	10.46 (7.23)	0.68
	Change**	3.03 (10.64)	0.65 (9.84)	0.48	0.10 (9.57)	2.45 (9.81)	0.51
TNF-α	Pre-Diet	2.28 (2.34)	2.65 (3.96)	0.95	2.04 (3.28)	2.64 (3.38)	0.82
	Post-Diet	3.05 (3.83)	2.80 (3.39)	0.50	3.21 (3.00)	1.89 (3.46)	0.15
	Change**	-1.72 (7.71)	0.34 (4.82)	0.46	-1.45 (6.78)	0.29 (3.70)	0.62

* Wilcoxon Rank Sum (Mann Whitney)

** These variables were normally distributed, and their values were checked with independent t-tests with pooled variance. The associated p-values of the t-tests led to the same conclusion as the non-parametric test.

The results for improvement based on the PGIC were further modeled using logistic regression (Table 3), where IL-1β was observed to predict improvement on the low glutamate diet based on the PGIC measure, after accounting for age and sex effects (OR (95% CL) of 1.18 (1.00, 1.38), p=0.05), but was shy of significance after the additional inclusion of change in BMI as a confounding variable (p=0.08). Multivariable models including all 4 inflammatory cytokines were also run and change in IL-1β remained significant in the model even after the addition of the other cytokines. Interestingly, this final model with all cytokines also resulted in the variable for change in TNF-α reaching significance (OR (95% CL) of 1.41 (1.00, 1.98), p=0.05).

Table 3. Results from the logistic regression models examining each inflammatory cytokine as a predictor of improvement on the diet based on the PGIC with adjustment for potential confounding factors.

N = 34	**Age and sex adjusted**		**Age, sex, and BMI change adjusted**		**Full model with all cytokines**		**Full model with all cytokines (age, sex, and BMI change adjusted)**	
	OR (95% CI)	**Pr > Chi Sq**	**OR (95% CI)**	**Pr > Chi Sq**	**OR (95% CI)**	**Pr > Chi Sq**	**OR (95% CI)**	**Pr > Chi Sq**
IL-1β Change (pg/mL)	1.18 (1.00, 1.38)	0.05	1.16 (0.98, 1.37)	0.08	1.26 (1.03, 1.55)	0.02	1.28 (1.01, 1.62)	0.04
IL-6 Change (pg/mL)	0.97 (0.67, 1.40)	0.87	1.01 (0.69, 1.47)	0.96	1.01 (0.65, 1.58)	0.95	1.13 (0.68, 1.88)	0.63
IFN-γ Change (pg/mL)	0.95 (0.86, 1.06)	0.38	0.96 (0.86, 1.07)	0.43	1.01 (0.88, 1.15)	0.91	1.02 (0.88, 1.18)	0.80
TNF-α Change (pg/mL)	1.07 (0.88, 1.30)	0.49	1.19 (0.93, 1.52)	0.17	1.20 (0.96, 1.50)	0.11	1.41 (1.00, 1.98)	0.05

Additionally, classification trees were used to examine how all four inflammatory cytokines may predict improvement on the low glutamate diet (Figure 1). In alignment with the above results, the classification tree for improvement based on the PGIC used both IL-1β and TNF-α as the two inflammatory cytokines with the highest predictive ability for improvement on the diet. This tree

shows that among those who do not meet the original improvement in IL-1β of ≥3.235, and who do not experience improvement in TNF-α, reduced IL-1β between 0 and 3.235 can still predict improvement for some individuals, although with less certainty. The other classification tree, based on improvement defined as ≥30% symptom remission, shows that in general, a larger drop in any of the three participating cytokines (IL-6, IL-1β, and TNF-α) enhances the chance of improvement. However, there are no pure nodes, so any classification rule based on this tree has exceptions. Thresholds are different from 0, allowing in principle that patients can improve on the diet (based on symptom remission) despite minimal *increases* levels of their cytokines.

Both classification trees had a misclassification error rate of 0.235, meaning that, based on IL-1β, IL-6, and TNF-α, improvement on the diet can be predicted correctly for 76.5% of subjects. This suggests that levels of other inflammatory cytokines are not irrelevant, even though change in these measures was not significantly different between those who did and did not improve on the diet.

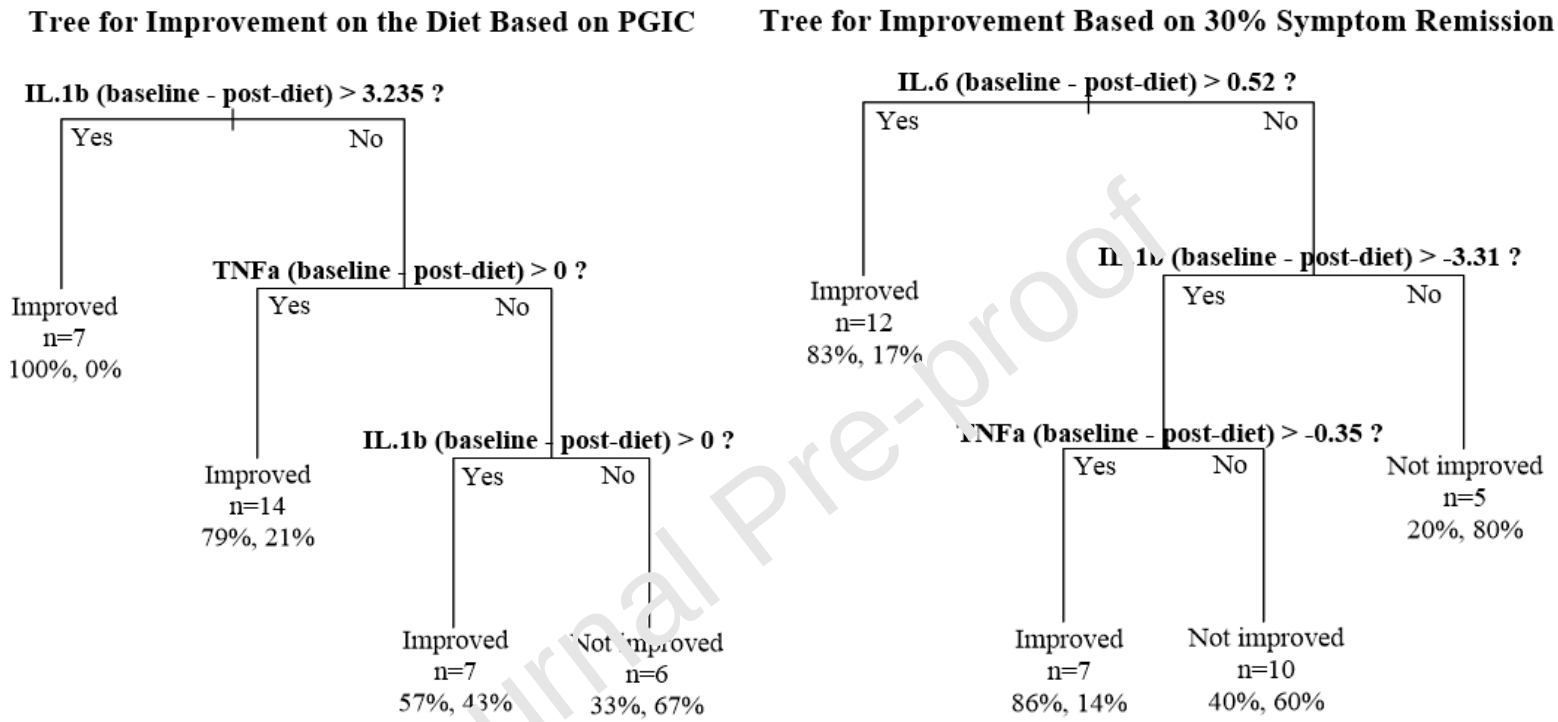

Figure 1. Classification trees for the ability of change in the inflammatory cytokines to predict improvement on the diet either by PGIC or based on ≥30% symptom remission. In each terminal node, the number of patients, n, is reported along with the percent of patients who improved and did not improve on the diet.

Discussion

To our knowledge, this is the first study to examine change in cytokine levels according to treatment response for GWI. These results suggest that the low glutamate diet may be able to reduce systemic inflammatory cytokine levels. Those with the greatest reduction in free glutamate consumption experienced significant reduction in TNF-α. Furthermore, those who improved on the diet (based on a report of "much" or "very much" improved on the PGIC) experienced an overall reduction in inflammatory cytokines, with significant reduction in IL-1β. Multivariable logistic regression models confirmed these findings, and also suggested a potential association between change in TNF-α and improvement based on the PGIC. Classification trees further suggested that change in IL-1β, TNF-α, and IL-6 could predict improvement on the diet with 76.5% accuracy.

Prior research has implicated the inflammatory cytokines in the pathophysiology of GWI. For example, Lacagnina and colleagues reported elevations in IL-1β and IL-6 in lumbar dorsal spinal cord, dorsal root ganglia, and the gastrocnemius of male rats treated with corticosterone, the sarin nerve agent surrogate diisopropylfluorophosphate (DFP), and saline; and this was associated with increased allodynia [18]. Other animal research has shown that GWI rats which were exposed to PB, DEET, permethrin, and moderate stress, had higher levels of IL-1β, IL-6 and TNF-α in their cerebral cortex, and this was linked to cognitive impairments in the animals [17]. Shetty and colleagues have similarly reported elevated oxidative stress in the hippocampus of male rats treated with PB, DEET, permethrin, and restraint stress; and these changes were associated with increased concentrations of IL-1β and TNF-α in the serum [8]. Similarly, a mouse model of GWI also demonstrated increased IL-1β and IL-6 levels in the ventral

hippocampus of animals treated with PB, DEET, corticosterone, and one exposure of DFP, as compared to control animals [26].

A few studies in veterans with GWI have examined inflammatory markers. A recent PET imaging study included systemic inflammatory cytokine measurement but did not find statistically significant differences between GWI patients (N=15) and healthy controls (N=33) [7]. Another study reported higher levels of IFN-γ and TNF-α in GWI veterans who had been diagnosed with chronic fatigue syndrome (CFS), as compared to controls who only had CFS [27]. TNF-α has also been reported as one of the five most important cytokines for differentiating GWI from CFS [6]. A very small study also suggested an association between IL-1β and higher fatigue severity days [5]. Thus, there is some human data to also suggest that inflammatory cytokines may be playing a role in the disorder.

Interestingly, no direct effects of IFN-γ were observed in this study, and this is in alignment with the omission of this inflammatory cytokine in the classification trees, which suggests that IFN-γ does not make a sufficient additional contribution, on top of IL-1β, TNF-α, and IL-6 in the prediction of improvement on the low glutamate diet. As mentioned above, prior research reported that IFN-γ is upregulated in GWI with CFS as compared to controls with CFS [27]; however, to our knowledge, little other data has implicated this cytokine in GWI.

The strongest effects observed in this study were for IL-1β. This cytokine has the ability to induce cyclooxygenase 2 (COX2) in the CNS, which is thought to contribute to pain hypersensitivity [28]. Importantly, this inflammatory cytokine can result in the release and

accumulation of glutamate, leading to excitotoxicity in the nervous system. Since the purpose of the low glutamate diet is to reduce excitotoxicity, these findings suggest that the ability of the diet to significantly reduce IL-1β could further reduce this over-excitation in the nervous system. In fact, both IL-1β and TNF-α have been shown to independently be able to stimulate the release of glutamate from cultured neurons, which is associated with neuronal cell death via excitotoxicity [29]. IL-1β also appears to reduce the protective glutamate uptake from the synaptic cleft by astrocytes, which would further potentiate this excitotoxic effect. This has been shown to result in oligodendrocyte death [30], which in turn impairs the brain's ability to maintain myelin on neuronal axons. Thus, emerging evidence suggests that inflammatory cytokines like IL-1β may be able to perpetuate inflammation in the CNS [31]. Therefore, the ability of the low glutamate diet to lower inflammatory cytokines like IL-1β and TNF-α, in addition to reducing exposure to dietary glutamate and increasing antioxidant consumption, may be one of the reasons for the striking reduction in symptoms noted in this group of veterans [19]. It should be noted that typically, dietary glutamate is limited in its transport at the BBB [32]; however, exposures common to military personnel such as stress [33, 34], neurotoxic exposures [35, 36], head injury [37], and infection,[38] can all cause increased permeability of the BBB, thus making veterans potentially more susceptible to dietary glutamate than healthy individuals without these exposures.

These findings are limited by the small sample size. The logistic regression models which included the other cytokines may have been overfit and unreliable due to the small sample size and larger number of variables included in those models. Future larger scale research will need to confirm these findings. No adjustments for multiple comparisons were made since this was a

pilot study meant to examine (1) whether any potential effects of the low glutamate diet on inflammatory cytokines exist, and (2) to provide preliminary data for future power calculations. Future larger clinical trials will be needed to further explore the effects of the low glutamate diet on inflammatory cytokines.

Conclusion

The results of this research suggest that the low glutamate diet may be able to beneficially reduce systemic inflammatory cytokines in veterans from the Gulf War. Reduction in free glutamate intake was significantly associated with reduction in TNF-α, and improvement on the low glutamate diet was associated with significant reduction in IL-1β, after one month on the diet. Three of the inflammatory cytokines (IL-1β, TNF-α, and IL-6) were also able to predict improvement or no improvement on the diet correctly in 76.5% of the cases. More research is needed to examine whether or not these findings hold in a larger clinical trial.

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References

1. Steele, L., et al., *Complex factors in the etiology of Gulf War illness: wartime exposures and risk factors in veteran subgroups.* Environ Health Perspect, 2012. **120**(1): p. 112-8.
2. Fukuda, K., et al., *Chronic multisymptom illness affecting Air Force veterans of the Gulf War.* JAMA, 1998. **280**(11): p. 981-8.
3. Johnson, G.J., et al., *Blood Biomarkers of Chronic Inflammation in Gulf War Illness.* PLoS One, 2016. **11**(6): p. e0157855.
4. Butterick, T.A., et al., *Gulf War Illness-associated increases in blood levels of interleukin 6 and C-reactive protein: biomarker evidence of inflammation.* BMC Res Notes, 2019. **12**(1): p. 816.
5. Parkitny, L., et al., *Evidence for abnormal cytokine expression in Gulf War Illness: A preliminary analysis of daily immune monitoring data.* BMC Immunol, 2015. **16**: p. 57.
6. Khaiboullina, S.F., et al., *Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis.* Cytokine, 2015. **72**(1): p. 1-8.
7. Alshelh, Z., et al., *In-vivo imaging of neuroinflammation in veterans with Gulf War illness.* Brain Behav Immun, 2020. **87**: p. 498-507.
8. Shetty, G.A., et al., *Chronic Oxidative Stress, Mitochondrial Dysfunction, Nrf2 Activation and Inflammation in the Hippocampus Accompany Heightened Systemic Inflammation and Oxidative Stress in an Animal Model of Gulf War Illness.* Front Mol Neurosci, 2017. **10**: p. 182.
9. Shetty, A.K., et al., *Monosodium luminol reinstates redox homeostasis, improves cognition, mood and neurogenesis, and alleviates neuro- and systemic inflammation in a model of Gulf War Illness.* Redox Biol, 2020. **28**: p. 101389.
10. Coughlin, S.S., *A Neuroimmune Model of Gulf War Illness.* J Environ Health Sci, 2017. **3**.
11. Trageser, K.J., et al., *The Innate Immune System and Inflammatory Priming: Potential Mechanistic Factors in Mood Disorders and Gulf War Illness.* Front Psychiatry, 2020. **11**: p. 704.
12. Macht, V.A., et al., *Pyridostigmine bromide and stress interact to impact immune function, cholinergic neurochemistry and behavior in a rat model of Gulf War Illness.* Brain Behav Immun, 2019. **80**: p. 384-393.
13. Torres-Altoro, M.I., et al., *Organophosphates dysregulate dopamine signaling, glutamatergic neurotransmission, and induce neuronal injury markers in striatum.* J Neurochem, 2011. **119**(2): p. 303-13.
14. Ganesan, K., S.K. Raza, and R. Vijayaraghavan, *Chemical warfare agents.* J Pharm Bioallied Sci, 2010. **2**(3): p. 166-78.
15. Joyce, M.R. and K.F. Holton, *Neurotoxicity in Gulf War Illness and the potential role of glutamate.* Neurotoxicology, 2020. **80**: p. 60-70.
16. Nguyen, D., et al., *A new vicious cycle involving glutamate excitotoxicity, oxidative stress and mitochondrial dynamics.* Cell Death Dis, 2011. **2**: p. e240.
17. Madhu, L.N., et al., *Neuroinflammation in Gulf War Illness is linked with HMGB1 and complement activation, which can be discerned from brain-derived extracellular vesicles in the blood.* Brain Behav Immun, 2019. **81**: p. 430-443.

18. Lacagnina, M.J., et al., *A role for neuroimmune signaling in a rat model of Gulf War Illness-related pain.* Brain Behav Immun, 2021. **91**: p. 418-428.
19. Holton, K.F., et al., *The Low Glutamate Diet Effectively Improves Pain and Other Symptoms of Gulf War Illness.* Nutrients, 2020. **12**(9).
20. Perrot, S. and M. Lanteri-Minet, *Patients' Global Impression of Change in the management of peripheral neuropathic pain: Clinical relevance and correlations in daily practice.* Eur J Pain, 2019. **23**(6): p. 1117-1128.
21. Turk, D.C., et al., *Core outcome domains for chronic pain clinical trials: IMMPACT recommendations.* Pain, 2003. **106**(3): p. 337-345.
22. Derry, S., et al., *Pregabalin for pain in fibromyalgia in adults.* Cochrane Database Syst Rev, 2016. **9**: p. CD011790.
23. Fletcher, M.A., et al., *Plasma cytokines in women with chronic fatigue syndrome.* J Transl Med, 2009. **7**: p. 96.
24. Friedman J, H.T., Tibshirani R., *Elements of Statistical Learning*. 2001, New York: Springer.
25. James G, W.D., Hastie T, Tibshirani R., *An Introduction to Statistical Learning*. 2013, New York: Springer.
26. Carpenter, J.M., et al., *Neurochemical and neuroinflammatory perturbations in two Gulf War Illness models: Modulation by the immunotherapeutic LNFPIII.* Neurotoxicology, 2020. **77**: p. 40-50.
27. Zhang, Q., et al., *Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome.* Clin Diagn Lab Immunol, 1999. **6**(1): p. 6-13.
28. Samad, T.A., et al., *Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity.* Nature, 2001. **410**(6827): p. 471-5.
29. Ye, L., et al., *IL-1beta and TNF-alpha induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase.* J Neurochem, 2013. **125**(6): p. 897-908.
30. Takahashi, J.L., et al., *Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity.* Ann Neurol, 2003. **53**(5): p. 588-95.
31. Becher, B., S. Spath, and J. Goverman, *Cytokine networks in neuroinflammation.* Nat Rev Immunol, 2017. **17**(1): p. 49-59.
32. Smith, Q.R., *Transport of glutamate and other amino acids at the blood-brain barrier.* J Nutr, 2000. **130**(4S Suppl): p. 1016S-22S.
33. Belova, I. and G. Jonsson, *Blood-brain barrier permeability and immobilization stress.* Acta Physiol Scand, 1982. **116**(1): p. 21-9.
34. Robinson, J.S. and R.A. Moody, *Influence of respiratory stress and hypertension upon the blood-brain barrier.* J Neurosurg, 1980. **53**(5): p. 666-73.
35. Sulhan, S., et al., *Neuroinflammation and blood-brain barrier disruption following traumatic brain injury: Pathophysiology and potential therapeutic targets.* J Neurosci Res, 2020. **98**(1): p. 19-28.
36. Abdel-Rahman, A., A.K. Shetty, and M.B. Abou-Donia, *Disruption of the blood-brain barrier and neuronal cell death in cingulate cortex, dentate gyrus, thalamus, and hypothalamus in a rat model of Gulf-War syndrome.* Neurobiol Dis, 2002. **10**(3): p. 306-26.

37. Kuriakose, M., et al., *Synergistic Role of Oxidative Stress and Blood-Brain Barrier Permeability as Injury Mechanisms in the Acute Pathophysiology of Blast-induced Neurotrauma.* Sci Rep, 2019. **9**(1): p. 7717.
38. Afonso, P.V., et al., *Human blood-brain barrier disruption by retroviral-infected lymphocytes: role of myosin light chain kinase in endothelial tight-junction disorganization.* J Immunol, 2007. **179**(4): p. 2576-83.

Highlights

- Reduced consumption of glutamate was associated with significantly reduced TNF-α.
- Those who improved on the low glutamate diet had significantly reduced IL-1β.
- Change in IL-1β, TNF-α, and IL-6 predicted improvement from diet in 77% of cases.
- Low glutamate diet appears to reduce inflammatory cytokines in veterans with GWI.