Author's response to reviews

Title: Impaired immune function in Gulf War Illness.

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Author's response to reviews: see over
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Dear Editor

Firstly, all the array data has been deposited (and accepted) into the ArrayExpress database (accession number E-MEXP-2069, for immediate release), and this has been added to the methods section.

Secondly, in regard payment, I have recently had the pleasure of reviewing a manuscript for BMC Medical Genomics and if this manuscript is accepted would like the 20% discount applied to this manuscript.

We wish to thank you for the review of our manuscript, and appreciate the very helpful comments provided by the reviewers, we believe they strengthen the manuscript. Below are our detailed responses to each of the reviewers:

Reviewer: Søren Bregenholt
No comments required

Reviewer: Shamgar Ben-Eliyahu

The authors report several significant and several marginally significant differences between the two groups, as well as interesting and important interactions between the groups and the effects of physical exercise. GWI veteran indeed exhibit different responses to physical exercise than controls. The authors seem to generalize their findings of the effects of physical exercise to other types of stressor, which is not indicated by the study. Other than that, I have no significant concerns regarding the study. I do wonder to what degree it is justified to name this syndrome GWI. Do we expect similar pattern of differences with respect to rape victims, various types of PTSD, or CFS (as suggested by the authors)? The authors may want to discuss this issue.

We do appreciate these insightful comments from this reviewer. These are all issues we are currently pursuing and hope to share with the research community soon. We feel this discussion is probably a little beyond the scope of this article, but we are very excited that it has raised these questions for this reviewer.
Reviewer: Yehuda Shoenfeld

(2) An attempt should be made to try to explore how NK cell defects are associated with the fatigue !!
The reviewer has summed up our research program for the next while.


1. Page 6: Exercise challenge
   Line 15 & 16- “diurnal variation” needs greater clarification in terms of “assessment” as there is an uncertainty as to whether the “assessments” refer to immune function.
   Wording changed to clarify meaning

   Line 17-“uniform breakfast” needs to be referenced from perhaps exercise journals and detail information on the major food groups that constitute the uniform breakfast.
   We believe the detail asked for here is important, but we feel a little beyond the scope of this study. We have remedied this by making some adjustments to the statement. We feel this clarifies our intent.

   Line 19 (page 6) to line 2 (page 7) - A reference for the exercise protocol was not provided.
   A reference has been added.

2. Page 7:
   Line 3: “urging” is not specified whether authors used vocal urgings
   Vocal added to text.

   Line 5: (approximately 30mins from the start)- Authors did not provide information on whether this was a uniform standard of 30mins post exercise. Authors need to consider immune function analysis post exercise is sampled immediately after exercise testing. However, questions are raised whether this paradigm is fully understood in terms of possible adverse consequences for participants. Many CFS/ME patients describe profound fatigue or post-exertional malaise and whether this occurred in the study participants is not stated. The subsequent recommendation suggesting extending the exercise challenge paradigm to other complex syndromes may be premature without patient acceptability validation and explicit ethical approval. However the findings of this study appear significant only after exercise testing and therefore the study suggest a legitimate application of the exercise paradigm.
   This is an important point and one which we were cognoscente of because of our CFS experience. We did note however that in this study that there were no adverse events experienced by the participants after the exercise challenge. A statement to that fact has been added.
Line 8: An expansion of the various cytokine abbreviations is necessary
These (and all abbreviations used in the manuscript) are detailed at the end of the manuscript in the “list of abbreviations used”. We feel this makes for easier reading of the manuscript.

Line 14: “high sensitivity ELISA”- is this in terms of pg and could authors clarify the sensitivity
This is an important point. The sensitivities have been added to the manuscript.

Line 17: before/ prior
Change made

3. Page 8
Line 1: Again “high sensitivity”
We have added the range of assay sensitivity.

Line 15-16: Need to increase font size
Done.

4. Page 9:
Line 1-2: Can the sentence be rephrased
Thank you, that was as clear as mud. We have made the wording far less cumbersome.

Line 14: “Two”
I am unclear as to the change required here. The paper reads “Two ug of total RNA”, as this is at the start of the sentence it is appropriate to be written in full.

5. Page 14
Line 8: “have” tense structure
Changed to had.

Line 12-13: Authors need to outline if this was significant
The p-values have been added for clarity. This definitely improves the interpretation.

IL-5 needs clarification and interpretation as it is referred to both as Th1 and Th2 cytokine
This was a typo not picked up by the authors, appreciate your finding it. Change has been made.

6. Page 15:
Line 12-13: Authors need to outline if this was significant
It is not clear to me what needs outlining. Do the reviewers wish us to add p-values to the fold changes given? For gene expression data fold-changes are generally not accompanied by a statistic. We believe the point to this statement relates more to the fact that there is a difference in responsiveness between certain genes.
7. Page 16  
Line 8-9: has been presented in the discussion  
It is unclear to me what is required. We believe this trend to significance is relevant and warrants mention in the discussion.

8. Page 17  
Line 5: “CFS” and reference is a bit confusing as no mention of CFS was made in the reference [6]. Thank you for pointing this out. With several re-writes the sentence structure had changed. We did not notice this error. We have added the relevant references to the text.

Line 18-19: Arguably, this is not a sports science paper. However, literature in exercise immunology clearly outlines post immune depression where these immune cells return to pre exercise. This was a most useful comment, we believe by adding a reference to this it clears up any possible confusion.

9. Page 19  
Line 4: “enhanced” font is different. This has been corrected.

10. The findings attributing changes in NK appear sound. There is a brief discussion regarding the findings of Th vs Th2 responses found in various studies and the significance of the findings to the present study are not explored in detail against other studies with contradictory findings. For example Table 3 appears to indicate more of a Th response in IL-6, plasma and PHA SN, TNFa and some impaired Th2 response with IL0 plasma in cases vs controls. There appears to be no comment on the findings regarding IL-5 and its context (see below). Hypofunction of the Hypothalamo-Pituitary-Adrenal (HPA) axis is suggested from the data. We had considered discussing this in greater detail. However, we found it to distract from the main message of the paper. We still believe it best to present the data and allow the reader to follow this as they see fit. We are currently looking at a larger sample number and will hopefully be publishing this aspect shortly.

11. Figures may benefit from better interpretation within the text of the article, although comments on Figures are reasonable. We are not exactly sure how to tackle this comment. We could give more in depth explanation/interpretation of the figures, however, we believe this will distract from the data. We feel the figures and the provided legends provide all that is needed to interpret the data.

Reviewer: Aristo Vojdani
6. Are limitations of the work clearly stated? No. The authors need to acknowledge that there is a weakness in their study in that they only have 9 subjects with GWI (2 African-American, 6 Hispanic, 1 white), and 11 controls (4 African-American, 4 Hispanic, and 3 white). With this type of demographic, it is very difficult to conclusively determine a trend regarding immune functions, including lymphocyte subpopulation, in particular CD4/CD8 ratio. Therefore, I would recommend that in addition to mean + SD individual data also be presented in the form of a scatter plot.

We believe we have tackled the issue of small sample size at every point possible through out this manuscript. We were well aware of this issue and wanted to keep it in the forefront of the readers mind. To do this we give the subject numbers in the abstract. It is reiterated in the methods, and by our wording using “preliminary exploration” and “pilot” study through out the manuscript. In the paragraph where we discuss the shortcomings of the study (page 21, paragraph 2), we have added “for this end we used a small subset of subjects from a much larger study cohort”, to once again emphasize this.

We appreciate the suggestion of using scatter plots for representation of the immune functions etc. We did try this and from our perspective we found the table with mean ±Sd to be more informative.

8. Do the title and abstract accurately convey what has been found? No. I recommend that the title be changed to indicate impaired immune function and not just NK cell function depression.

Title has been changed.

Yours sincerely,

Toni Whistler, PhD