Author’s response to reviews

Title: Disrupting prolonged sitting reduces IL-8 and lower leg swell in active young adults

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REVIEWER 1: Katsuhiko Suzuki

1. Title: swell => swelling? There are also mixed usage like in the lines 191 and 192. However, it is all right if each usage is appropriate.

RESPONSE: We have gone through the manuscript carefully and assessed use of the term swell and swelling, and have edited where necessary.

2. L33: bodyweight => body weight

RESPONSE: We have made this change.


RESPONSE: Thank you for pointing us to these papers. We have added reference to the paper on oxidative stress to our background section.
4. **L102:** Participants: The subject number and sex should be included.

RESPONSE: Thank you. We have added this information to line 109.

5. **L178:** Although the authors noted "TNF-α and IL-6 were not detected in any of the saliva samples" but are they usually detected? Measurements of these cytokines are usually done by high-sensitivity (HS) ELISA kits but it is all right if their detection limits are mentioned in the Results or Methods section.

RESPONSE: This discrepancy may be due to the fact that we used saliva. The limit of quantification of TNF-α was 15.6 pg/mL and IL-6 was 9.38 pg/mL. We have reworded this line to read as follows:

Concentrations of TNF-α and IL-6 within the saliva samples were below the limit of quantification (TNF-α: 15.6 pg/mL and IL-6: 9.38 pg/mL), while IL-1β was not significantly different between PS and DS.

6. **L218:** swelling,(29); space is needed instead of a comma (,)

RESPONSE: Thank you. We have made this edit.

7. **L226:** Although the authors noted "a systemic increase in IL-8 is typically observed in response to intense exercise 226 (31)" , the IL-8 kinetics had been better described in an earlier review: Suzuki K, et al. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8, 6-48, 2003.

RESPONSE: We have added this reference.

8. **L233:** Although the authors noted "Concentrations of IL-1β, IL-6, and TNFα remained unchanged," IL-1β and IL-6 were not detected, and authors cannot say "remained unchanged" and need to reword.

RESPONSE: The sentence has been edited to read as follows:

Concentrations of IL-1β remained unchanged, while IL-6 and TNFα were undetectable; this may reflect the nature of the intervention, as impact on these pro-inflammatory cytokines varies greatly with exercise duration and intensity.

9. **L312:** There are no volume information of the Journal.

RESPONSE: This has been added.

10. **L315:** The American journal of clinical nutrition => The American Journal of Clinical Nutrition

RESPONSE: This has been changed.
11. L317, 326: There are no journal and other information. As such, please check carefully throughout the references.

RESPONSE: Thank you for pointing this out. We have made edits throughout the reference list.

REVIEWER 2: Ian Walshe

1. The study aimed to compare disrupted sitting to prolonged sitting on salivary IL-8 and leg swell. It is an interesting, relevant topic, and is generally well written. However, I do have some comments for consideration. Abstract: Line 33 - you state the exercise load is 0.05% bodyweight. Is this correct? For a 70 kg male, this would be a load of 3.5 g.

RESPONSE: Thank you for pointing this out to us. The units were incorrect. We have changed this to 5% of body weight.

2. It would be useful to include p values in the results of this abstract.

RESPONSE: Thank you for the suggestion. We have added p-values to the abstract.

3. Background: The background is well written. It would be useful to include more information on the specific role of IL-8 in prolonged sitting or sedentary activity.

RESPONSE: Unfortunately little work has been done to look specifically at the role of sedentary physiology with regards to IL-8. However several promising studies indicate that it may play a critical role in exercise and exercise disruptions. We have tried to highlight this point better in the introduction with the following addition:

In a study of young sedentary (n=10) and trained men (n=10), 30 minutes of high intensity treadmill running was found to increase IL-6 and IL-8 significantly in both groups. While there is some research on the response of such cytokines to acute and chronic exercise among in adults, little work has been done to determine the impact of disrupting sitting on these inflammatory biomarkers.

4. Methods: Line 110-111 - Was water consumption recorded? There is some evidence to suggest that hydration status is associated with edema. This may influence your results if water intake was not controlled for or recorded.

RESPONSE: The reviewer raises an interesting point. Unfortunately, we did not record water consumption during the session. We have added the following limitation to the discussion to address this issue.

Third, we did not record water intake during the session. This may have influenced leg swell, and should be considered in future research.
5. Line 118 - Could you change height and weight to stature and mass?

RESPONSE: This change has been made.

6. Line 121 - It is not clear why there is some discrepancy between collection times.

RESPONSE: We collected samples before and after the 4 hour period. We have edited the sentence to improve clarity.

Blood and saliva were sampled, and heart rate and blood pressure were assessed prior to commencement of the experiment (T1) and then again at the end of the four hour session (T2).

7. Line 159 - Could you include what type of post hoc analysis was done?

RESPONSE: Thank you for raising this. We have edited the sentence to read as follows:

Pairwise comparisons with a bonferroni correction applied were conducted to detect where the differences were. All statistics were conducted in SPSS (v25, IBM).

8. Line 161 - The term 'effect size' is confusing as it could be construed as the size of the effect of your results. Using another term such as 'sample size estimation' would be preferable.

RESPONSE: We had initially used G Power to calculate effect sizes (cohens d) for our data. We have updated the manuscript with partial eta squares from the ANCOVA output and have removed the effect sizes as well as the line mentioned above.

9. Results: It is not clear how many participants took part in the study. Please include information on how many males and females took part in the study. Including the characteristics of each sex.

RESPONSE: Thank you for pointing out this omission. We have added this information to line 109.

10. Do you have the results for power output during the cycle exercise?

RESPONSE: Yes, these data were automatically collected through our software; however, we did not include or analyze these data. Sprint workloads were prescribed based on body weight, thus each participant would have achieved a different power output during their all out effort, and variability would be significant between male and female participants. Given the sample size, we would be unable to account for this variability in our statistical analysis. The purpose was to assess the effect of high intensity bursts or “exercise snacking” on sedentary physiology. We were not looking for a specific dose, but simply the effect of disrupting the sitting session with a high intensity burst. As such we decided not to include these data in the paper. We hope the reviewer agrees with our approach.
11. Please check the effect sizes in Table 1. There appears to be very large effects, which are not supported by the results.

RESPONSE: We had initially used G Power to calculate effect sizes (cohens d) for our data. We have updated the manuscript with partial eta squares from the ANCOVA output and have removed the effect sizes.

12. The error bars in Figure 1 appear to cross zero at baseline. Is this just the fact that the error bar is negative?

RESPONSE: Thank you for bringing this to our attention. We have changed the SD bars to standard error bars. There was an error in our original graph.

13. There are no error bars for figure 2.

RESPONSE: Thank you for this suggestion. We have now added error bars to the figure.

14. Discussion: The discussion is well written and acknowledges the limitations of the study. Line 203 - You have not reported an increase in leg swell in your results. Could you amend this?

RESPONSE: Thank you! We have edited this sentence.