COVID-19 is getting on our nerves: Sympathetic neural activity and hemodynamics in young adults recovering from SARS-CoV-2

Nina L Stute, Jonathon L Stickford, Valesha M. Province, Marc A. Augenreich, Stephen M. Ratchford, and Abigail S.L. Stickford

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Tye Dawood (Referee #1); Erin Joan Howden (Referee #2)

Review Timeline:

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|------------------------------|----------|
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Dear Dr Stickford,

Re: JP-RP-2021-281888X "COVID-19 is getting on our nerves: Sympathetic neural activity and hemodynamics in young adults recovering from SARS-CoV-2" by Nina L Stute, Jonathon L Stickford, Valesha M. Province, Marc A. Augenreich, Stephen M. Ratchford, and Abigail S.L. Stickford

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

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EDITOR COMMENTS

Reviewing Editor:

Dear Dr Stickford,

Thank you for submitting a revised version of your manuscript to The Journal of Physiology, for incorporating MSNA data obtained during HUT, including the vascular transduction data and tempering your conclusions about HRV. There a few outstanding issues the Reviewers raise
that you will need to attend to. Moreover, please adjust the Key Points summary to reflect the changes in MSNA during HUT; your conclusion that there was an exaggerated HR response during HUT doesn't match the observations reported in Results.

Senior Editor:

Comments for Authors to ensure the paper complies with the Statistics Policy:
Please include P values in Figure 1.

Please include data points in Figure 3.

Please include supplemental table into the manuscript.

Comments to the Author:

While the authors make the comment that the American Statistical Association’s Statement on P-Values asserts that practices that reduce data analysis or scientific inference to mechanical “bright-line” rules (such as “p < 0.05”) can result in erroneous beliefs, the standards also importantly highlight the importance of assessing other types of error analysis. This would include type I error along with type II error when assessing interpretation of statistical comparisons. Thus, while the Journal does not explicitly state the importance of reporting type I error, it is incumbent on the authors to closely assess both, when the type II error is in question. The ASA statement also asserts: “Researchers should recognize that a p-value without context or other evidence provides limited information. For example, a p-value near 0.05 taken by itself offers only weak evidence against the null hypothesis...For these reasons, data analysis should not end with the calculation of a p-value when other approaches are appropriate and feasible.” The ASA stance also recommends other statistical approaches that are available when determining the relevance of a data comparison. The Journal does not mandate a path of approaches that one needs to follow to verify statistical interpretations. We do, however, make clear that P values >0.05 are NOT to be interpreted as a trend. The Journal policy also suggests that inclusion of 95% confidence intervals are appropriate and useful. (please see https://physoc.onlinelibrary.wiley.com/doi/10.1113/jphysiol.2011.205062)

In regard to statistics in figures, the Journal wants to see actual P values included in figure and tables and not use of asterisks. This should not be interpreted to mean no statistics should be shown. Actual P values can and should be shown in figures when possible. This is particularly true for bar graphs such as Figure 1.

The Journal also requires the inclusion of data points when possible in figures. It is possible to
show the actual data points in Figure 3 using open and solid squares or triangles. Please include them.

The supplemental table needs to be incorporated into the manuscript as a table.

REFEREE COMMENTS

Referee #1:

Thank you for addressing the previous comments. The inclusion of the MSNA HUT data is a significant and important addition to the manuscript. My concern remains, though, that the groups are not really matched: one group tested prior to the pandemic, and the other tested during the pandemic. Moreover, the sex distribution between the two groups does not match. I realise that a chi square test was conducted and no differences were found, but that does not allay my concern.

Referee #2:

The authors have thoughtfully addressed my suggestions. The authors note the challenges of performing this work in the midst of the pandemic and have documented these limitations clearly. I have a very minor suggestion to further clarify the HUT data collection within in the methods section - Could the authors clarify if the MSNA data collected during tilt is the average of the 5 minutes or last minute? Should recovery data be included for HR and BP (figure 4), to contextualize the MSNA findings more completely?
END OF COMMENTS
We sincerely thank the editors and reviewers for their initial and subsequent feedback to strengthen this paper. We are excited to share our findings with the readers of *The Journal of Physiology*.

EDITOR COMMENTS

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Dear Dr Stickford,

Thank you for submitting a revised version of your manuscript to The Journal of Physiology, for incorporating MSNA data obtained during HUT, including the vascular transduction data and tempering your conclusions about HRV. There a few outstanding issues the Reviewers raise that you will need to attend to. Moreover, please adjust the Key Points summary to reflect the changes in MSNA during HUT; your conclusion that there was an exaggerated HR response during HUT doesn't match the observations reported in Results.

We have updated the Key Points summary to reflect the higher MSNA in COV+ compared with CON throughout HUT. We did see a significant group-by-position interaction in HR during HUT, and so we have continued to include this in the Key Points summary.

Senior Editor:

Comments for Authors to ensure the paper complies with the Statistics Policy:

Please include P values in Figure 1.

*We have updated Figure 1 to include p values.*

Please include data points in Figure 3.

*We have updated Figure 3 to be similar to Figure 1 in that individual data points (distinguished by sex) are included.*

Please include supplemental table into the manuscript.
The supplemental table has been relabeled as Table 2, and included/referred to within the manuscript instead of as a supplement.

Comments to the Author:

While the authors make the comment that the American Statistical Association's Statement on P-Values asserts that practices that reduce data analysis or scientific inference to mechanical "bright-line" rules (such as "p < 0.05") can result in erroneous beliefs, the standards also importantly highlight the importance of assessing other types of error analysis. This would include type I error along with type II error when assessing interpretation of statistical comparisons. Thus, while the Journal does not explicitly state the importance of reporting type I error, it is incumbent on the authors to closely assess both, when the type II error is in question. The ASA statement also asserts: "Researchers should recognize that a p-value without context or other evidence provides limited information. For example, a p-value near 0.05 taken by itself offers only weak evidence against the null hypothesis...For these reasons, data analysis should not end with the calculation of a p-value when other approaches are appropriate and feasible." The ASA stance also recommends other statistical approaches that are available when determining the relevance of a data comparison. The Journal does not mandate a path of approaches that one needs to follow to verify statistical interpretations. We do, however, make clear that P values >0.05 are NOT to be interpreted as a trend. The Journal policy also suggests that inclusion of 95% confidence intervals are appropriate and useful. (please see https://physoc.onlinelibrary.wiley.com/doi/10.1113/jphysiol.2011.205062)

We thank the Senior Editor for these comments and agree that multiple statistical approaches help with transparency in reporting results. We understand that The Journal of Physiology must have statistical standards and thus p values > 0.05 should not be interpreted as a trend; we have removed all language of “trends,” etc. throughout the manuscript to ensure we align with The Journal’s standards.

In regard to statistics in figures, the Journal wants to see actual P values included in figure and tables and not use of astericks. This should not be interpreted to mean no statistics should be shown. Actual P values can and should be shown in figures when possible. This is particularly true for bar graphs such as Figure 1.

We apologize for not including the p values in the figure (as opposed to just in the legend). We have updated Figure 1 accordingly. However, if the editors feel this is redundant and would prefer the version of Figure 1 with p values just in the legend and not figure, we are happy to send that as well.

The Journal also requires the inclusion of data points when possible in figures. It is possible to show the actual data points in Figure 3 using open and solid squares or triangles. Please include them.

Thank you for this suggestion. We have updated Figure 3 to be similar to Figure 1 in that individual data points (distinguished by sex) are included. We hope this transparency will allow readers to make informed interpretations of our findings.
The supplemental table needs to be incorporated into the manuscript as a table.

The supplemental table has been relabeled as Table 2, and included/referred to within the manuscript instead of as a supplement.

REFEREE COMMENTS

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We appreciate the reviewer’s feedback and concerns. It is certainly possible that confounding factors associated with the pandemic may have impacted our findings. And thus, we agree with the reviewer that the control group is not ideal, but we hope that the inclusion of these details and transparency in the limitations will allow readers to make a more informed interpretation of our findings. Indeed, many studies in which a diseased/sick population is studied are limited by other factors (e.g., anxiety/stress associated with disease and/or treatment) in comparison with control groups.

We also recognize and agree that it is unfortunate we were unable to more closely match the sex distribution of the two groups (though, again, as you point out, a chi square analysis indicates no significant difference in distributions). However, while studies have indicated sex differences in MSNA (and this consequently seems to be the default assumption of many in our field), we maintain that many studies – including a number of quite recent investigations – have not observed differences in resting MSNA between sexes, especially in young adults like this cohort (Carter & Ray, 2009; Jarvis et al., 2011; Best et al., 2014; Adler et al., 2019; Holwerda et al., 2019; Miller et al., 2019; Coovadia et al., 2020; Keller-Ross et al., 2020). An exceptional recent publication by Keir et al. compiled MSNA data from >600 adults from four institutions; they found that at age 20 (which is similar to the average age of participants in our study), MSNA was similar between males and females (Keir et al., 2020). While underpowered, we nonetheless also found no effect of sex on MSNA. And, in fact, the absolute values of our female COV+ participants are actually higher than the males (20 vs 17 bursts/min), which is in contrast to what would be expected if the male>female-presumed-MSNA-sex-difference were driving the difference between our CON (more females) and COV+ (more males) groups.

We have updated Figure 3 to be similar to Figure 1 in that individual data points (distinguished by sex) are included. We again hope this transparency will allow readers to make informed interpretations of our findings!
Referee #2:

The authors have thoughtfully addressed my suggestions. The authors note the challenges of performing this work in the midst of the pandemic and have documented these limitations clearly. I have a very minor suggestion to further clarify the HUT data collection within in the methods section - Could the authors clarify if the MSNA data collected during tilt is the average of the 5 minutes or last minute? Should recovery data be included for HR and BP (figure 4), to contextualize the MSNA findings more completely?

We appreciate your feedback!

Thanks for requesting this clarification; it was oversight to not include those details. We have updated the methods to include over what time periods data was averaged during each test.

We certainly understand the basis for your second question. We agree that the reported data between hemodynamics and MSNA should be consistent and apologize that it was not. When thinking about what should be displayed to best illustrate our findings as they relate to our research question, we have actually removed the recovery data from the MSNA graphs (rather than add to the hemodynamics graphs). We are really interested in discussing what occurs during the orthostatic challenge; as a result, including the recovery data - especially for the HR response, which dropped quickly during recovery in all subjects – is not as representative as the HUT data alone in showing the subjects’ response to orthostatic challenge. This is also more consistent with our discussion. We have updated the graphs and corresponding statistics accordingly. Below, we give the statistical results (p values) for each variable including/excluding recovery data.

| Variable | WITH Recovery (i.e., 4 “position” points) | WITHOUT Recovery (i.e., 3 “position” points) |
|----------|------------------------------------------|---------------------------------------------|
| HR       | Position p <0.001 Group p=0.908 Interaction p=0.077 | Position p <0.001 Group p=0.868 Interaction p=0.044 |
|          |                                          |                                             |
| SBP      | Position p=0.451 Group p=0.088 Interaction p=0.088 | Position p=0.412 Group p=0.073 Interaction p=0.065 |
|          | Position p<0.001 Group p=0.427 Interaction p=0.106 | Position p<0.001 Group p=0.253 Interaction p=0.360 |
| MSNA BF  | Position p<0.001 Group p=0.006 Interaction p=0.137 | Position p<0.001 Group p=0.007 Interaction p=0.234 |
| MSNA BI  | Position p<0.001 Group p=0.005 Interaction p=0.033 | Position p<0.001 Group p=0.003 Interaction p=0.012 |
| MSNA Tot | Position p<0.001 Group p=0.022 Interaction p=0.128 | Position p<0.001 Group p=0.019 Interaction p=0.967 |
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Miller AJ, Cui J, Luck JC, Sinoway LI & Muller MD. (2019). Age and sex differences in sympathetic and hemodynamic responses to hypoxia and cold pressor test. *Physiol Rep* **7**, e13988.
Dear Dr Stickford,

Re: JP-RP-2021-281888XR1 "COVID-19 is getting on our nerves: Sympathetic neural activity and hemodynamics in young adults recovering from SARS-CoV-2" by Nina L Stute, Jonathon L Stickford, Valesha M. Province, Marc A. Augenreich, Stephen M. Ratchford, and Abigail S.L. Stickford

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:
Dear Dr Stickford,

Thank you for addressing the remaining concerns raised by the two reviewers, both of whom are now satisfied with your amendments. I am pleased to endorse publication in The Journal of Physiology.

------------------------------------------------------------------------------

REFEREE COMMENTS

Referee #1:

All comments have been addressed.

Referee #2:

I have no further comments for the authors.

END OF COMMENTS