**Author’s response to reviews**

**Title:** THE EFFECT OF LOW LEVEL LASER IRRADIATION ON OXIDATIVE STRESS, MUSCLE DAMAGE AND FUNCTION FOLLOWING NEUROMUSCULAR ELECTRICAL STIMULATION. A DOUBLE BLIND, CROSS-OVER TRIAL

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**Author’s response to reviews:**

Dear Editor,

Dear Reviewers,

Thank you very much for the thorough analysis of our manuscript, for valuable and helpful comments and for giving us the opportunity to revise and improve our submission. We hope that our replies and explanations, as well as the amendments to the manuscript, fully address your concerns. We keep the change tracking as highlighted in yellow for the first revision and as highlighted in green for the second, and blue for the third revision.

In the following, please find our answers to your comments.

Reviewer reports:

Angus Lindsay (Reviewer 1):

Reviewer’s comment:

Thank you for responding to my suggestions. A few points to consider.
- 45 contractions was stated in the methods but why is the new Figure 3A and B only showing contractions from 6 - 45?

Response:

We would like to thank the Reviewer for his systematic and thorough analysis of our paper, and for his further suggestions. We believe that all remaining concerns are now fully addressed.

First five contractions were not included in the analysis. This is explained in the manuscript, in the ‘Methods’ section (lines 186 – 189): We recorded and analysed moments of force of each EEC, except first five contractions, which were excluded from the analysis, as first stimulation bouts are presumed initial and allowing the current to fully penetrate. We performed NMES based on Aldayel et al. [9] and with a typical electrical stimulation technique [11].

Also, please see the ‘Results’ section, line 237 (with reference to Fig. 3A, added in the revised manuscript). The statement ‘The EEC values of the sixth and the last contractions (Fig. 3A) were ..’ also informs that we did not include first five contractions in the analysis.

Reviewer’s comment:

- "The differences between EECs in part I and part II (Fig. 3A) in the whole group, as well as between LLLT and sham-LLLT interventions (Fig. 3B), were not significant in each contraction." I am unsure what you mean by each contraction? Was a simple repeated measures ANOVA completed with post-hoc analysis to determine a group difference?

Response:

Thank you for this point.

In terms of the conduct of the intervention (not in terms of the research questions and/ or the results) it was important to test whether NMES was equally (comparatively) strenuous in both study periods (parts) as well as in LLLT and sham-LLLT interventions.

Therefore, we performed a two-way ANOVA (group and time) with repeated measures to determine the differences in EECs, between LLLT and sham-LLLT interventions, as well as between part I and part II of the study. We found that the groups did not differ significantly since the main group effect and the group x time interaction were p=0.29 and p= 0.91, respectively. In the same manner, the differences between the parts of the study were also insignificant since the main group effect and the group x time interaction were p=0.74 and p= 0.98, respectively. In both cases, only main time effect was significant. Nonetheless, the latter is not relevant and not applicable in terms of the study questions and interpretation of the results.

We supplemented the ‘Statistical analysis’ section of the paper (lines 227-229) with relevant information (we had overlooked it when preparing the requested figures 3A and 3B):
‘A two-way ANOVA (group and time) with repeated measures was completed to determine the differences between EECs of the quadriceps muscle in part I and part II of the study, in the whole group, as well as between LLLT and sham-LLLT interventions’.

Also, we added a further explanation in the ‘Results’ section (line 241):

‘The differences between EECs in part I and part II (Fig. 3A) in the whole group, as well as between LLLT and sham-LLLT interventions (Fig. 3B) were not significant in each contraction. (no main effects of group or group and time interaction were observed)’, as well as in the figure legend (lines 653-654):

‘differences between each contractions not significant in part I and part II (Fig. 3A) as well as in between LLLT and sham-LLLT interventions (Fig. 3B) were not significant (p>0.05 for group or group and time interaction).

Reviewer’s comment:

- Figure 4A and B - the figures are easier to understand, thank you. The use of "0" at baseline is far more informative. Can the authors please provide the actual statistics to these new analyses rather than simply stating there is no difference between groups or treatment?

Response:

According to previous comments of the Reviewer, we prepared (in the previous revision) additional figures (3A, 3B) or modified the originally submitted figures (4B, 5B) to improve the visual presentation of the data, specifically to highlight the baseline value. We did not perform any new analyses.

Hence, Figure 4A (presenting absolute changes in MVC of the quadriceps femoris muscle following single NMES sessions) remains unchanged since the original submission (except the figure number). The same applies for the statistical analyses regarding data presented in Figure 4A (as described in the paper; please see lines 241-249).

Following former comments (first review), we prepared Figures 4B and 5B in revision 1. In Figures 4B and 5B, we address relative (%) change from baseline (0 level) for each group (LLLT and sham-LLLT) separately. The baseline is shown as ‘0 level’ and the changes in MVC are shown in correspondence to baseline. Horizontal line marked as ‘0 level’ (Y-axis) is the baseline for each group (please note that 0 level represents different absolute values in the LLLT and sham-LLLT groups). Following the previous comment (review 2), we have only added the baseline point (legend) at the X-axis, to underline the ‘0 level’ as baseline. We have not performed any new statistical analyses. Moreover, due to the lack of main effects (i.e. intervention or time and intervention interaction), we decided that there is no need to describe the time effects in detail, as this does not provide any new information as to the main objective of the study (the effect of LLLT irradiations).
Also, we understand that the figures ought to be self-explanatory; therefore we supplemented the figures and tables with relevant legends (lines 646 – 719). The manuscript submission system, with separate figure and figure legends placement in the submission file, might probably have caused some confusion.

Reviewer’s comment:

- As reviewer two has pointed out, it still looks at first glance that the LLLT group started off with lower torque and also lost less torque. Can the authors please address this properly providing statistics.

Response:

We agree that the baseline difference appears considerable. Nonetheless, as we found no statistically significant differences between the interventions, we assume that it did not influence the overall interpretation of our findings.

We did report on baseline differences, both in the original submission, and in the subsequent amendments (specifically lines 427-440, but the remaining part of the ‘Strengths and limitations of the study’ subsection also addresses this issue).

Because of the noticeable difference in MVC between interventions at baseline, and following the Reviewer’s comments, we prepared in the revised manuscript (revision 1) an additional Figure 4C, showing relative changes in MVC, where average baseline values from two interventions were taken into account.

Please see the ‘Results’ section as well (lines 251 – 253): ‘in case of changes in MVC when the values in both LLLT and sham-LLLT interventions were normalised on baseline values from two interventions combined, with standard deviation (Z-score; p<0.01; Fig. 4B 4C).’

We believe that we made use of the benefits of the cross-over design, in opposition to parallel group trials, in reducing inter-group differences and controlling for confounding factors. We address it broadly in the ‘Study design’ section (lines 101 – 129).

The study is registered at the ANZCTR trials registry (lines 42 – 44), which was also preceded by a strict, step-by-step methodological review process. We supplement the manuscript with a CONSORT checklist (line 59, Supplementary file 1).

Again, we are grateful to the Reviewers for taking their time and sharing with us their expertise, and for their very careful analyses of our paper.