Reviewer’s report

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

Version: 1 Date: 04 Jun 2019

Reviewer: Angus Lindsay

Reviewer's report:

The authors have examined the effect of LLLT on indices of NMES-induced skeletal muscle stress. Below are broad and specific comments regarding the design, analyses and interpretation of the data.

Major comments

1. There is concern over the length of time between the crossover. The referenced paper (Mackey et al. 2008) shows muscle damage following isometric contraction significantly elevates CK activity up to seven days post-exercise. While the authors have stated that eight days is a long enough washout period based on their own preliminary studies, the current study design did not measure MVC or CK activity beyond 96 hours and it looks like there are differences between interventions at baseline even though it is a crossover design. Therefore, the eight-day duration between bouts may not allow full recovery and probably affected the response of the second bout to injury.

2. The statistical analyses have been conducted on absolute values. Given the MVC between the two groups at baseline looks to be large (~240 vs 280 Nm) and indicative of a group effect, it would be interesting to know whether the same statistical differences provided are also evident when compared as a % change from baseline in all parameters.

3. The statistical analyses conducted on the repeated measures is questionable. Post-hoc analyses are only to be used when a main effect is observed. T-tests can be used if an interaction is found. Because a two-way ANOVA was conducted and no time, group or interaction effect was measured, then I am unsure where the authors have got their statistical values from. For example, for TAC and TAC-UA in Table 3, no time, intervention or interaction effect is found. Therefore, post-hoc analyses cannot be conducted. So where has the statistical values presented in the table come from?

4. The inference that LLLT had an effect on several indices of muscle damage, redox status and inflammation is based on statistics that were not conducted correctly. Furthermore, the authors make several inferred statements about the possible protective effect of LLLT before stating that no difference existed between groups. It is suggested the authors re-evaluate their discussion based on statistical analyses. For example, there was no
intervention effect for changes in MDA concentration, yet the authors have detailed the possible benefit of LLLT on lipid peroxidation.

5. The authors need to consider the design or at least comment on the design with regard to the repeated bout effect. Because a single bout of exercise can provide protection from a subsequent bout of the same exercise (normally eccentric contractions), it is possible the crossover design here did not account for this effect. The authors might consider presenting or analysing data from the group as a single parallel design and/or as percent changes from baseline for each group.

6. The article requires grammatical consideration throughout for better readability.

Minor comments

1. The authors should consider plotting the torque tracings of the NMES intervention.

2. Line 62 - lack of desmin staining? Do you mean low protein levels of desmin following contraction?

3. Line 68 - why are oxidant/antioxidant status among top priorities?

4. Line 77 - what are vague findings? Subject number? Statistical analysis?

5. Line 79 - no need to BOLD your objective

6. Line 103 - what is the period effect?

7. Line 118 - abstract says 20-23 year olds.

8. Line 179 - Did subjects complete a VAS after the squat as well?

9. Line 224 - These data would be better presented in a table.

10. Table 3 (GPx) - All the values seem to be consistent for this measurement except for 48 hours in the sham-LLLT group. Can this potentially be explained by something other than the intervention? It seems unusual that it suddenly drops and rebounds again 24 hours later.

11. Line 256 - CRP did not change compared to baseline until 96 hours so the language "increased throughout" should be changed.

12. Line 312 - there is minimal evidence of secondary damage to muscle (see Warren et al. Minimal evidence for a secondary loss of strength after an acute muscle injury: a systematic review and meta-analysis)
13. Line 312 - "increased ROS production" - the authors never actually measured ROS production

14. Line 315 - When was muscle fatigue measured in this study?

15. Line 317 - Because no time or intervention effect was observed, the authors should limit the inference that LLLT might affect the antioxidant system.

16. Line 327 - can the authors provide a reference for this please.

17. Line 325 - 333 - This paragraph goes back and forth between satellite cells, muscle repair and ROS before circling back to the antioxidant defence system of this study. Can the authors please adjust the structure for readability please.

18. Line 357 and 375 - similar to above, muscle fatigue was not measured…or at least not presented.

19. Line 376 - This statement is not corroborated by the statistical analyses. See comment in "major comments" section.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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