The Journal of Physiology

Altered muscle mitochondrial, inflammatory and trophic markers and reduced exercise training adaptations in type 1 diabetes

Dean Minnock, Giosuè Annibalini, Giacomo Valli, Roberta Saltarelli, Maurício Krause, Elena Barbieri, and Giuseppe De Vito
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The referees have opted to remain anonymous.

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Reviewing Editor: Bettina Mittendorfer

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision **26-Nov-2021**

Dear Mr Valli,

Re: JP-RP-2021-282433 "Altered muscle mitochondrial, inflammatory and trophic markers and reduced exercise training adaptations in type 1 diabetes" by Dean Minnock, Giosuè Annibalini, Giacomo Valli, Roberta Saltarelli, Maurício Krause, Elena Barbieri, and Giuseppe De Vito

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

Please advise your co-authors of this decision as soon as possible.

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- Statistical Summary Document

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If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

The reviewer and I myself found considerable merit in the work

Enclosed are specific comments to help further improve this already strong paper

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REFEREE COMMENTS

Referee #1:

In the present study, those with and without type 1 diabetes were evaluated before and after 12 weeks of COMB exercise training. Measures were IG variability, hypoglycaemic event number and gene expression of a number of muscle specific signalling pathways, as well as Western blotting for OXPHOS and AMPK.

The inclusion of muscle biopsies pre and post training provide an novel insight into potential changes that are happening in those with T1D. The glycemic data, while a focus of this work, is less novel but does serve to demonstrate an important patient focused outcome.

Major Comments:

(1) Authors briefly discuss the low N for males and females prohibited a more detailed investigation. While this may be true, the results of the present study would be more important and novel for the research community (and the lay reader) if data points were coded to denote female and male participants. This would allow the reader to observe sex differences between those with type 1 diabetes and those without.

(2) The primary outcome of interest of this work was noted as the changes in glycemia. This outcome has been studied extensively (and, as the authors noted, has a consensus report written on the subject). The novel aspect of this work (muscle changes) should be the primary outcome of interest with the changes in glycemic a secondary outcome. This would also make the introduction more relevant (which focuses primarily on skeletal muscle)

(3) Given the recent work of Dial, Grafham et al (2021- AJP Cell), do you think that the reduced benefits of RE in those with type 1 diabetes was related to a reduced ability to repair from the previous bouts of exercise?

Minor Comments;

- [ ] Might be more impactful if the subject dots (pre and post) were connected together to show the change for each subject.

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- [ ] Figure 3B- Y axis has a spelling mistake (length, not lenght)

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- [ ] L440: Monaco et al 2021 is the appropriate reference for this statement

- [ ] L443: should not be the beginning of a new paragraph

END OF COMMENTS

Confidential Review **29-Sep-2021**

Dear editor and reviewers, we the authors very much appreciated the opportunity to publish in *The journal of Physiology* and are grateful for the suggestions to improve our paper. Whether you might not be satisfied with any of the answers or changes provided, we remain available for further clarification.

Please see our reply below:

REQUIRED ITEMS:

-Author photo and profile. First (or joint first) authors are asked to provide a short biography (no more than 100 words for one author or 150 words in total for joint first authors) and a portrait photograph. These should be uploaded and clearly labelled with the revised version of the manuscript. See Information for Authors for further details.

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This point was changed in the text

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*p<0.05, **p<0.01, ***p<0.001 compared to pre;

† p<0.05, †† p<0.01, ††† p<0.001, compared to control.

This should make the identification of the statistical differences easier.

The reviewer and the editor should note that during the filling of the statistical summary document and the careful revision of the manuscript, we realised 2 mistakes:

1. we have incorrectly reported MCP-1 and IGF-1 mRNA expression (in the Methods, Statistical Analysis section) as not normally distributed variables. We corrected this error in the revised manuscript.
2. We noticed that carbohydrates intake was higher in type 1 diabetes compared to the control group (but no difference between pre and post-training was observed) and this was not reported in the manuscript. This is now reported in the results section.

Please note that none of these changes modified the results described in the first version of the manuscript.

We remain available for any additional clarification.

-Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the research and should summarise the main conclusions. If possible, the image should be easily 'readable' from left to right or top to bottom. It should show the physiological relevance of the manuscript so readers can assess the importance and content of its findings. Abstract Figures should not merely recapitulate other figures in the manuscript. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion(s). Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures should be created using BioRender. Authors should use The Journal's premium BioRender account to export high-resolution images. Details on how to use and access the premium account are included as part of this email.

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Major Comments:

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We agree with this comment as the gender-specific response is an important topic. At first, we decided not to distinguish between males and females because of the small sample size that prevented the application of appropriate statistical analysis on the two separated groups. However, in response to this comment, male and female participants were separated in the figures (different colours and shapes) in order to allow the reader to clearly distinguish between the two.

(2) The primary outcome of interest of this work was noted as the changes in glycemia. This outcome has been studied extensively (and, as the authors noted, has a consensus report written on the subject). The novel aspect of this work (muscle changes) should be the primary outcome of interest with the changes in glycemic a secondary outcome. This would also make the introduction more relevant (which focuses primarily on skeletal muscle)

We really agree with this comment. It was changed in the text

(3) Given the recent work of Dial, Grafham et al (2021- AJP Cell), do you think that the reduced benefits of RE in those with type 1 diabetes was related to a reduced ability to repair from the previous bouts of exercise?

This is a very good point. In our previous study (Minnock et al., 2020; Eur J Appl Physiol. 2020 Dec;120(12):2677-2691), we demonstrated that an acute bout of combined resistance and aerobic exercise (the same as used in the present study) did not increase muscle markers of muscle damage (serum creatine kinase and LDH) in type 1D subjects. Thus, the exercise modality adopted in our study differed completely from that reported in Dial et al (2021- AJP Cell), which used an exercise protocol specifically designed to induce muscle damage (eccentric quadriceps contractions). In fact, Dial et al. observed a large increase 96 hours post-exercise of serum CK (75 times higher than resting levels). The exercise protocol proposed in our study was well tolerated by all participants and no injuries events were recorded during the training period. Moreover, only minor differences were observed for the session RPE assessed across the 12 weeks of training. Obviously, the experimental design of the present study, which considers only pre and post-training muscle biopsies, did not allow us to draw conclusive remarks on this issue. Further studies, which may consider multiple biopsies during the training sessions, might be useful to clarify this point. If the reviewer and the editor believe that this aspect should be added in the discussion section, we can add a sentence on that.

Minor Comments;

- [ ] Might be more impactful if the subject dots (pre and post) were connected together to show the change for each subject.

We consider this a good suggestion, the figures were changed

- [ ] It is admittedly surprising to see significant changes in so many measures of aerobic and resistance exercise fitness yet no change in measures that subjects would care about (LBM, BMI, BW). Why do the authors think this happened?

No physical characteristic differences were found between type 1 diabetes and control participants at baseline. Moreover, no significant improvements in lean mass, and body fat, were observed following the intervention period. This, in part, can be explained by the brief duration of the exercise sessions (40mins),

resulting in an overall low volume of total exercise performed over 36 exercise sessions. It should be noted that we monitored carbohydrate intake in both groups at Pre- and Post-intervention week (as it might heavily affect glucose control) but did not control for energy intake across all the study duration. This could have also affected body composition adaptions. These findings, however, confirm previous research, indicating that HIT exercise training may not be the most effective modality towards improving lean body mass, body fat (kg) and body fat percentage (Sultana et al., 2019), mostly due to their short duration and overall shortened volume of exercise when compared to traditional training methods.

- [ ] L109: 'muscle to' not 'to muscle'

This was corrected

- [ ] was there a significant differential between people (in METs) before an during the exercise training sessions. Essentially, subjects were doing less than 120 min and <500METs before. Now they are doing 120 min and 500 METs. Not a huge difference possibly? Also, why did you choose training only 3 times a week as this would not reach the recommendations of most diabetes organizations worldwide?

The primary reason for training 3 times weekly was for the convenience of the participants. Our aim was to mimic a real-world scenario and our belief was that by offering a manageable and realistic protocol our participants who were not habitual exercisers would adhere to the programme. Moreover, the aim was not to reach the desired recommended training durations set out by diabetes organisations. Based on our previous research (Minnock et al., 2020) which indicated that 40 min combined exercise resulted in the safest glycaemic response in the 24 hour post exercise. We wished examine the physical and molecular outcomes of this training mode when applied to a chronic training intervention.

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All queries at proof stage should be sent to TJP@wiley.com

Yours sincerely,

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PEER REVIEW COORDINATOR COMMENTS:

Note: at the publication proofing stage, please could authors add a statement on database registration and compliance with Clause 35 (of the Helsinki Declaration), or else state "except for registration in a database."

EDITOR COMMENTS

Reviewing Editor:

No further comments

REFEREE COMMENTS

Referee #1:

The authors were very attentive to the recommendations suggested. No further comments.

This research will be a welcome addition to the muscle research field in those with Type 1 Diabetes.

END OF COMMENTS

1st Confidential Review **17-Dec-2021**