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Methodological considerations for the determination of mitochondrial ADP sensitivity in skeletal muscle

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Reviewer 2: Beatrice Chabi
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Manuscript reviewed 2024-03-15: *Only major points included.*

Reviewer 2

In this technical communication, Brunetta and Holloway offer a practical update on the use of ADP sensitivity measurement when assessing mitochondrial oxidative phosphorylation (OXPHOS) in skeletal muscle fibers using high-resolution respirometry.

They particularly highlight the relevance of oxygen tension, substrates choices and skeletal muscle fibers states (contracting versus relaxed) on this measurement. The impact of sample replicates and weight normalization on the assay variability is also addressed, with insightful comments based on their expertise.

They also provide few examples where ADP sensitivity is of interest to evidence subtle regulation of mitochondrial bioenergetics in skeletal muscle in physiological and pathological conditions.

This technical communication is well written and documented, and will definitely be of use for readers willing to implement this method in their exploration of mitochondrial function in skeletal muscle.

It is suitable for publication with these few minor revisions:

Methods:

1. Could you specify C57/Bl6 substrain?
2. The amount and the recovery process for the fibers could be indicated in this section and this point still be discussed line 311 to 334.

Authors

We thank the reviewer for the kind words regarding our work.

1. Yes, we have used C57/Bl6N strain. We have now added this information to the main manuscript.
2. Thank you for this suggestion. We have now added these details into the manuscript.

Reviewer 2

Experimental design

1. In table 1: the first volume of ADP added should be 4 µl to achieve a final concentration of 100 µM.
2. Line 116: even if rotenone and uncouplers are not added, are mitochondrial respiratory values corrected with the addition of antimycin A? If so, could you add this information in the method section?

Authors

1. We apologize for this oversight. We have now corrected the volume added.
2. We thank the reviewer for this insight. We have now performed an experiment where we added oligomycin and antimycin A at the end of the ADP titration protocol to determine the coupling degree of our fibers preparation. In summary, we observed a full reduction in mitochondrial respiration following oligomycin addition. Antimycin A decreased mildly respiration, which is expected when added after oligomycin in coupled mitochondria. We have added this information to the methods and results section (Fig. 2E, F). Of note, we do not normalize respiration to inhibitors given this is not part of our routine. In this respect, we subtract PM JO_2 values (leak respiration) to consider zero ADP in the media to calculate Km. This information can be found in the methods section of our study.