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

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Reviewer 1: Mario Ost

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Manuscript reviewed 2024-03-11: *Only major points included.*

Reviewer 1

This is a very straight-forward manuscript introducing the concept of a mitochondrial ADP sensitivity assay, where mitochondrial bioenergetic responses in murine skeletal muscle to a wide range of ADP concentrations have been studied. Moreover, they carefully dissected potential confounding factors of oxygen tension, substrate-dependency, muscle contraction and inter-experimental variability. Overall, this technical communication is positioned to make a solid contribution to the field.

In Figure 3, experiments have been conducted with (or without) blebbistatin, as the apparent ADP K_m for PM in Figure 3C is similar to Figure 4C (+) blebbistatin?

Authors

We thank the reviewer for the compliments regarding our work.

We apologize for the lack of clarity. The reviewer is right, the experiment in Figure 3 was done in the presence of blebbistatin, given the relaxed fibers give a greater range of ADP titration. We have now added this information to the figure's legend to make it as clear as possible to the reader.

Reviewer 1

The data used for the correlations in figure 6 are based on previous, published analyses by the group. But this represents mixed sample from mouse and human muscle, which at least should be mentioned in results part and the figure legend.

Authors

Although we have already demonstrated modulation of mitochondrial ADP sensitivity in other conditions in humans (i.e. Holloway et al. 2018, Cell Reports), the correlation graphic in Figure 6 is based only on mouse data. We have added this information to the text to avoid misleading the reader.

Reviewer 1

Would the authors expect differences in the presented methodology and ADP sensitivity between (a) oxidative vs. glycolytic muscle fibers or (b) mouse vs. human skeletal muscle permeabilized fibers? This could be added to the discussion and add important value resource for researchers interested in incorporating this skeletal muscle analysis.

Authors

We thank the reviewer for this insight. Yes, ADP sensitivity between oxidative and glycolytic muscle fibers was assessed by us and independent groups in the past. We have now added this to our manuscript alongside the references for the original work.