

## Open peer review and authors' responses

### The influence of adenylate cycling on mitochondrial calcium-induced permeability transition in permeabilized skeletal muscle fibers

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Reviewer 1: Vilma Borutaite

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Manuscript reviewed 2022-11-21: *Only major points included.*

#### Reviewer 1

The study by C.A. Bellissimo et al. investigates how mPTP sensitivity to Ca<sup>2+</sup> in permeabilized skeletal muscle fibers is regulated by adenylates added as exogenous ADP or in the presence of hexokinase ATP recycling system. The results of the study point that it is important to consider the impact of physiological adenylate recycling system when analyzing factors influencing mPTP regulation in permeabilized muscle fibers. The methodology is appropriate, study is carefully performed, however, methods must be described in more details.

#### Authors

We thank the reviewer for their comments and have directly answered the concerns below:

#### Reviewer 1

P.4, line71-73: It is stated that for the experiments male CD1 mice were used, but nothing is said about DBA/2J WT mice.

#### Authors

We apologize for this uncertainty, and we have added that only male DBA/2J WT were used (line 76).

#### Reviewer 1

What was the complete composition of the buffer for mPTP measurements?

#### Authors

We thank the reviewer for this comment and we apologize if buffer composition is unclear. The buffer composition did change pending on the question being investigated, but the core composition of the buffer are sucrose 250 mM, Tris-HCl 10mM, Tris Base 20 mM, KH<sub>2</sub>PO<sub>4</sub> 10mM, BSA 0.5 mg/mL, 1 μM Calcium Green-5N (Invitrogen), 2 μM

thapsigargin, 5  $\mu$ M blebbistatin, and 40  $\mu$ M EGTA). We have now updated the methods section to include this in lines 109-112. In cases where ATP was depleted, 5 mM 2-deoxyglucose and 2 U/mL hexokinase were included in the buffer, such as in Figures 1B and 2B. Ranges of ADP and 20mM creatine (Figure 2 and 3) were utilized and noted in each figure (below graph) and noted in the figure caption.

### Reviewer 1

What was the concentration of hexokinase and 2-deoxyglucose used in the experiments?

### Authors

We apologize for this missing information and have added that 5 mM 2-deoxyglucose and 2 U/mL hexokinase were added (lines 111-112).

### Reviewer 1

In the Abstract, it would be reasonable to describe shortly the results on transgenic mice.

### Authors

We have updated the summary to include the specific results of the D2.*mdx* experiments.