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Fighting against reproducibility crisis: Inter-laboratory harmonization of protocols for mitochondrial function evaluation in permeabilized muscle fibers

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Despite of the growing interest in mitochondrial research in many physio-pathological conditions and consequently the exponentially increase of scientific literature in the mitochondrial field, currently there is no database which provides information about mitochondrial function. Moreover, an appropriate evaluation and interpretation of mitochondrial respiration is required for understanding mitochondrial physiology and bioenergetics in health and disease. In the framework of the MitoEAGLE COST Action, the MitoEAGLE network is a powerful tool for improving our knowledge on mitochondrial function, and for establishing a mitochondrial database mapping different species, tissues, and sample preparations. However, the comparison of data sets on mitochondrial function from scientific published data and inter/intra-laboratory studies is very complicated. Additionally, in studies performed in permeabilized muscle fibers, other factors such as the quality of the sample preparation play a key role which makes difficult intra and inter-laboratory comparisons. In the MitoEAGLE project, one of the main goals of the WG2 (muscle tissues) is to generate reference values which allow the evaluation of the skills preparing high-quality permeabilized fibers. To generate these reference values, a pilot study collecting mitochondrial respiratory data (permeabilized fibers from soleus in female and male mice, following the same experimental procedure) was performed in different laboratories (7 laboratories, 5 European countries). In this pilot study, we observed heterogeneous results between the groups involved [1]. During a MitoEAGLE Short-Term Scientific Mission, we addressed some potential aspects which could trigger this variability. For this purpose, mitochondrial respiration was evaluated by high-resolution respirometry in permeabilized fibers from soleus muscle from *N*=15 C57BL6/J male mice using the SUIT-008 O2 pfi D014 protocol (Fig. 1) [2]. Experiments were performed in parallel in the same laboratory by two researchers from different research groups (who participated in the pilot study). Our results did not exhibit significant differences on mitochondrial respiration by comparing individual mechanical sample preparations and, the effect of chemicals prepared for this study in both laboratories. On the other hand, we compared the results obtained for each researcher in the former and present study. We detected significant differences in oxygen consumption between both studies. Our findings demonstrate two sources of variability in mitochondrial respiration. First, ADP storage (time and/or temperature)

seems to be a critical aspect affecting oxygen fluxes. Wet weight is the other important issue that must be considered and well-explained for generating reliable results in the future. Once the critical factors producing variability in the pilot study were identified, the open call for an international ring test was performed. Other 8 international laboratories have participated in the current study for inter-laboratory harmonization of protocols for mitochondrial function evaluation in permeabilized muscle fibers. This study will allow to set specific and detailed SOPs and provide reference values which will be used by researchers for evaluating technical skills for permeabilized skeletal muscle preparations. Taken together, this work will contribute to face the lack of reproducibility and will benefit the elaboration of a public mitochondrial database in muscle permeabilized tissues.

Affiliations and Support

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Figures

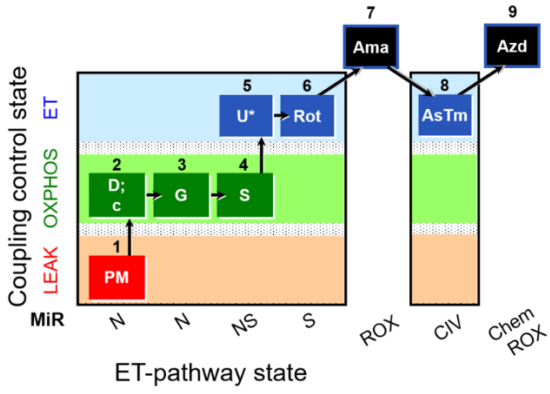

Figure 1. Representative diagram for the SUIT-008 O2 pfi D014 protocol. Briefly, 1PM, pyruvate and malate as substrates of NADH-linked pathway;

2D, saturated ADP to evaluate OXPHOS state in NADH-linked pathway; 2c, cytochrome c to assess the integrity of the outer mitochondrial membrane; 3G, glutamate for the NADH-pathway; 4S, succinate for NADH&Succinate pathway in OXPHOS state; 5U, stepwise titration of an uncoupler for obtaining ET-state with NADH&Succinate linked-substrates; 6Rot, rotenone for inhibiting Complex I; 7Ama, antimycin A for blocking Complex III.

References

1. Garcia-Roves Pablo M, Chabi B, Doerrier C, Dubouchaud H, Grefte S, Irving B, Ost M, Pesta D (2017) Mitochondrial respirometry reference values from permeabilized mouse soleus muscle fibers. MitoEAGLE WG2 pilot study. Garcia-Roves_2017_MiP2017
2. MitoPedia: SUIT-008 O2 pfi D014