

RESEARCH ARTICLE | Molecular Pathways in Cell Signaling

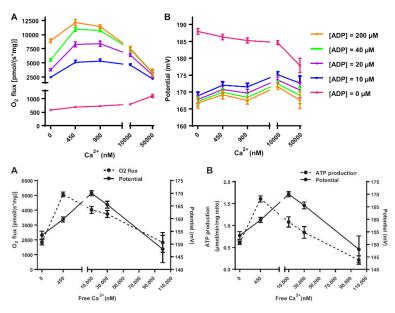
High-resolution respirometry and calcium effect on mitochondria



Regulation of ATP production: dependence on calcium concentration and respiratory state

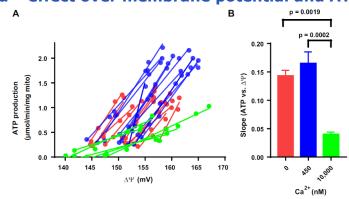
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## **High-resolution respirometry of mouse skeletal muscle**



**Figure 1. A)** High-resolution respirometry in mouse muscle isolated mitochondria, 5 mM glutamate, 1 mM malate. Mean  $\pm$  SE; N=8 (each data point), N=5 for 50,000 nM  ${\rm Ca}^{2+}$ . **B)** Membrane potential measured in parallel with a TPP $^+$  electrode. **C)** O<sub>2</sub> flux and membrane potential measured with TPP $^+$  electrode in different concentrations of  ${\rm Ca}^{2+}$ , 5 mM glutamate, 1 mM malate, 20  $\mu$ M ADP with a 2-deoxyglucosehexokinase clamp. **D)** ATP production was measured from samples acquired from the O2k chambers (2-deoxyglucosephosphate detected by NMR).

Ca<sup>2+</sup> effect over membrane potential and ATP production



**A.** Membrane potential measured with the O2k/TPP $^+$  electrode in different concentrations of glutamate and malate to reach different  $\Delta\Psi$  values, with 0, 450 and 10,000 nM free Ca $^{2+}$ . ATP production was measured from samples acquired from the O2k chambers. **B.** Slope of ATP vs.  $\Delta\Psi$ , mean  $\pm$  SE; n=11 (Ca $^{2+}$  0 and 450 nM), n=5 (10,000 nM Ca $^{2+}$ ), oneway ANOVA.

Figure 2.

Free Ca<sup>2+</sup> induced changes on mitochondrial respiration and ATP production irrespective to the changes in membrane potential and without promoting the opening of the mPTP (no decrease in  $\Delta\Psi$ )

Reference: Fink BD, Bai F, Yu L, Sivitz WI (2017) Regulation of ATP production: dependence on calcium concentration and respiratory state. Am J Physiol Cell Physiol 313(2): C146-C153.

Text slightly modified based on the recommendations of the COST Action MitoEAGLE CA15203. Doi:10.26124/mitofit:190001.v4

O2k-brief communicated by L Cardoso Oroboros Instruments



