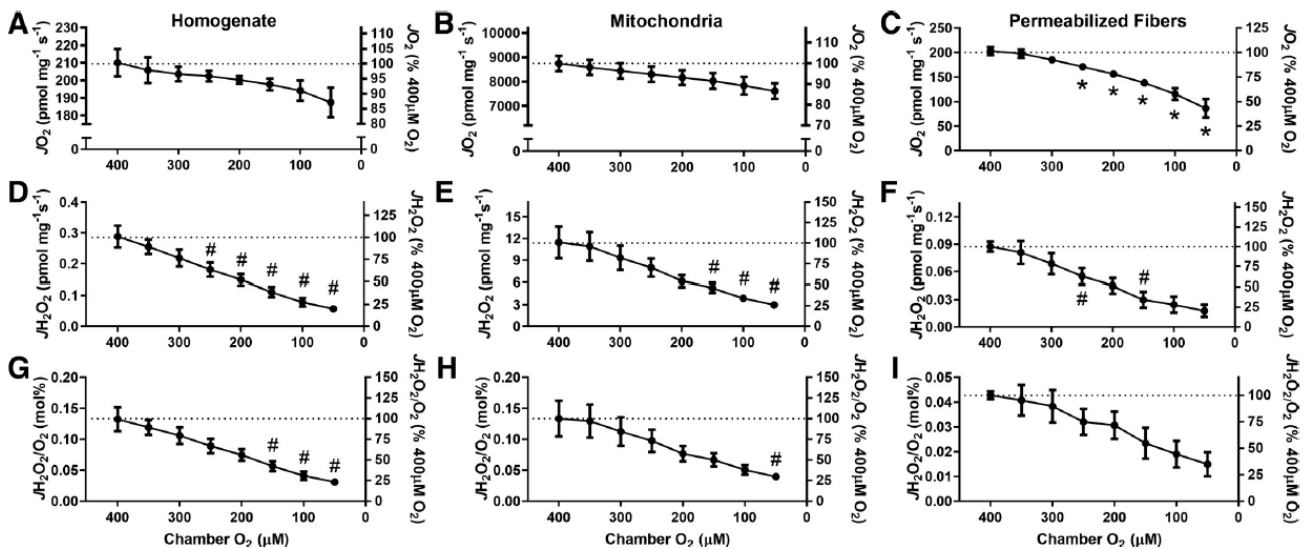


## Experimental oxygen concentration influences rates of mitochondrial hydrogen peroxide release from cardiac and skeletal muscle preparations

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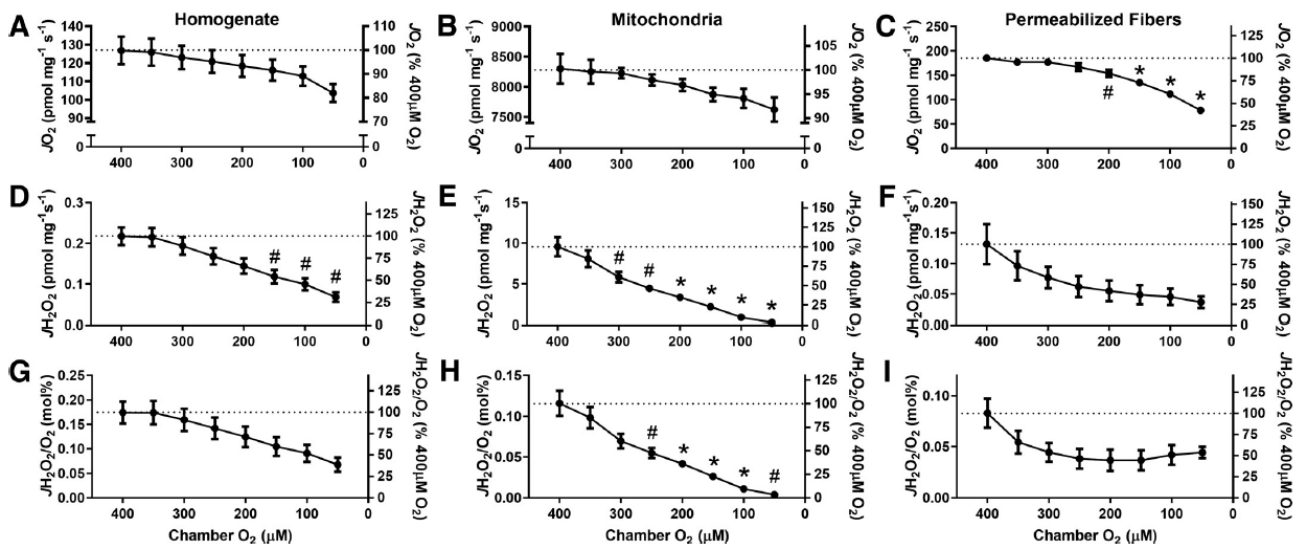


### Oxygen dependence of oxygen and hydrogen peroxide fluxes in the OXPHOS state in cardiac sample preparations



**Figure 1. Influence of chamber oxygen concentration [O<sub>2</sub>] on oxygen flux ( $J_{O_2}$ ) and hydrogen peroxide flux ( $J_{H_2O_2}$ ) in the OXPHOS state in cardiac sample preparations.**  $J_{O_2}$  of homogenates (A) and isolated mitochondria (B) declined slightly (10–15%) as chamber [O<sub>2</sub>] decreased from 400 to 50  $\mu$ M, whereas  $J_{O_2}$  of permeabilized fibers (C) decreased significantly for every 50  $\mu$ M decline in chamber [O<sub>2</sub>] below 300  $\mu$ M.  $J_{H_2O_2}$  of all sample preparations declined ~75 % as chamber [O<sub>2</sub>] decreased from 400 to 50  $\mu$ M when expressed as pmol H<sub>2</sub>O<sub>2</sub>·mg<sup>-1</sup>·s<sup>-1</sup> (D–F) or as a proportion of  $J_{O_2}$  (G–I). Data are means  $\pm$  SE ( $n = 4$ –12/group) presented as absolute flux (left y-axes) and as a percentage of the 400  $\mu$ M [O<sub>2</sub>] value (right y-axes). Statistics: unpaired  $t$  test, with \* $p < 0.05$  for  $J_{O_2}$  between 50  $\mu$ M [O<sub>2</sub>] intervals. # $p < 0.05$  for  $J_{H_2O_2}$  between 100  $\mu$ M [O<sub>2</sub>] intervals.

## Oxygen dependence of oxygen and hydrogen peroxide fluxes in the OXPHOS state in skeletal muscle sample preparations



**Figure 2. Influence of chamber oxygen concentration on oxygen flux ( $J_{O_2}$ ) and hydrogen peroxide flux ( $J_{H_2O_2}$ ) during oxidative phosphorylation (OXPHOS) in skeletal muscle sample preparations.**  $J_{O_2}$  of homogenates (A) and isolated mitochondria (B) declined slightly as chamber  $[O_2]$  decreased from 400 to 50  $\mu M$ , whereas  $J_{O_2}$  of permeabilized fibers (C) declined significantly for every 50  $\mu M$  decrease in chamber  $[O_2]$  below 250  $\mu M$ .  $J_{H_2O_2}$  of all sample preparations declined markedly as chamber  $[O_2]$  decreased from 400 to 50  $\mu M$  when expressed as pmol  $H_2O_2 \cdot mg^{-1} \cdot s^{-1}$  (D-F) or as a proportion of  $J_{O_2}$  (G-I). Data are means  $\pm$  SE ( $n = 4-12/group$ ) presented as absolute flux (left y-axes) and as a percentage of the 400- $\mu M$   $O_2$  value (right y-axes).

Statistics: unpaired Student's  $t$  test, with  $*p < 0.05$  for  $J_{O_2}$  between 50  $\mu M$   $[O_2]$  intervals. # $p < 0.05$  for  $J_{H_2O_2}$  between 100  $\mu M$   $[O_2]$  intervals.

**H<sub>2</sub>O<sub>2</sub> flux declined by ~75% from 400  $\mu M$  to 50  $\mu M$  O<sub>2</sub> in all cardiac and skeletal muscle sample preparations in NS-OXPHOS state. In contrast, decline in the O<sub>2</sub> flux was more pronounced in permeabilized fibers (~70%) and negligible in isolated mitochondria and tissue homogenate (~10-15%) in the range of 400  $\mu M$  to 50  $\mu M$  O<sub>2</sub>.**

**The linear dependence of H<sub>2</sub>O<sub>2</sub> flux on O<sub>2</sub> concentrations from 400  $\mu M$  to 50  $\mu M$  O<sub>2</sub> in all sample preparations indicates that mtROS-production sites are significantly sensitive to changes in O<sub>2</sub> concentration. Furthermore, this study demonstrates negligible O<sub>2</sub> sensitivity of O<sub>2</sub> flux in isolated mitochondria and tissue homogenate, while O<sub>2</sub> flux of permeabilized fibers declines linearly with O<sub>2</sub> concentrations below ~ 250  $\mu M$ .**

Reference: Li Puma LC, Hedges M, Heckman JM, Mathias AB, Engstrom MR, Brown AB, Chicco AJ (2020) Experimental oxygen concentration influences rates of mitochondrial hydrogen peroxide release from cardiac and skeletal muscle preparations. *Am J Physiol Regul Integr Comp Physiol* 318:R972-80.

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