



Mitochondrial Respiration with Frozen Bovine Heart Mitochondria: Diagnostic Tests

O2k-DemoExperiment - Diagnosis of Disease: Workshop on HRR at Mitochondrial Medicine 2007, UMDF, San Diego, USA, June 2007 ([IOC40](#))

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1 The O2k-Demo experiment

The following demo experiment was performed in preparation for the lecture hall experiment, presented at the Mitochondrial Medicine conference. For explanation of symbols, see [[MiPNet12.15](#)].

1. MiR06 [[MiPNet14.13](#)] in both O2k chambers; equilibrate at air saturation for air calibration of the oxygen sensor (R1; previous file; see [[MiPNet12.08](#)]), close chamber, add succinate (S, 10 mM final, 20 µl) and rotenone (Rot, 1 µM final, 1 µl). Start DatLab file.
2. Observe stability of apparent flux, as a measure of instrumental background close to air saturation [[MiPNet12.09](#)].
3. Add 10 µl of mitochondrial suspension (frozen bovine heart mitochondria = BHM, 5 mg/ml stock; 0.025 mg/ml final). Measurement of LEAK respiration, S(Rot)_N.
4. Add ADP (D, 2 mM final, 8 µl). Measurement of OXPHOS capacity, S(Rot)_D. Frozen BHM are uncoupled and do not show any stimulation and no OXPHOS capacity under these conditions.
5. Add cytochrome c (c, 10 µM final, 5 µl). Measurement of OXPHOS capacity, Sc(Rot)_D. A large cytochrome c

- effect in frozen BHM indicates injury of the outer mitochondrial membrane and loss of cytochrome c.
6. Not shown in this protocol is the simultaneous loss of NADH from frozen mitochondria, and the fact that sub-mitochondrial particles with inverted membranes may be formed. These SMPs may be (partially) re-coupled, and require external NADH for respiratory activity with Complex I substrates.
 7. Open chamber stopper by c. 1 cm, flush gas phase with a few ml of pure oxygen gas, close chamber when experimentally chosen level of oxygen is reached. Wait for c. 10 min for signal stabilization. The independence of mitochondrial respiration is thus shown extending into the high oxygen range, in contrast to respiration with permeabilized fibres which is oxygen limited even at air-saturation levels due to artificially large diffusion distances for oxygen to the mitochondria in the fibre bundles. Further levels of oxygen can be set by flushing the gas phase again with O₂ or N₂ after partial opening the chamber.
 8. Flux declines steeply during the aerobic-anoxic transition, after which the zero calibration of the oxygen sensor can be made (R0).
 9. Flux declines steeply during the aerobic-anoxic transition, after which the zero calibration of the oxygen sensor can be made (R0); see [MiPNet12.09].

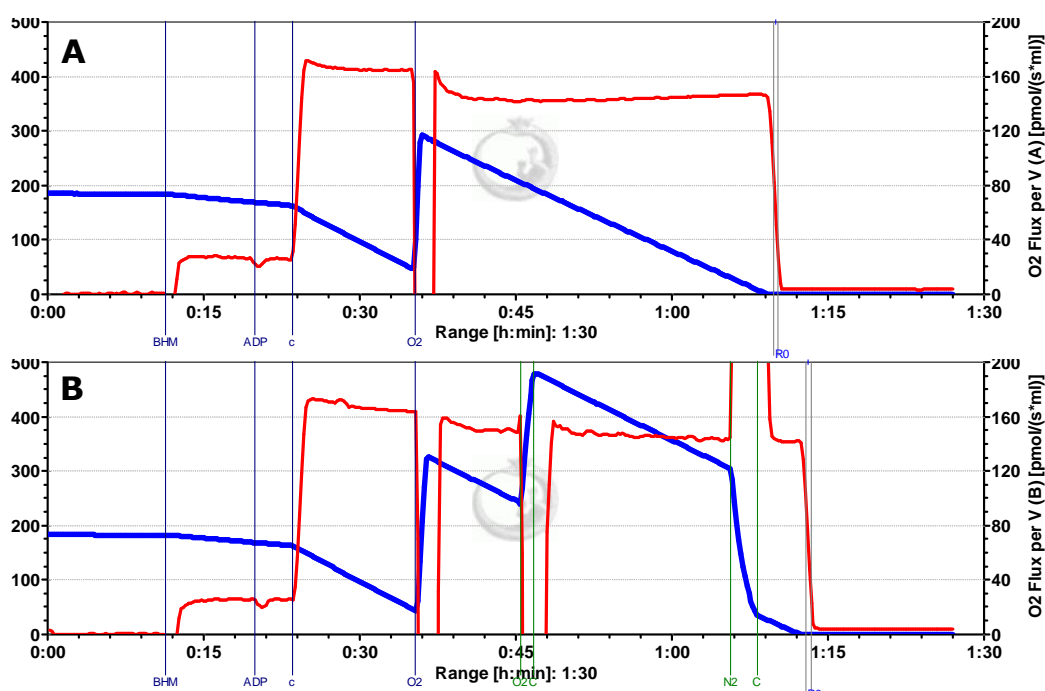


Fig. 1 Simultaneous display of oxygen concentration (blue lines) and oxygen flux (respiratory rate, red lines; negative time derivative of oxygen concentration) in chamber A and B.

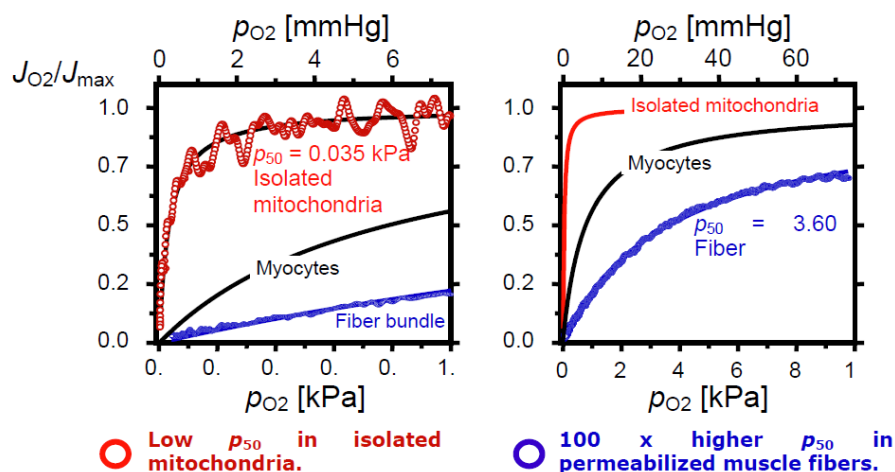


Fig.2 Hyperbolic relation of mitochondrial active respiration, J_{O_2} , and oxygen pressure, p_{O_2} , in isolated heart mitochondria at state 3; isolated cardiomyocytes and permeabilized rat skeletal muscle fibres (*M. soleus*) at state 3 (from Gnaiger 2003, Fig 6).

2 References

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O2k-Manual

MiPNet12.08
MiPNet12.09

Oxygen and pX (pH) calibration.
Oxygen flux analysis: on-line.

Protocols

MiPNet12.15
MiPNet14.13

MitoPathways: Respiratory States.
Mitochondrial respiration medium – MiR06.