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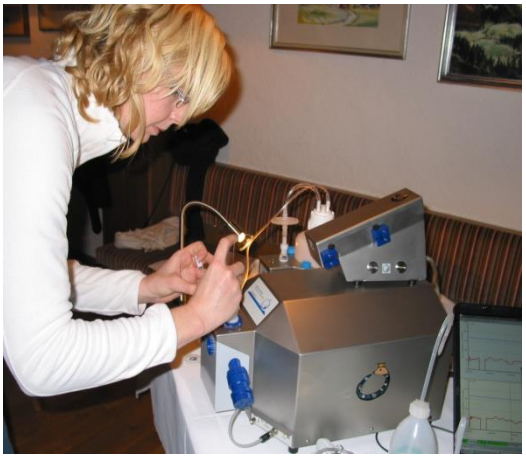

Mitochondrial respiration in permeabilized fibres: needle biopsies from horse skeletal muscle

Oxygraph-2k Workshop Protocol (IOC44),
December 2007, Schroecken, Austria.

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Section

1. Introduction

Methodological and conceptual features of High-Resolution Respirometry (HRR) are illustrated in an experiment with permeabilized fibres in the Oroboros Oxygraph-2k (O2k). A mitochondrial substrate-uncoupler-inhibitor titration (SUIT) protocol with manual titrations was applied to study physiologically relevant maximum mitochondrial respiratory capacity and coupling/pathway control. Experiments were carried out

by participants of an O2k-Workshop in December 2007 (IOC44; Schroecken, Austria; Votion et al 2012).

2. The SUIT protocol and respiratory states

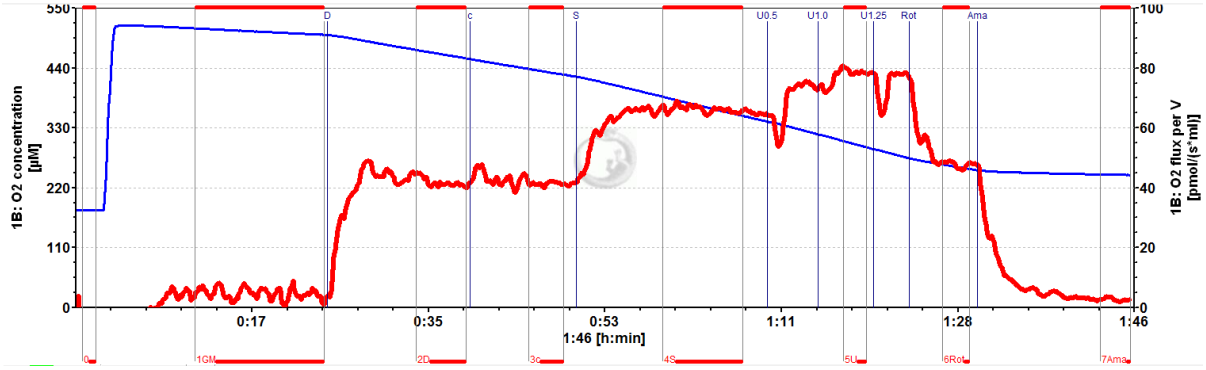


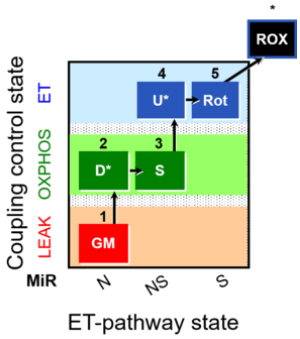
Figure 1. Oxygen concentration ([µM] blue line) and oxygen flux per mg wet weight of muscle ([pmol·s^{-1}·mg^{-1}] red line) in permeabilized fibres from horse skeletal muscle.

\3.O2k-Procedures\MiPNet12.23_FibreRespiration\ MiPNet12.23_Pfi_2007-03-21 P1-01.DLD

2.1. The O2k-Demo experiment

Permeabilized fibres from horse skeletal muscle (*Triceps branchii*) were prepared (Pesta and Gnaiger 2012) and incubated at 37 °C in the Oxygraph-2k, with 2 mL of mitochondrial respiration medium (MiR05 or MiR06 [MiPNet14.13]).

2.2. SUIT events, marks, and respiratory states



In the SUIT protocol (Fig. 1) a sequence of respiratory states is induced experimentally by stepwise titrations (Events, E). As a consequence of the titrated compounds, respiration reaches a new steady-state, and a mark (M) is set for numerical evaluation of the corresponding respiratory state.

1GM;2D;2*c*;3S;4U;5Rot;6Ama

E **1G,1M** 10 mM glutamate & 2 mM malate was added to the chambers before adding the fibres (1.5 to 2.5 mg wet weight), resting state.

M **1GM** **GM$_L$ or GM_L: LEAK state with type N substrates, N$_L$:** NADH-linked substrates glutamate&malate (type N; CI-linked pathway to Q). Non-phosphorylating resting state (LEAK state *L*; in the absence of ADP; no adenylates).

E	**2D**	After titration of 2.5 mM ADP (D), flux increases to active respiration.
M	**2D**	**GM_P or GM_P: OXPHOS capacity with type N substrates, N_P:** Respiratory capacity in the active coupled OXPHOS state (with saturating [ADP], *P*).
E	**2c**	10 µM cytochrome *c* is added as a test for the intactness of the mitochondrial outer membrane (mtOM).
M	**2c**	**GMc_P or GMc_P: Cytochrome *c* test for quality control:** Addition of cytochrome *c* yields a test for integrity of the outer mitochondrial membrane (loss of cytochrome *c* would be indicated by a stimulation of respiration).
E	**3S**	Respiration is further stimulated by succinate (10 mM).
M	**3S**	**GMS_P or GMS_P: OXPHOS capacity with type NS substrates (CI&II-linked pathway to Q), NS_P:** Respiratory stimulation by further addition of succinate (S) to type N substrates, with convergent electron flow through CI and CII at the Q-junction, in the coupled state, as an estimate of OXPHOS capacity with reconstitution of the TCA cycle (Gnaiger 2009); *P*, OXPHOS capacity of the NS-pathway.
E	**4U***	Subsequently, FCCP (uncoupler, U) is titrated in steps of 0.125 µM, to test for a possible increase of noncoupled flux compared to state GMS_P.
M	**4U***	**GMS_E or GMS_E: Electron transfer (ET) capacity with type NS substrates, NS_E:** Uncoupling by FCCP titration (avoiding inhibition by high FCCP concentrations), as a test for limitation of OXPHOS-capacity by the phosphorylation system (ANT, ATP synthase, phosphate transporter) relative to ET-capacity (ADP activated, noncoupled state, *E*, ETS capacity or the NS-pathway).
E	**5Rot**	Inhibition of CI by rotenone (0.5 µM).
M	**5Rot**	**S_E or S_E: ET capacity with type S (CII) substrate:** S-pathway ET-capacity after blocking CI with rotenone.
E	**6Ama**	Inhibition of CIII by Antimycin A (Ama; 2.5 µM) or myxothiazole.
M	**6Ama**	**Residual oxygen consumption (ROX)** due to oxidative side reactions, estimated after addition of Antimycin A (inhibitor of CIII). Respiration of uncoupled mitochondria might be inhibited slightly further by cyanide (KCN; 1 µM). ROX is subtracted from oxygen flux as a baseline for all respiratory states, to obtain mitochondrial respiration.

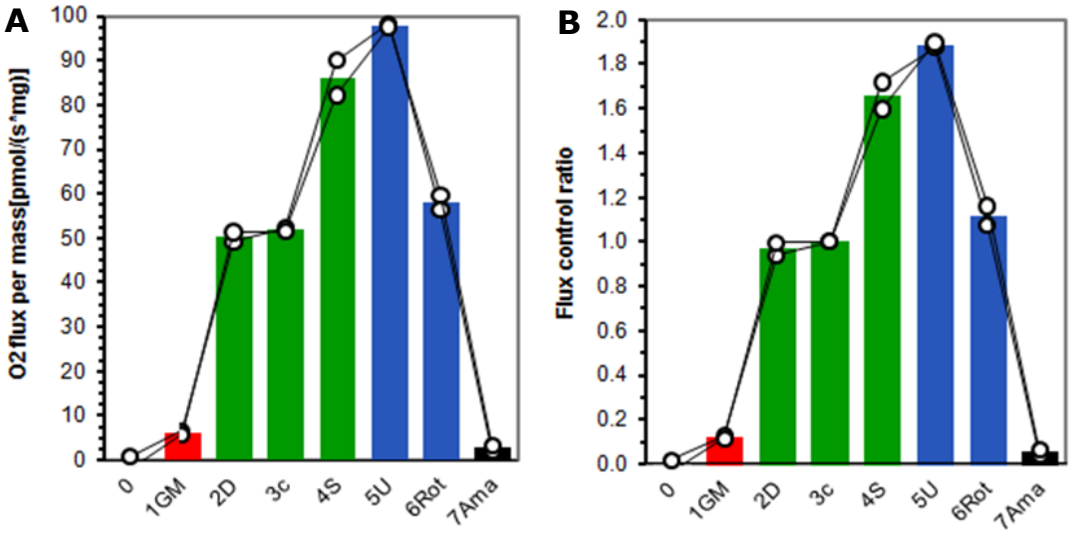


Figure 2 A: Mitochondrial O_2 flux corrected for ROX. **B:** Flux control ratios normalized to ETS capacity.

Excel demo file:
\3.O2k-Procedures\MiPNet12.23_FibreRespiration\
MiPNet12.23_Pfi_O2k-Analysis.xls

3. References

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. Int J Biochem Cell Biol 41:1837–45. »Biolast link«

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. Methods Mol Biol 810:25-58. »Bioblast link«

Votion DM, Gnaiger E, Lemieux H, Mouithys-Mickalad A, Serteyn D (2012) Physical fitness and mitochondrial respiratory capacity in horse skeletal muscle. PLoS ONE 7(4):e34890. »Bioblast link«