

SUIT-011(01):1-4 (2019)

Version 01: 2019-01-11 ©2019 Oroboros
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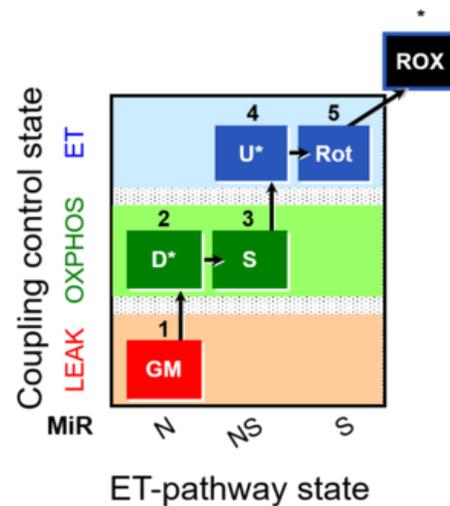
SUIT-011

SUIT-category: NS(GM)

Mitochondrial preparations, mt

- Physiologically relevant maximum mitochondrial respiratory capacity,
- Additivity at the Q-junction,
- Coupling/pathway control.

Experimental time: 1.5 to 2 h



1. The O2k-Demo experiment

Permeabilized fibres from horse skeletal muscle (*Triceps branchii*; 1.5 to 2.5 mg wet weight) were prepared (Pesta and Gnaiger 2012) and incubated at 37 °C in the O2k with 2 mL MiR05.

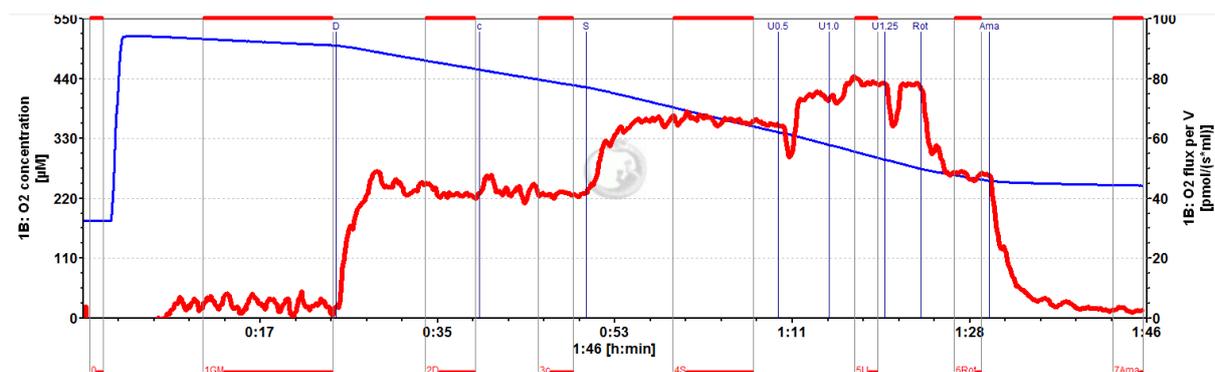


Figure 1. SUIT-011: Oxygen concentration ($[\mu\text{M}]$ blue line) and oxygen flux per mg wet weight of muscle ($[\text{pmol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}]$ red line) in permeabilized fibres from horse skeletal muscle (from Lemieux et al 2019).

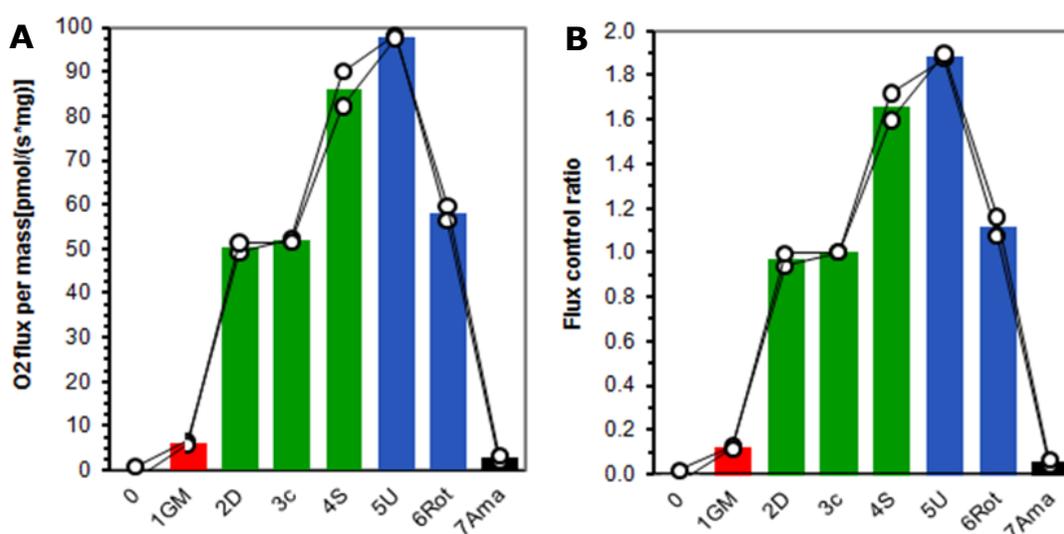


Figure 2 A: Mitochondrial O₂ flux corrected for Rox. **B:** Flux control ratios normalized to ET-capacity.

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;2c;3S;4U;5Rot;6Ama

- | | | |
|---|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E | 1G,1M | 10 mM glutamate & 2 mM malate. |
| M | 1GM | GM_L or GM_L: N-LEAK respiration, N_L
NADH-linked substrates glutamate&malate (type N; CI-linked pathway to Q). Non-phosphorylating resting state (LEAK state); L _n in the absence of ADP, ATP, AMP (no adenylates). |
| E | 2D | After titration of a saturating concentration of ADP (D), flux increases to active respiration. |
| M | 2D | GM_P or GM_P: N-OXPHOS capacity, N_P
OXPHOS capacity, P (with saturating [ADP]), with NADH-linked substrates glutamate&malate. |
| E | 2c | 10 μM cytochrome c is added as a test for the intactness of the mitochondrial outer membrane (mtOM). |
| M | 2c | GM_{cP} or GM_{cP}: Cytochrome c test for quality control
Addition of cytochrome c yields a test for integrity of the mtOM. Stimulation by added cytochrome c would indicate an injury of the mtOM and limitation of |

		respiration in state GM_P due to loss of cytochrome <i>c</i> (P , OXPHOS capacity with type N substrates). Cytochrome <i>c</i> is added immediately after the earliest ADP-activation step.
E	3S	Respiration is further stimulated by succinate, S .
M	3S	GMS_P or GMS_P: NS-OXPHOS capacity, NS_P Respiratory stimulation by further addition of succinate, S , to type N substrates, with convergent electron flow in the NS-pathway (CI&II-linked pathway to the Q-junction) for reconstitution of TCA cycle function, in the coupled state as an estimate of OXPHOS-capacity, P (Gnaiger 2009).
E	4U*	An uncoupler, U , is titrated stepwise to test for a possible increase of noncoupled flux compared to state GMS_P .
M	4U*	GMS_E or GMS_E: NS-ET capacity, NS_E Uncoupler titration (avoiding inhibition by high uncoupler concentrations) to obtain electron transfer (ET) capacity (noncoupled ET-state), as a test for limitation of OXPHOS-capacity by the phosphorylation system (ANT, ATP synthase, phosphate transporter) relative to ET-capacity, E .
E	5Rot	Inhibition of CI by rotenone.
M	5Rot	S_E or S_E: S-ET capacity S-pathway ET-capacity after blocking CI with rotenone.
E	6Ama	Inhibition of CIII by Antimycin A or myxothiazole.
M	6Ama	ROX: residual oxygen consumption Rox is due to oxidative side reactions, estimated after addition of Antimycin A (inhibitor of CIII). Rox is subtracted from oxygen flux as a baseline for all respiratory states, to obtain mitochondrial respiration (mt).
*		SUIT extension: CIV activity.

3. Strengths and limitations

- * A succinate concentration of >10 mM may be required for saturating S_E capacity.
- * Rox might be inhibited slightly further by inhibition of CIV by cyanide (KCN; 1 μ M). But cyanide inhibits not only CIV, but also catalase and other oxygenases involved in ROX.
- + NS-OXPHOS capacity provides a physiologically relevant estimate of maximum mitochondrial respiratory capacity.
- + Comparison of GM- with PM-capacity yields important information on N-pathway respiratory control upstream of CI (Lemeux et al 2017; Votion et al 2012).

- + Glutamate is easier to prepare compared to pyruvate.
- + Application of the cytochrome c test early in the protocol ensures comparability of all states in case of any effect of "c".
- + Reasonable duration of the experiment.

- GM and PM yield typically identical fluxes in human skeletal muscle fibres. However, PM is the superior alternative to GM: the fraction of the N-pathway is lower and of the S-pathway is higher with GM compared to PM (GM_P is inhibited by the CII inhibitor malonic acid to a larger extent than PM_P). PM, therefore, yields a more sensitive assay for the diagnosis of injuries in the N-pathway, since an impairment of N-pathway capacity can be compensated partially by activation of the S-pathway. This is a disadvantage compared to SUIT-004 and SUIT-008 for diagnosis of N-capacity.
- To detect an additive effect of P after GM_P , pyruvate would have to be added as step 3 (before S). However, inhibition of respiration was observed after titration of P (5 mM) in horse skeletal muscle fibres (Votion et al 2012), which was not the case when P was titrated in steps of 1 mM.
- When evaluating the additive effect of the N- and S-pathway, it has to be considered that NS_{P-} and NS_{E-} capacities can only be compared with N_{P-} and S_{E-} capacities. This is not a problem when $NS_P = NS_E$ (Gnaiger 2009). Otherwise, it may be assumed that $S_P = S_E$ (Votion et al 2012), such that NS_P can be compared with $N_P + S_P$. SUIT-004 should be chosen for the additive effect in the ET-state.
- *Rox* may be lower in substrate states earlier in the SUIT protocol. Therefore, this *Rox* measurement is frequently taken as a methodological control rather than as the final basis of *Rox* correction of mitochondrial respiration (mt).
- Careful washing is required after the experiment to avoid carry-over of inhibitors and uncoupler.
- CIV activity is not measured, to save experimental time.

4. References

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