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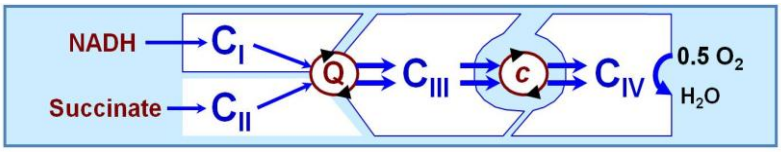
MitoPathways at the Q-junction: mouse skeletal muscle fibres.

O2k-Workshop Report, IOC39, Schroecken, Austria.



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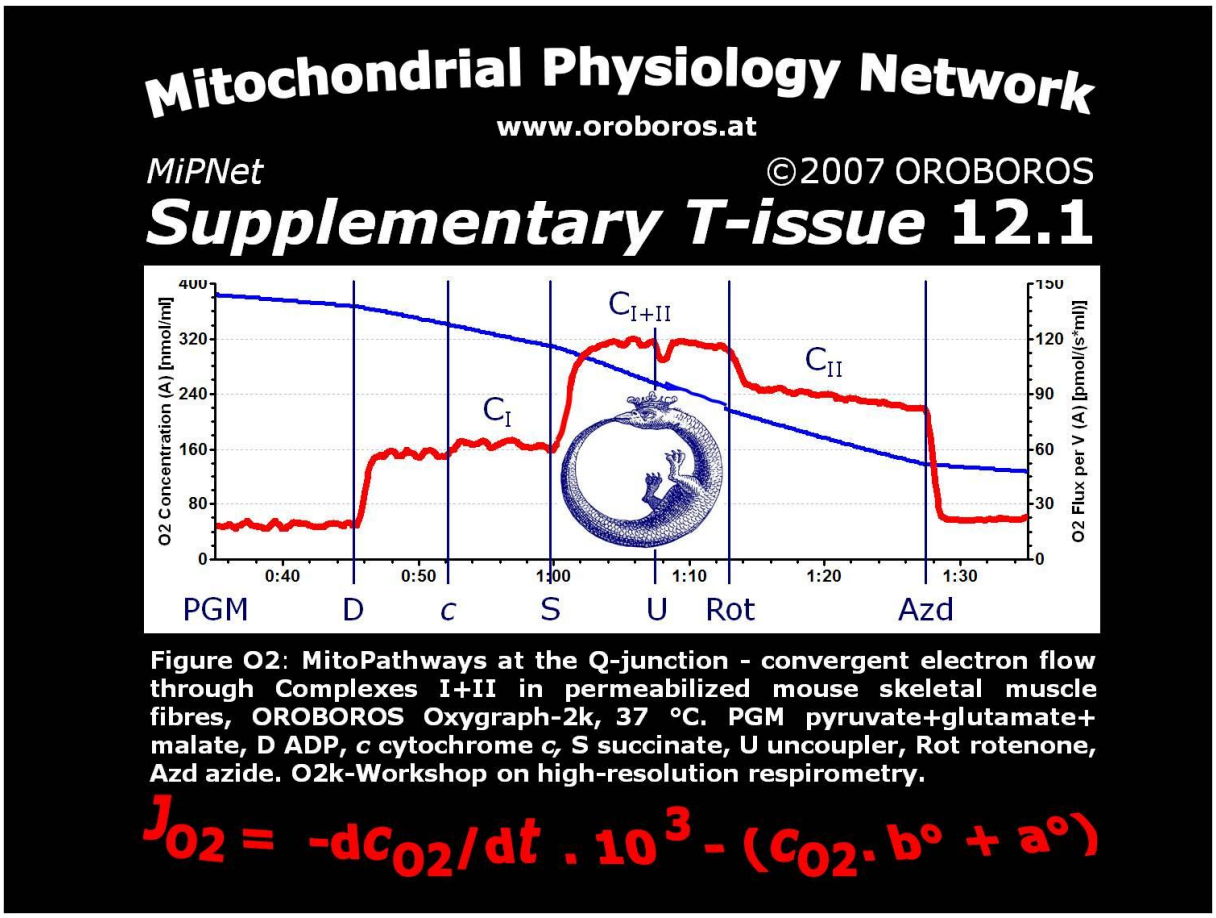
High-resolution respirometry with a SUIT protocol[1] for OXPHOS analysis[2] is presented as supplementary ***T-issue*** (OROBOROS T-shirt).

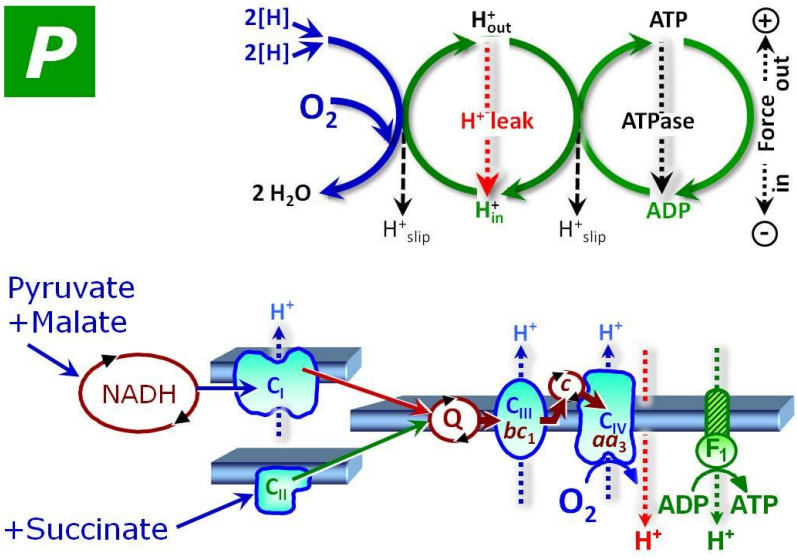
1. The SUIT protocol

Pyruvate+glutamate+malate (PGM) were used in combination to induce C_I-linked LEAK respiration in permeabilized mouse skeletal muscle (IOC39; Fig. O2).[3,4] Saturating ADP (D; 2.5 mM final concentration) stimulated respiration to the level of OXPHOS capacity (*P* state), with a small effect of 10 µM cytochrome c (*c*), expressed as the cytochrome *c* control factor (FCF_c<0.01; indicating integrity of the outer mt-membrane). Without correction for residual oxygen consumption (ROX), the biochemical coupling efficiency, (*P-L*)/*P*, was 0.68 (RCR=3.1). Addition of succinate (S) stimulated respiration by convergent e-input through the Q-junction. The corresponding succinate control factor was (C_{I+II}-C_I)/C_{I+II}=0.47, i.e. succinate increased respiration by 47%. C_{I+II} OXPHOS capacity was not stimulated further by uncoupler titration (U). Therefore, the capacity of the phosphorylation system matched the ETS capacity (*E* state). At *E*=*P* the *E-P* coupling control factor is zero, indicating that there is no ETS excess capacity over *P*, in striking contrast to human skeletal and cardiac muscle mitochondria.[1,5,6] Inhibition of C_I by rotenone (Rot) inhibited respiration to the level of C_{II}-linked ETS capacity. The corresponding C_I-control factor is (C_{I+II}-C_{II})/C_{I+II}=0.25. C_{II}- was higher than C_I-linked respiratory capacity (*E*=*P*). C_{I+II}-linked respiratory capacity was higher than respiration with any single e-input substrate state,

indicating an additive effect at the Q-junction. However, since $C_{I+II} < C_I + C_{II}$, the additive effect was incomplete, which indicates that any electron channelling through supercomplexes to C_{IV} was incomplete. Addition of azide (Azd; 10 mM) inhibited respiration to the level of residual oxygen consumption (ROX). ROX was 0.18 of C_{I+II}-linked respiratory capacity.



OXPHOS capacity: saturating [ADP]



http://wiki.oroboros.at/index.php/OXPHOS_capacity

2. Limitations of the SUIT protocol

2.1. Maximum OXPHOS and ETS capacity

Evaluation of maximum respiratory capacities requires titration of further substrates activating additional respiratory complexes at the Q-junction (C_{ETF} and C_{GpDH}).

2.2. Malate concentration

The malate concentration was 2 mM, to saturate C_I-linked respiration. However, at 2 mM malate, the fumarate concentration is increased to a level which inhibits succinate dehydrogenase. Then C_{I+II}- and C_{II}-linked respiratory capacities are underestimated. A malate concentration of 0.5 mM, which saturates C_I-linked respiration and inhibits C_{II}-linked respiration to a lesser extent, represents and improved standard. »Optimum malate concentration in SUIT protocols

2.3. ROX correction

The fact that ROX was higher in the C_{I+II} substrate state compared to C_I-linked LEAK respiration indicates that ROX is partially controlled by the substrate state. Therefore, a single measurement of ROX cannot be applied for correction of total oxygen consumption in the different substrate states. Total respiration, therefore, represents apparent coupling states *L´*, *P´* and *E´* (Fig. 1). ROX correction is possible in the present experiment only for C_{I+II}- and C_{II}-linked respiration. Azide inhibits not only C_{IV} but other heme-based oxidases and peroxidases, and therefore may interfere with ROX beyond blocking respiratory electron transfer. Based on this argument, a combination of C_{II}- and C_{III}-inhibitors (malonic acid, antimycin A, myxothiazol) may yield more consistent results, although any ROS scavenged by cytochrome *c* may in the absence of a C_{IV}-inhibitor result in respiratory oxygen consumption through C_{IV}.

3. References

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» Product: OROBOROS Oxygraph-2k, O2k-Catalogue

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