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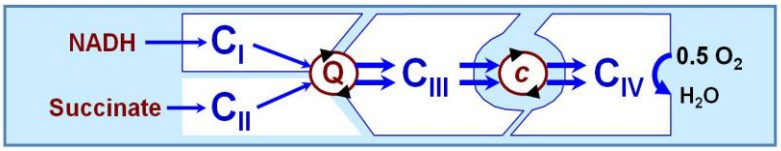
MitoPathways at the Q-junction: mouse skeletal muscle fibres.

O2k-Workshop Report, IOC39, Schroecken, Austria.



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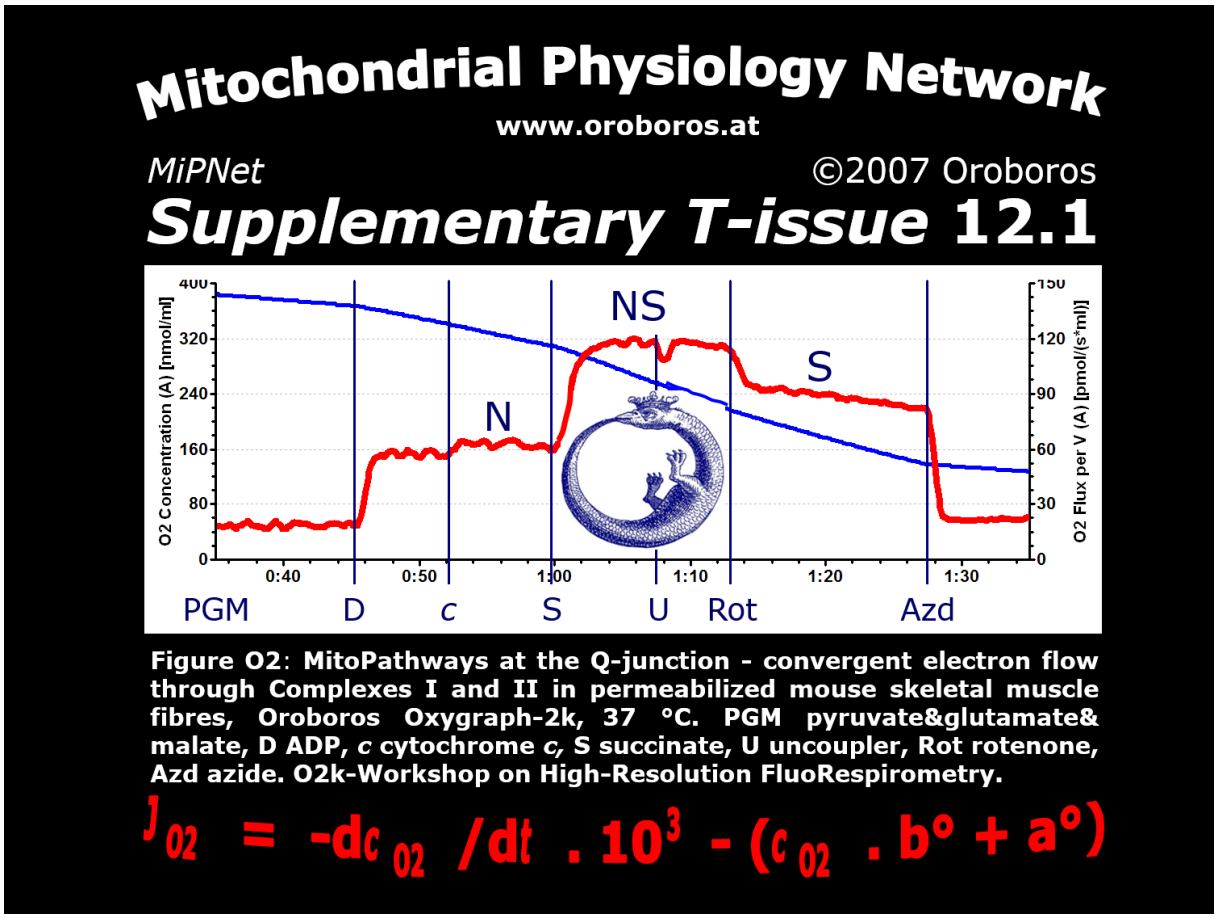
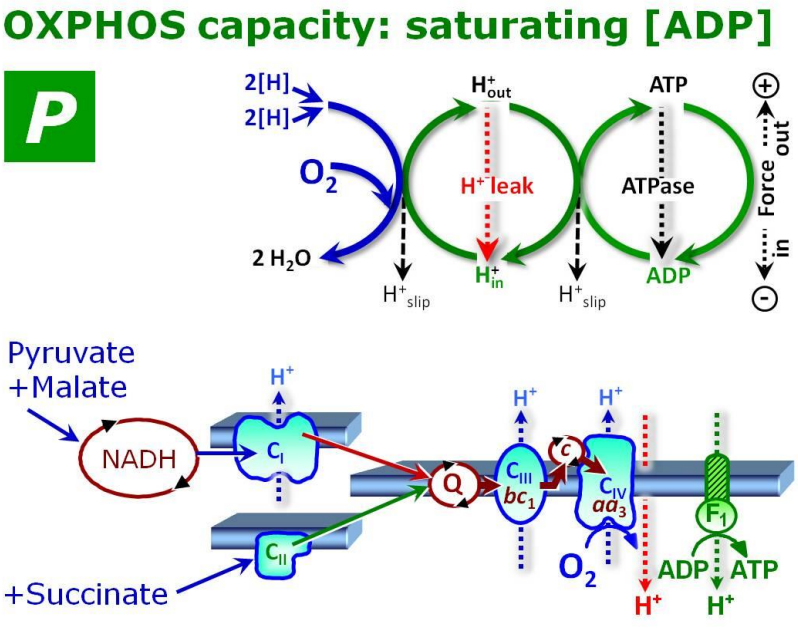
High-Resolution FluoRespirometry with a SUIT protocol[1] for OXPHOS analysis[2] is presented as supplementary ***T-issue*** (Oroboros T-shirt).

1. The SUIT protocol

Pyruvate&glutamate&malate (PGM) were used in combination to induce N-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 mt-outer membrane, mtOM). 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 (NS-N)/NS=0.47, *i.e.*, succinate increased respiration by 47%. NS-OXPHOS capacity was not stimulated further by uncoupler titration (U). Therefore, the capacity of the phosphorylation system matched the ET-capacity (*E* state). At *E=P* the *E-P* coupling control factor is zero, indicating that there is no ET excess capacity over *P*, in striking contrast to human skeletal and cardiac muscle mitochondria.[1,5,6] Inhibition of CI by rotenone (Rot) inhibited respiration to the level of S-linked ET-capacity. The corresponding N-control factor is (NS-S)/NS=0.25. S- was higher than N-linked respiratory capacity (*E=P*). NS-linked respiratory capacity was higher than respiration with any single e-input substrate state, indicating an additive effect at the Q-junction. However, since NS < N+S, the additive effect was incomplete, which indicates that any electron channelling through supercomplexes to

CIV was incomplete. Addition of azide (Azd; 10 mM) inhibited respiration to the level of residual oxygen consumption (ROX). ROX was 0.18 of NS-linked ET-capacity.





www.bioblast/index.php/OXPHOS_capacity

2. Limitations of the SUIT protocol

2.1. Maximum OXPHOS- and ET-capacity

Evaluation of maximum respiratory capacities requires titration of further substrates activating additional respiratory complexes at the Q-junction (CETF and CGpDH).

2.2. Malate concentration

The malate concentration was 2 mM, to saturate N-linked respiration. However, at 2 mM malate, the fumarate concentration is increased to a level which inhibits succinate dehydrogenase. Then NS- and S-linked respiratory capacities are underestimated. A malate concentration of 0.5 mM, which saturates N-linked respiration and inhibits S-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 NS substrate state compared to N-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 NS- and S-linked respiration. Azide inhibits not only CIV 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 CII- and CIII-inhibitors (malonic acid, antimycin A, myxothiazol) may yield more consistent results, although any ROS scavenged by cytochrome *c* may in the absence of a CIV-inhibitor result in respiratory oxygen consumption through CIV.

3. References

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