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FEBS PRESS science publishing by scientists

Oxidative phosphorylation and mitochondrial function differ between human prostate tissue and cultured cells

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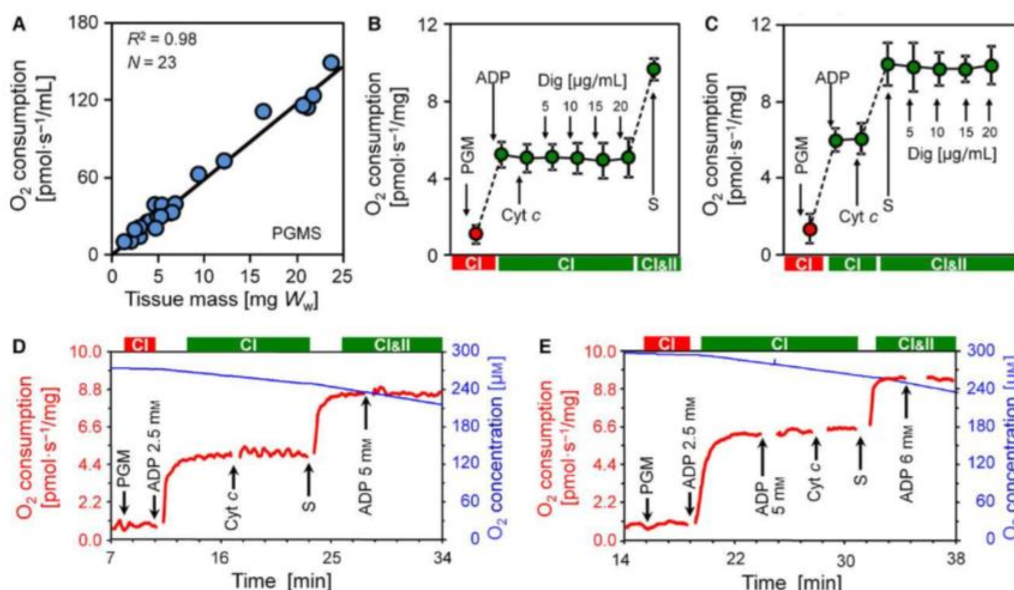
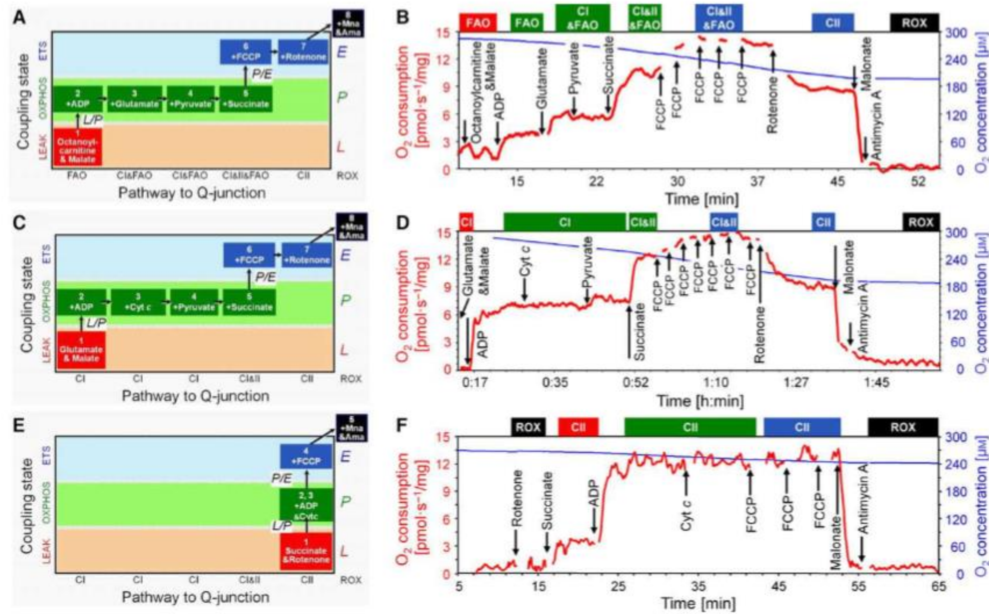
Analysis protocols for investigating mitochondrial metabolic pathways and different segments of the electron transfer system in permeabilized prostate tissue biopsies.

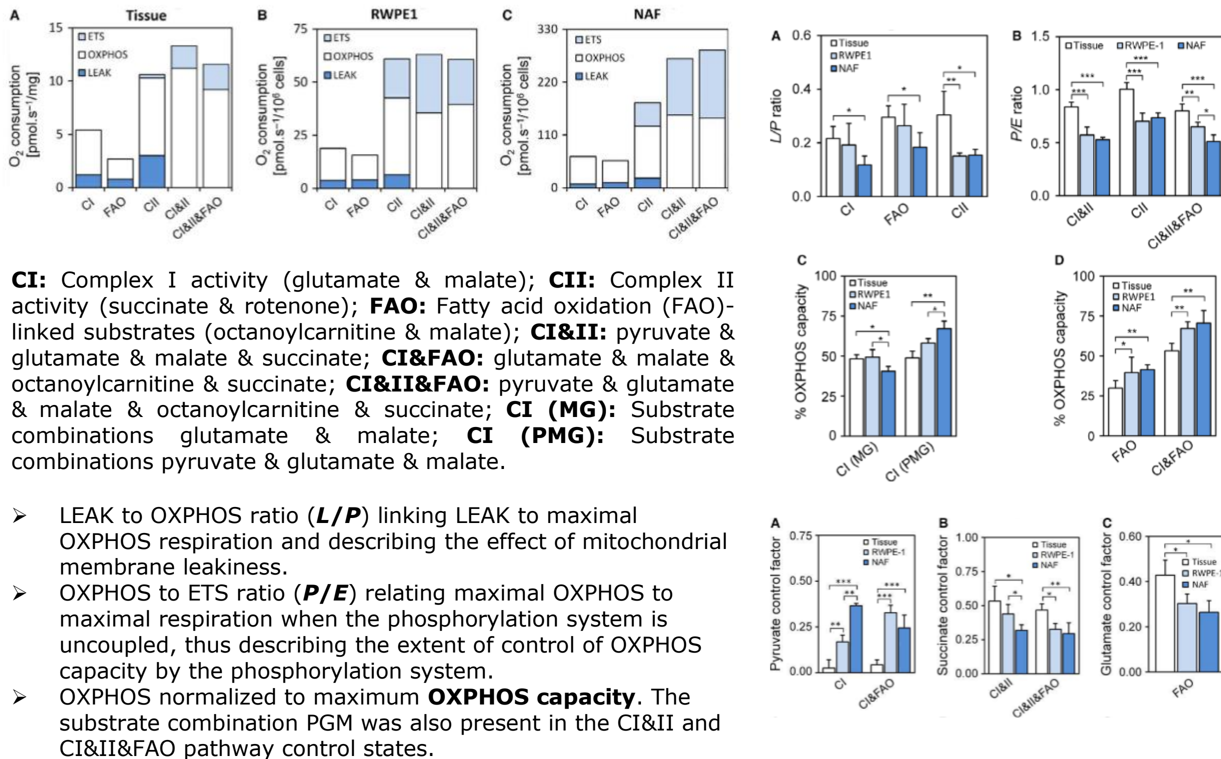


Conditions and quality controls. (A) Volume-specific oxygen consumption as a directly proportional function of tissue mass (wetweight, Ww). Values represent single experiments on ETS capacity with pyruvate & glutamate & malate & succinate; PGMS). **(B)**, **(C)** Stepwise titration of digitonin (Dig) induced no increase in respiration with either CI-linked (panel B) or CI&II-linked substrates (panel C), indicating that mechanical plasma membrane permeabilization was complete. **(D)**, **(E)** Tissue mass-specific oxygen consumption and ADP titration in mechanically permeabilized prostate tissue. A cytochrome c test resulted in no increase in respiration, demonstrating intactness of the outer mt-membrane after mechanical permeabilization.



Coupling/pathway control diagrams (left panels) indicate the coupling states LEAK (read), OXPHOS (green) and ETS (blue), and residual oxygen consumption (ROX, black). Representative respirometric traces (right panels) show oxygen consumption (red plots) calculated as the negative slope of oxygen concentration (blue plots), corrected for instrumental background oxygen flux and normalized for tissue mass.
(A), **(B)** Protocol for analysis of fatty acid ß-oxidation (FAO), and convergent pathways from Complex I and FAO (CI&FAO) or Complex I, II, and FAO (CI&II&FAO) to the Q-junction. **(C)**, **(D)** Analysis of CI and CI&II. **(E)**, **(F)** CII-linked respiration with succinate & rotenone, without the other substrates present in protocols 1 and 2. The same protocols were used in cell lines.

Respiratory capacities of tissue biopsies and cell lines related to internal reference states for direct comparison of tissue and cell lines.



CI: Complex I activity (glutamate & malate); **CII:** Complex II activity (succinate & rotenone); **FAO:** Fatty acid oxidation (FAO)-linked substrates (octanoylcarnitine & malate); **CI&II:** pyruvate & glutamate & malate & succinate; **CI&FAO:** glutamate & malate & octanoylcarnitine & succinate; **CI&II&FAO:** pyruvate & glutamate & malate & octanoylcarnitine & succinate; **CI (MG):** Substrate combinations glutamate & malate; **CI (PMG):** Substrate combinations pyruvate & glutamate & malate.

- LEAK to OXPHOS ratio (***L*/*P***) linking LEAK to maximal OXPHOS respiration and describing the effect of mitochondrial membrane leakiness.
- OXPHOS to ETS ratio (***P*/*E***) relating maximal OXPHOS to maximal respiration when the phosphorylation system is uncoupled, thus describing the extent of control of OXPHOS capacity by the phosphorylation system.
- OXPHOS normalized to maximum **OXPHOS capacity**. The substrate combination PGM was also present in the CI&II and CI&II&FAO pathway control states.

Reference: Schöpf B, Schäfer G, Weber A, Talasz H, Eder IE, Klocker H, Gnaiger E (2016) Oxidative phosphorylation and mitochondrial function differ between human prostate tissue and cultured cells. FEBS J 283:2181-96.