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

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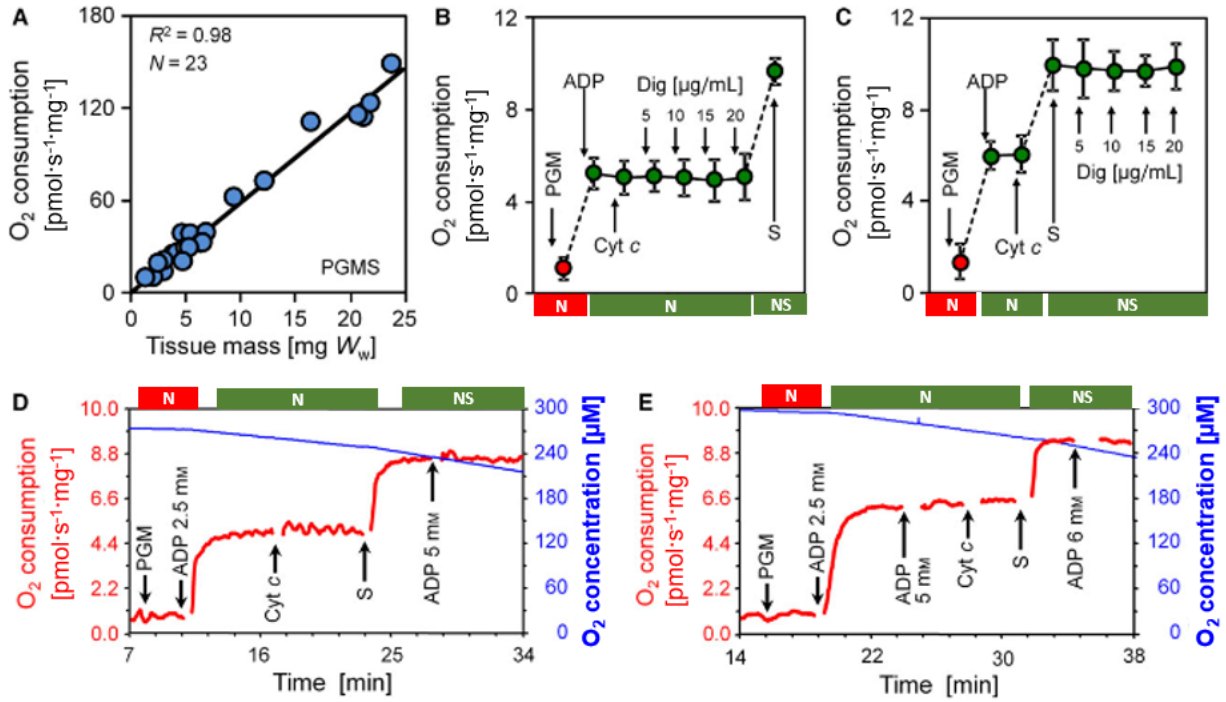
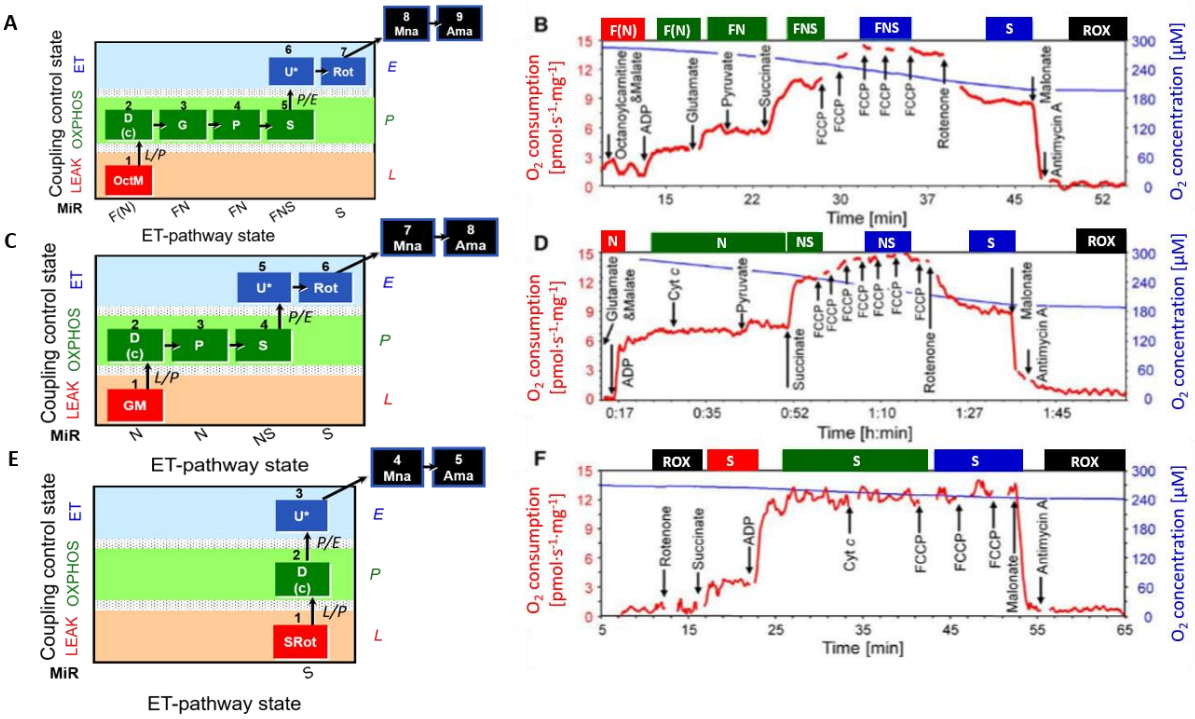
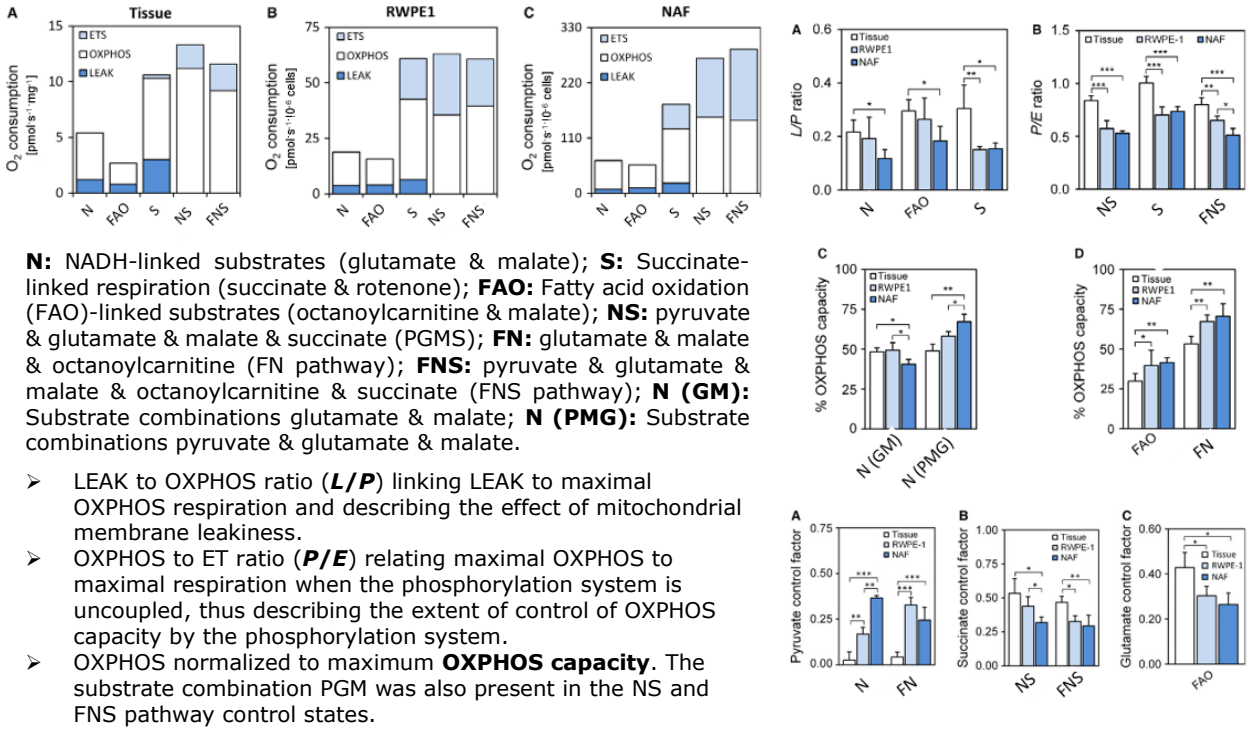


Conditions and quality controls. (A) Volume-specific oxygen consumption as a directly proportional function of tissue mass (wet weight, W_w). Values represent single experiments on electron transfer (ET) capacity with Pyruvate & Glutamate & Malate & Succinate; PGMS). **(B)**, **(C)** Stepwise titration of digitonin (Dig) induced no increase in respiration with either NADH-linked (panel B) or NADH and Succinate-linked substrates (panel C), indicating that mechanical plasma membrane permeabilization was complete. **(D)**, **(E)** 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 mitochondrial outer membrane after mechanical permeabilization.



Coupling/pathway control diagrams (left panels) indicate the coupling states LEAK (read), OXPHOS (green) and ET (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 NADH-linked substrates through Complex I and from FAO (FN-pathway) or through Complex I, II, and FAO (FNS-pathway) to the Q-junction. **(C)**, **(D)** Analysis of N-pathway and NS-pathway. **(E)**, **(F)** Succinate-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.



N: NADH-linked substrates (glutamate & malate); **S:** Succinate-linked respiration (succinate & rotenone); **FAO:** Fatty acid oxidation (FAO)-linked substrates (octanoylcarnitine & malate); **NS:** pyruvate & glutamate & malate & succinate (PGMS); **FN:** glutamate & malate & octanoylcarnitine (FN pathway); **FNS:** pyruvate & glutamate & malate & octanoylcarnitine & succinate (FNS pathway); **N (GM):** Substrate combinations glutamate & malate; **N (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 ET 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 NS and FNS 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.