pH-Induced Regulation of Cardiac Mitochondrial Respiration in Naked Mole-Rats (*Heterocephalus glaber*)

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Introduction

1. The energy demands of hypoxia/exercise/cancer favour anaerobic glycolysis and lactate buildup. ATP hydrolysis from these conditions or lactate itself, from lactic acid, results in acidification of cellular pH (Robergs et al., 2004). This pH change inhibits healthy cell processes, including metabolism, if beyond their buffering capacity.

2. Naked mole-rats (**NMRs**) appear to tolerate these conditions and their accompanying pH change. NMRs live in variable and intermittently **hypoxic burrows** while **exercising**/working, and they are **resistant to cancer** (Tian et al., 2013). NMRs putatively upregulate anaerobic glycolysis during hypoxia as well (Pamenter et al., 2019).

3. Mitochondrial respiration is a hub for adaptive plasticity and resistance in NMRs. The link between pH change and mitochondrial respiration remains incompletely understood.

Hypothesis and Objectives

Hypothesis: pH regulates mitochondrial respiration to maintain metabolic function in NMRs.

Objectives

1. Explore the pH range of a possibly potent buffering capacity of NMRs.
2. Determine the effect of pH at different levels of mitochondrial respiration across the pH range.
3. Compare the range of pH modulation and mitochondrial sensitivity with mice.

Materials and Methods

Ethics: The study was approved by the University of Ottawa Animal Care committee. Animals were housed according to approved Animal Care protocols and in CCAC guidelines.

Respiration media: A standard respiration medium (MiR05) was used to contain cardiac fiber samples. To test different pH, separate batches of MiR05 were calibrated to pH 6.6, 6.9, 7.1 (baseline), 7.3, or 7.6 at physiological temperature (32°C for NMRs and 37°C for mice).

Permeabilized cardiac muscle fiber: Animals were rapidly euthanized with cervical dislocation and immediate decapitation. The heart was excised and cardiac muscle fibers from the left ventricle were permeabilized using saponin (50 µg ml^{-1}) to access mitochondria.

Substrate-uncoupler-inhibitor-titration (SUIT) protocol: A single protocol was repeated using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments). Samples were weighed (wet weight) prior to placement in the respirometer. Different substrates, inhibitors and uncouplers of mitochondrial electron transport system (ETS) enzymes were used to test respiration pathways.

Statistics: Data were analyzed using two-way ANOVA ($p < 0.05$) followed by Bonferroni testing. Data are mean ± SEM from n = 10 for NMRs and n = 7-9 for mice.

SUIT protocol sequence	Respiration level
Malate (M) and Pyruvate (P)	TCA stimulated and complex I fueled (CI-CIV)
ADP (D)	**CI-CIV** maximally stimulated
Rotenone (ROT)	Complex I inhibited
Succinate (S)	**CII-CIV** maximally stimulated
Antimycin A (AMA)	Complex III inhibited
Ascorbate (ASC)/TMPD	**CIV** maximally stimulated
CCCP	Uncoupled (**total ETS capacity**)

Figure 1. pH modulates respiration primarily at complex IV and uncoupled respiration

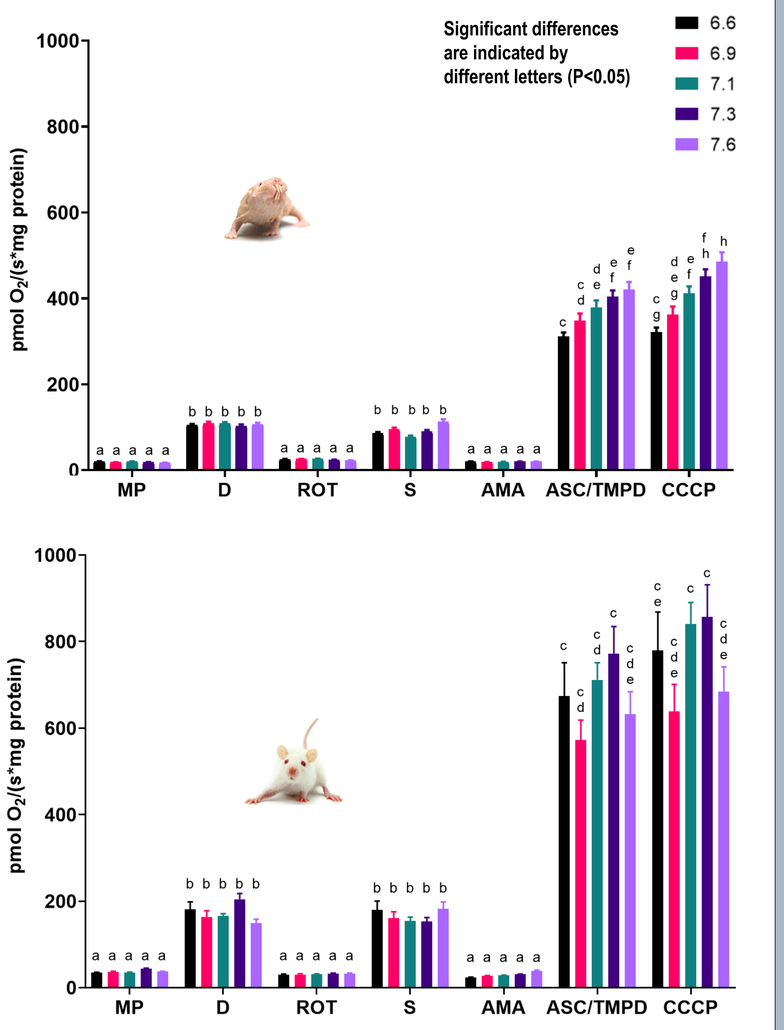


Figure 2. Normalizing to uncoupled respiration, complex IV regulation may be an underlying mechanism in both animals

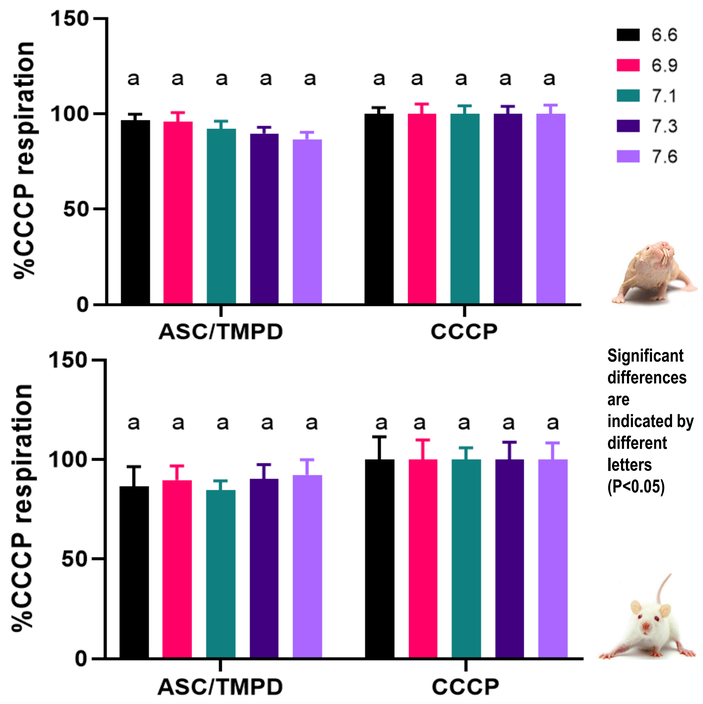


Figure 3. NMRs demonstrate a more robust tolerance to the effects of pH change than mice

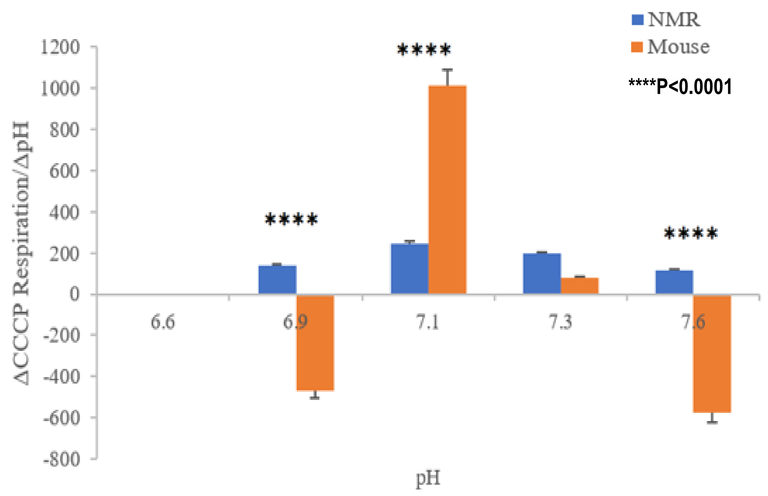
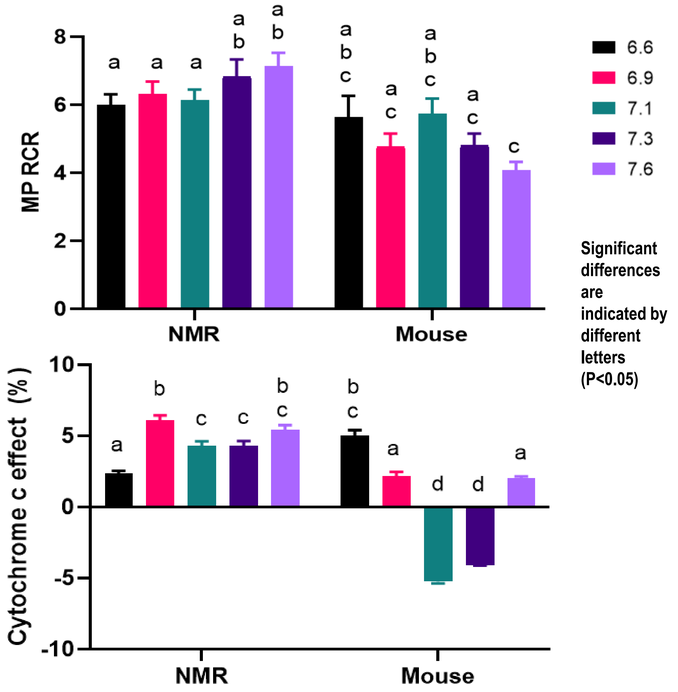


Figure 4. pH has a limited effect on coupling efficiency and membrane integrity



Conclusions

1. pH modulates mitochondrial respiration primarily at complex IV respiration and total ETS capacity, which are commonly modulated in other hypoxia-tolerant species and tissues.
2. NMR cardiac mitochondria appear to be less sensitive to inhibitory effects of pH compared to mice.
3. Functional changes in mitochondria mediate a unique adaptation to acidity associated with metabolic stressors including hypoxia, cancer, and intense exercise.

References

- Pamenter, M. E., Dzal, Y. A., Thompson, W. A., & Milsom, W. K. (2019). Do naked mole rats accumulate a metabolic acidosis or an oxygen debt in severe hypoxia? *Journal of Experimental Biology*, 222(3).
- Robergs, R. A., Ghiasvand, F., & Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 287(3 56-3), 502–516.
- Tian, X., Azpurua, J., Hine, C., Vaidya, A., Myakishev-Rempel, M., Ablaeva, J., Mao, Z., Nevo, E., Gorbunova, V., & Seluanov, A. (2013). High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature*, 499(7458), 346–349.

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