The Effect of Anoxia on Mitochondrial Performance in a Hibernator (*Ictidomys tridecemlineatus*)

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THE THIRTEEN-LINED GROUND SQUIRREL

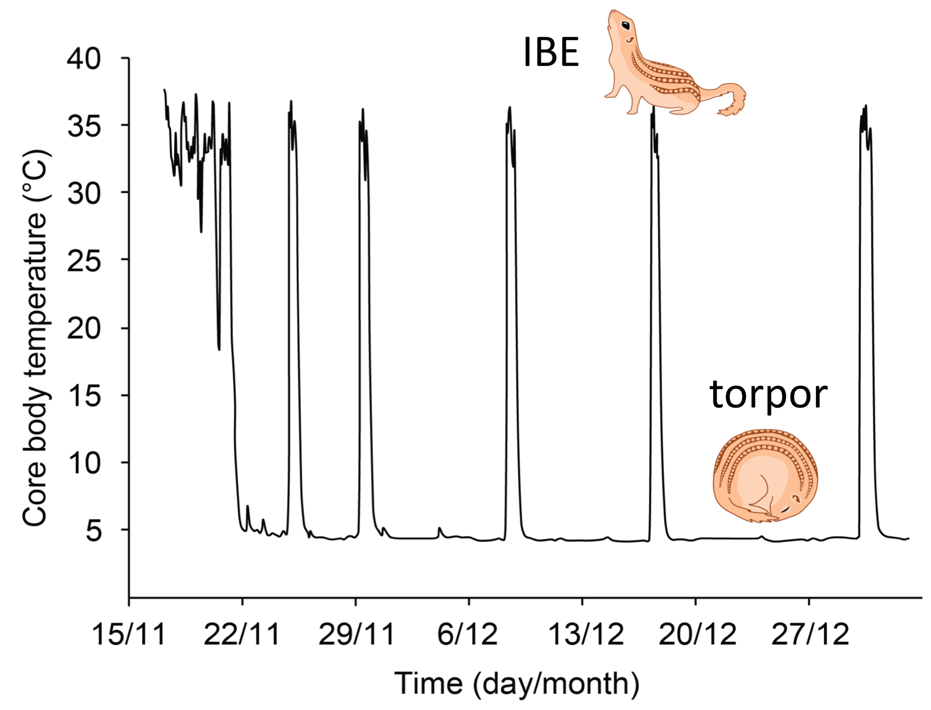


Figure 1. Body temperature (T_b) fluctuations of a thirteen-lined ground squirrel during the hibernation season. Ground squirrels spend most of the hibernation season in torpor—a state of reduced T_b (and metabolic rate). Between these torpor bouts, spontaneous arousals increase T_b and metabolic rate rapidly to euthermic levels. These periods of interbout euthermia (IBE) are maintained for several hours.

Figure 2. Geographic range of the thirteen-lined ground squirrel (*I. tridecemlineatus*). The range of the 13-lined ground squirrel encompasses regions that experience harsh winters. To reduce the energetic costs of thermoregulation, this species hibernates from ~November to April.

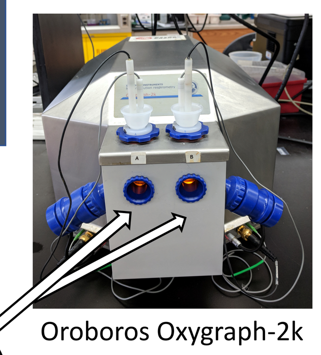
- Small hibernators fluctuate between 2 metabolic extremes during winter: torpor and interbout euthermia (IBE; Fig.1)
- The rapid transition from torpor to IBE may cause transient hypoxia in certain tissues.
- Some hibernator tissues are hypoxia tolerant[1,2,3] and this tolerance improves during the hibernation season[1]. The mechanisms that confer this tolerance are unknown.

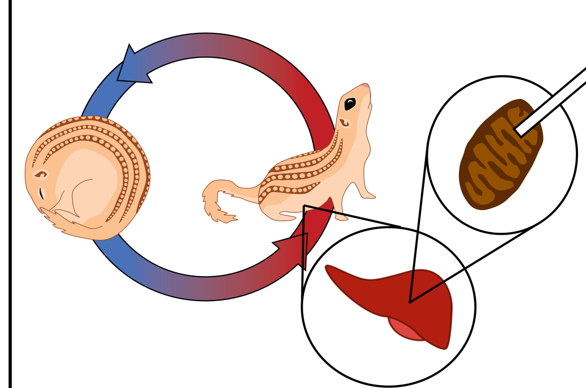
RESEARCH QUESTIONS

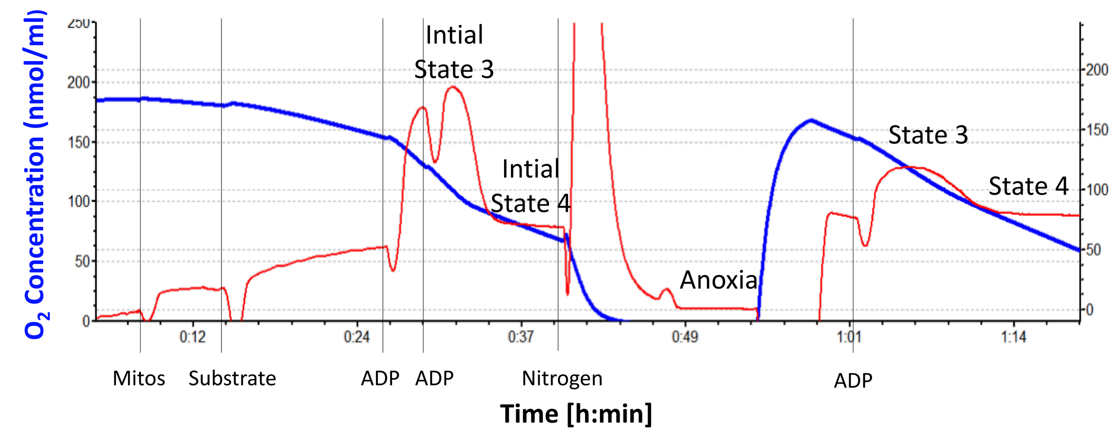
(1) Does mitochondrial anoxia tolerance differ between hibernators and non-hibernators, and/or seasonally in hibernators?

(2) What biochemical mechanisms underlie any differential tolerance?

METHODS







1. Isolate liver mitochondria from summer, torpid & IBE ground squirrels and rats
2. Quantify mitochondrial performance before and after 5 minutes of anoxia:
   - state 3, state 4, membrane potential
3. Recover mitochondrial samples and measure activities of key enzymes

STATE 3 (MAX) RESPIRATION

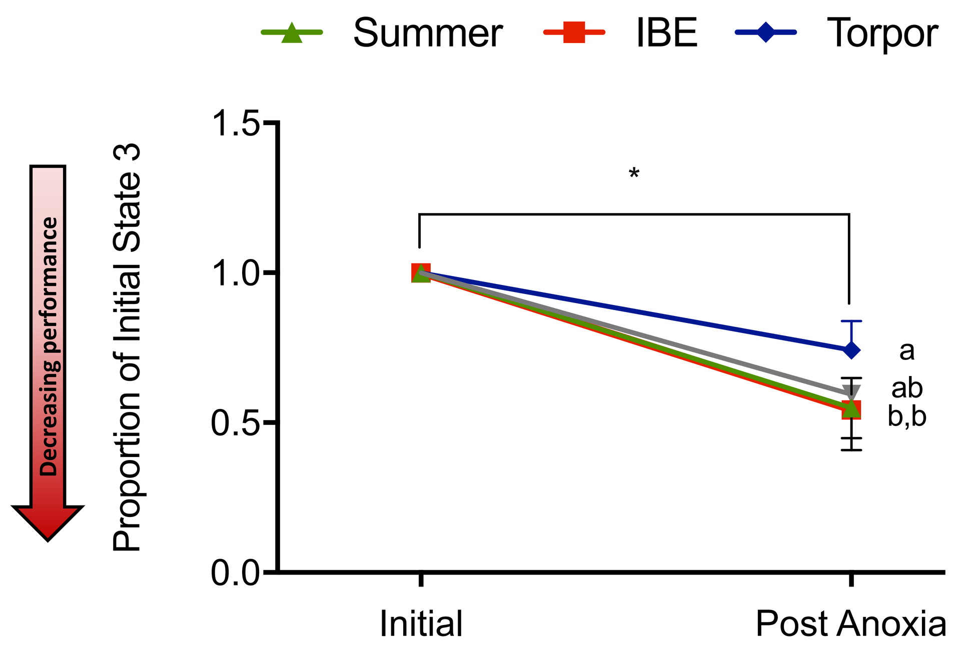


Figure 3. Anoxia decreases state 3 respiration. In all groups, 5 minutes of anoxia decreases maximal respiration rates of liver mitochondria relative to the initial rate. Data are presented as mean ± SE. All groups show significant (P≤0.05) decreases following anoxia (indicated by "*"). Within the post-anoxia group, differential lettering represents statistical significance (P≤0.05). N=8 for summer, N=6 for IBE and torpor, and N=5 for rat.

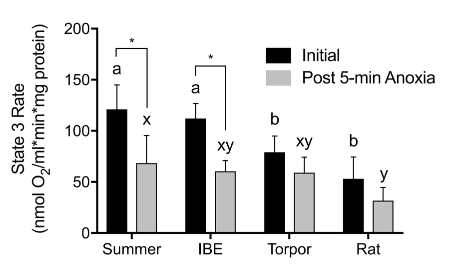


Figure 4. Absolute state 3 respiration rates.

Anoxia reduces state 3 more in summer and IBE than in torpor.

STATE 4 (LEAK) RESPIRATION

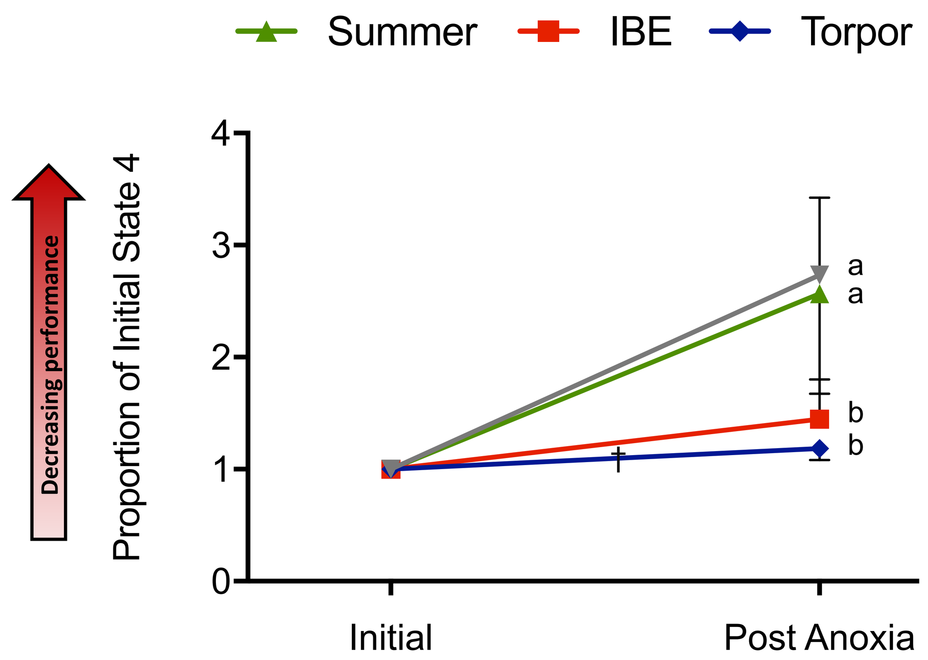


Figure 5. Anoxia increases state 4 respiration in all groups except torpor. When compared to initial state 4 respiration rates, anoxia does not affect leak respiration in torpid liver mitochondria. Data are presented as mean ± SE. Within the post-anoxia group, differential lettering represents statistical significance (P≤0.05). In torpor, leak respiration did not change significantly following anoxia (indicated by "†"). N=7 for summer, N=5 for IBE, N=6 for torpor, and N=4 for rat.

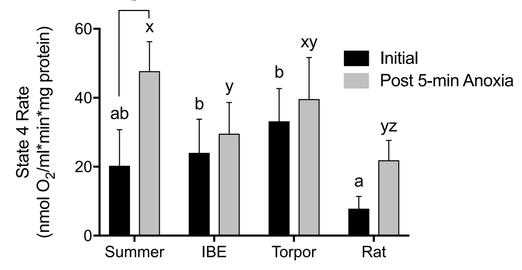


Figure 6. Absolute state 4 respiration rate.

Anoxia increases leak respiration, especially in rats and summer ground squirrels, but has no effect in torpor.

MEMBRANE POTENTIAL

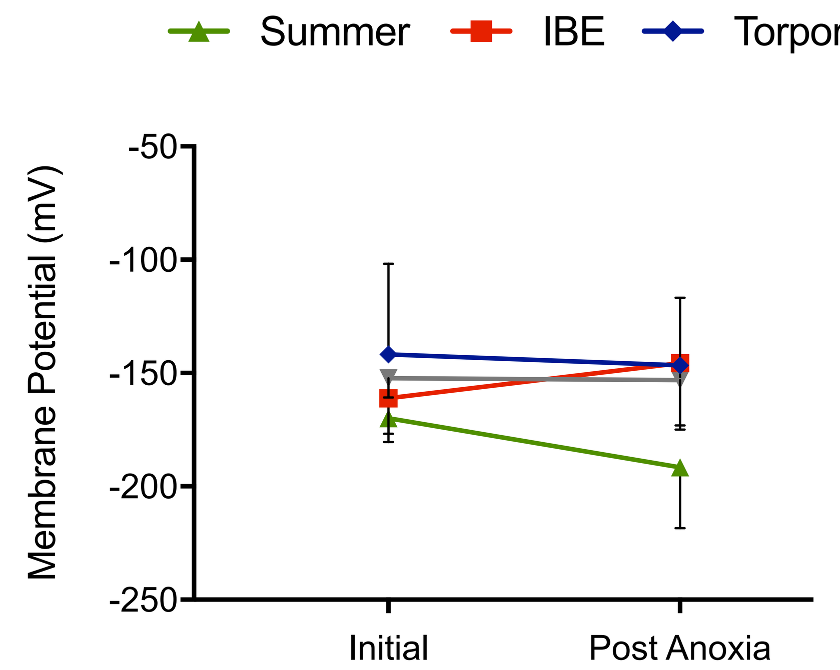


Figure 7. Anoxia does not affect membrane potential. When measured in the leak state, membrane potential does not differ between initial conditions and following 5 minutes of anoxia. Data are presented as mean ± SE. N=3 for summer, N=3 for IBE, N=5 for torpor, and N=4 for rat.

Anoxia does not affect membrane potential of liver mitochondria in the leak state.

ETS COMPLEX ACTIVITY

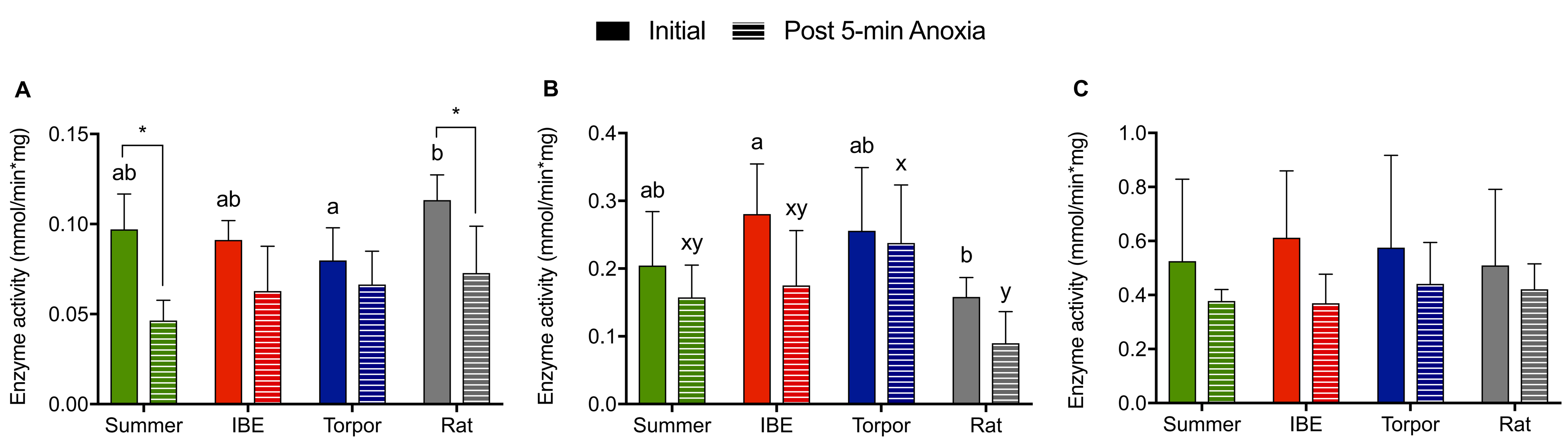


Figure 8. Anoxia reduces Complex I enzyme activity in summer squirrel and rat liver mitochondria. The effect of anoxia on maximal enzyme activity (mmol/min*mg protein) of Complex I (A), Complex II (B), and Complex V (C). Complex I activity is significantly reduced following anoxia in summer ground squirrel and rat liver mitochondria (*P≤0.05). Anoxia does not affect Complex II or Complex V activity in any group. Data are presented as mean ± SE of both the original mitochondrial sample, and the sample recovered following respiration measurements. Among experimental groups, differential lettering represents statistical differences (P≤0.05), and is expressed for the original mitochondrial sample and the recovered samples separately. N=5 for summer, IBE, and rat original mitochondrial samples, N=6 for torpor, and N=4 for rat recovered mitochondria.

Anoxia does not affect ETS Complex I activity in winter (torpor and IBE) ground squirrel liver mitochondria, but decreases it in summer ground squirrels and rats. Anoxia does not affect Complex II or Complex V activity in any group.

In summer ground squirrel and rat liver mitochondria:
Increased leak respiration and decreased enzyme activity suggests anoxia-associated damage (vs. regulatory changes). **ROS-mediated damage could mechanistically explain these findings.**

SUMMARY AND CONCLUSIONS

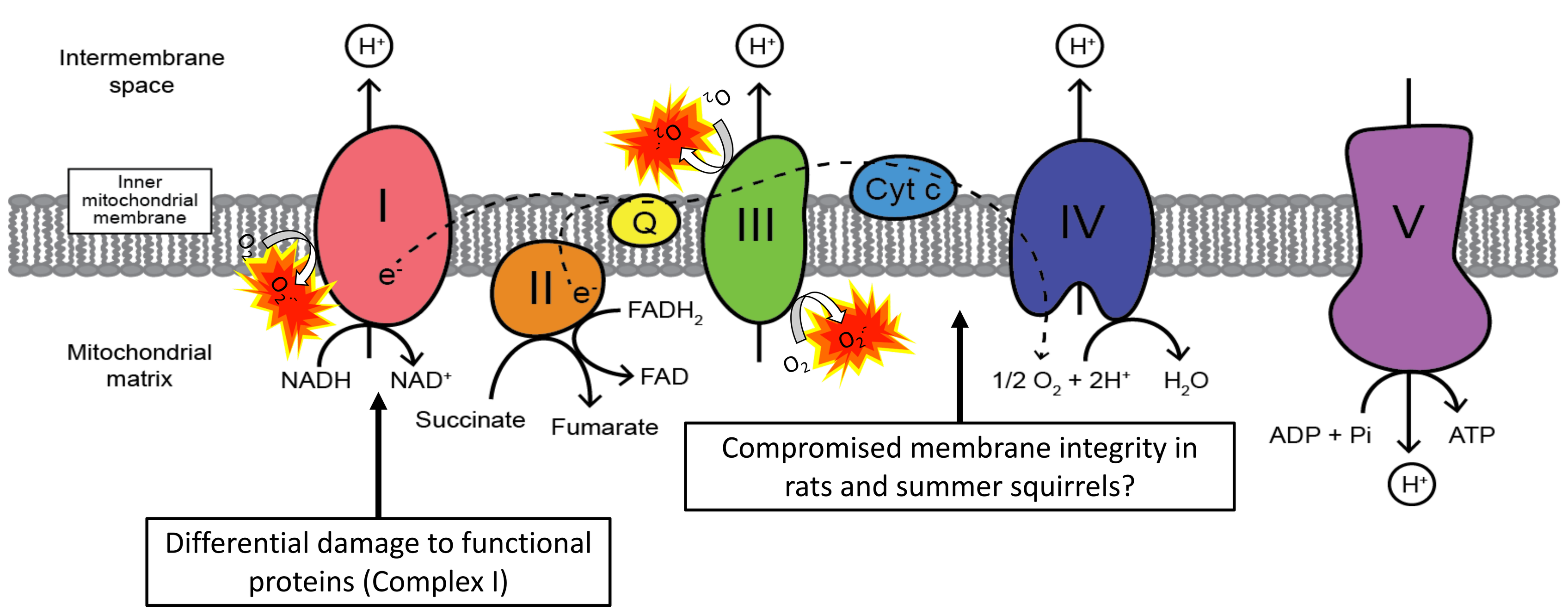


Figure 9. Differential effect of anoxia on liver mitochondria of squirrels and rats. Anoxia halts electron flux through the electron transport system (ETS). This leads to an overall reduction of complex enzymes, which can promote reactive oxygen species (ROS) production (shown as superoxide $O_2^{\cdot -}$). ROS can damage mitochondrial components, such as ETS complex enzymes[4], which may explain reduced enzyme activity of Complex I in summer squirrels and rats following anoxia. The increase in leak respiration after anoxia in summer squirrels and rats may also be explained by higher ROS-related damage compared to IBE and torpor (perhaps through increased lipid peroxidation).

- Enhanced anoxia tolerance in liver mitochondria of torpid and IBE ground squirrels
  - minimal (IBE) or no (torpor) anoxia effect on leak respiration
  - no anoxia effect on Complex I activity
- Lower ROS production or improved capacity for ROS detoxification may mitigate anoxia-associated decreases in performance

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References

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