Fatty acid-stimulated uncoupling mitochondrial metabolism increases during arousal from hibernation in the 13-lined ground squirrel

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1. Hibernation: Torpor and arousal

In winter, 13-lined ground squirrels hibernate to conserve energy. In torpor, body temperature (T_b) is maintained at ~5°C, while metabolic rate (MR) is suppressed by ~95%, periodically interrupted by rapid arousals into interbout euthermia (IBE).

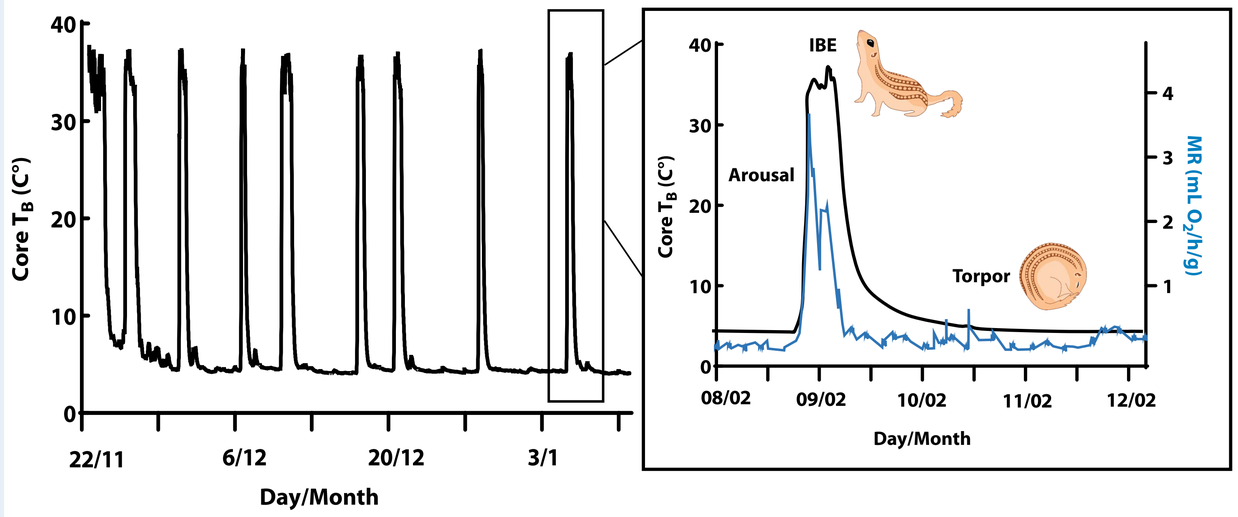


Fig. 1. T_b and MR of a 13-lined ground squirrel through multiple torbor bouts. Modified with permission from ref. 1

2. Uncoupling mitochondrial metabolism

During arousals, up to 60% of the increase in MR is attributed to activation of ETS function in brown adipose tissue mitochondria through uncoupling protein 1 (UCP1)[2]. Free fatty acids (FFA) may also uncouple mitochondria in other tissues during arousal, and hibernators rely on lipid oxidation.

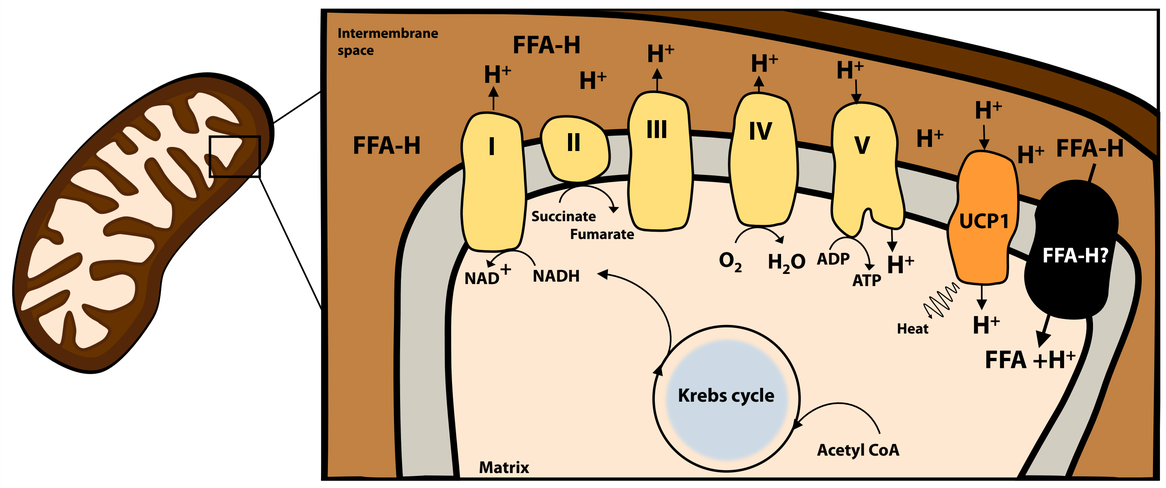


Fig. 2. In brown adipose tissue, UCP1 uncouples substrate oxidation by the mitochondrial electron transport system, resulting in the futile cycling of protons between the mitochondrial matrix and intermembrane space, releasing energy from substrate oxidation as heat.

3. Research questions

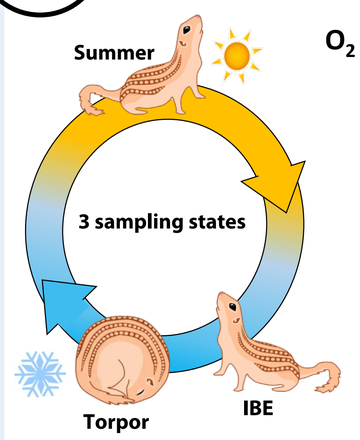
Do FFA uncouple oxidative metabolism in liver mitochondria?

- ❑ **FFA-uncoupling may be mediated by UCP homologues, the *A*deninine *N*ucleotide *T*ransporter or the *P*ermeability *T*ransition *P*ore**

Does any uncoupling differs among hibernation states?

- ❑ **Thermogenesis by the large, metabolically active liver could facilitate arousal**

4. Experimental design

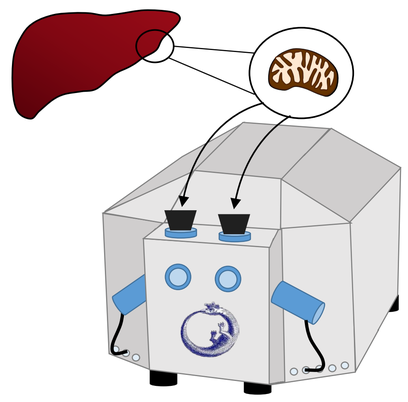


O_2 consumption of isolated liver mitochondria

- ❑ State II respiration w/succinate (S) and rotenone (R)
- ❑ Uncoupling with palmitate (16:0 FFA)

Controls: Bovine serum albumin (BSA; binds FFA)
Ethanol (solvent for palmitate)
CCCP (well-characterized synthetic uncoupler)

Blockers[3]: Guanosine-5-diphosphate (GDP; blocks UCPs)
Atractyloside (Atra; blocks ANT)
Cyclosporin A (CSA; blocks PTP)



Oroboros Oxygraph-2k

5. FFA uncouple mitochondrial metabolism through the permeability transition pore

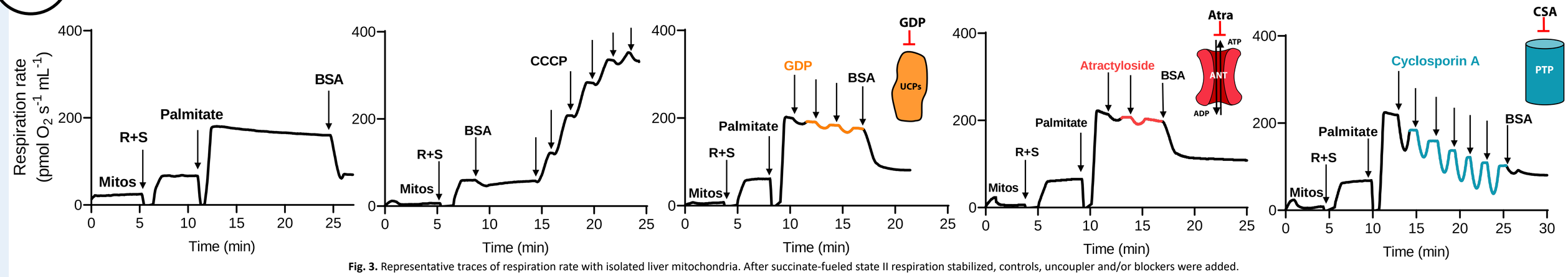


Fig. 3. Representative traces of respiration rate with isolated liver mitochondria. After succinate-fueled state II respiration stabilized, controls, uncoupler and/or blockers were added.

6. FFA uncoupling is significantly higher during IBE compared to torpor

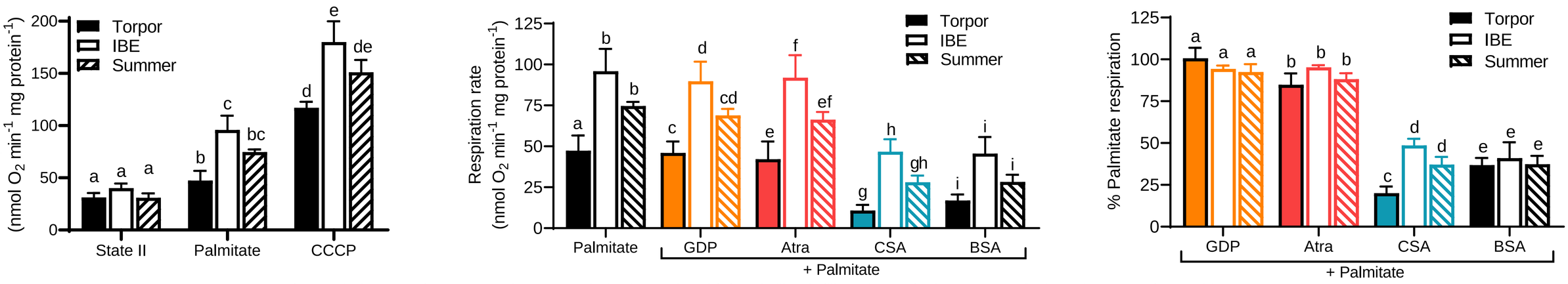


Fig 4. Respiration rates. Values are mean + SEM. Bars not sharing the same letter label are significantly different from each other (two-way ANOVA). n = 7 for torpor and IBE, n = 8 for summer.

7. Conclusion: Liver thermogenesis by PTP-mediated FFA-uncoupling could facilitate arousal

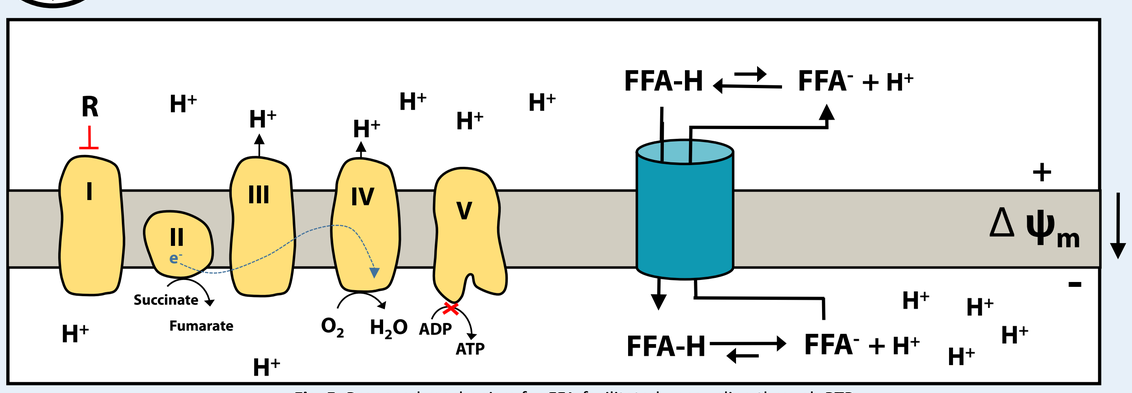
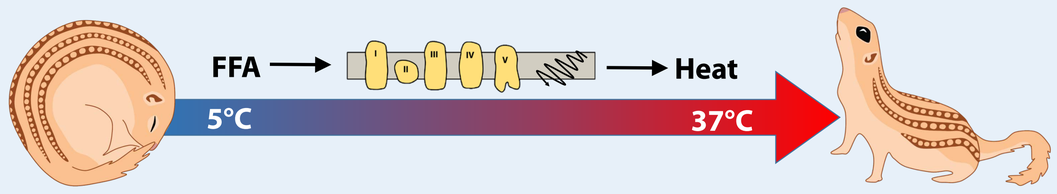


Fig. 5. Proposed mechanism for FFA-facilitated uncoupling through PTP.



Significantly higher FFA-induced uncoupling in IBE mitochondria could facilitate thermogenesis in the liver during arousal. BSA eliminated palmitate uncoupling in all hibernation states, indicating that the uncoupling was mediated entirely by FFA. Cyclosporin A also completely reversed the uncoupling, suggesting that FFA-uncoupling is facilitated through PTP.

Future direction: Does PTP expression or amount differ between torpor and IBE?

References

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Abbreviations

ANT: adenine nucleotide transporters
Atra: Atratyloside
BSA: Bovine serum albumin
CCCP: Carbonyl cyanide m-chlorophenyl hydrazine
CSA: Cyclosporin A
FFA: Free fatty acids
GDP: Guanosine-5-diphosphate
IBE: interbout euthermia
PTP: mitochondrial permeability transition pore
MR: Metabolic rate
R: Rotenone
S: Succinate
UCP: uncoupling protein

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