Mitochondrial Capacity in Brown Adipose Tissue of the 13-Lined Ground Squirrel

Charles Sieberg
Swenson College of Science and Engineering
University of Minnesota Duluth
Sieb0158@d.umn.edu

Introduction

Brown adipose tissue (BAT) is an organ found in mammals that functions to produce heat via non- shivering thermogenesis. Heat generation occurs within BAT mitochondria due to the presence of uncoupling protein 1 (UCP1) which uncouples ATP synthesis from the oxidation of fuels. For the 13- lined ground squirrel (*Ictidomys tridecemlineatus*), a hibernating mammal, this heat production is important when body temperature increases from 5^0C during torpor (TOR) to 37^0C during interboutal arousal (IBA). BAT is less active during the fall and spring and the long term purpose of this experiment is to determine how BAT mitochondrial capacity changes during the hibernation season. The immediate purpose of this experiment is to successfully isolate mitochondria, develop a protocol for the measurement of mitochondrial capacity, and determine the mitochondrial capacity of BAT during the fall time point of the hibernation season. Recently, appreciable deposits of BAT have been found in adult humans, and the ability to increase the mitochondrial capacity within BAT and therefore the overall metabolic rate may prove to be an effective target for weight loss therapy.[1]

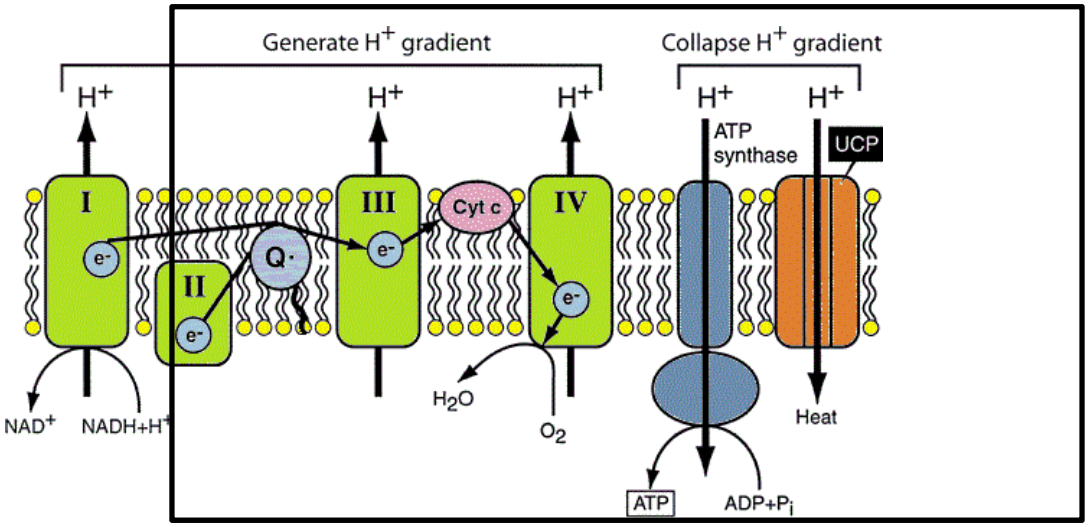

Figure 1. UCP1 in BAT mitochondria uncouple ATP synthesis from the oxidation of fuels in the electron transport chain (ETC)

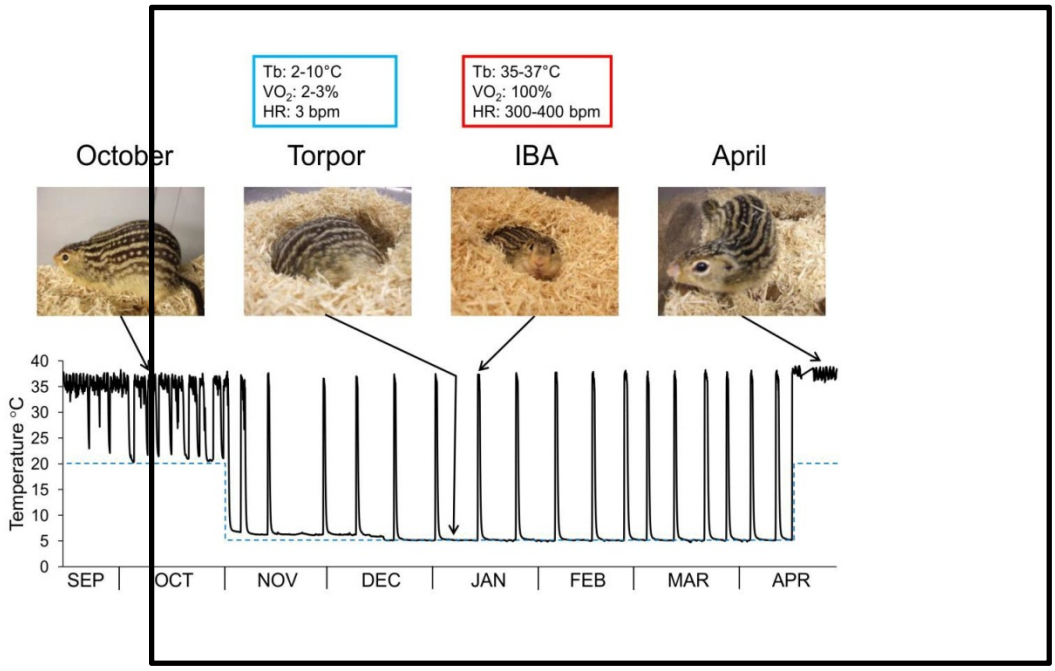

Figure 2. Seasonal body temperature of the 13-lined ground squirrel.

Methods

State 1 respiration was obtained with only mitochondria present. State 2 respiration was obtained with the addition of a substrate, such as glutamate/malate, at a saturating concentration. State 3 respiration was obtained with the addition of a saturating concentration of ADP, and this rate represents the oxidative phosphorylation capacity of the mitochondria. State 4 respiration was obtained after the addition of ADP had been converted to ATP in coupled mitochondria. For uncoupled mitochondria such as BAT mitochondria, the oxidative phosphorylation capacity is not limited by ADP. Oligomycin was added to inhibit ATP synthase and FCCP was added to completely uncouple the mitochondria. Rotenone was used to inhibit substrates of Complex I of the ETC, malonate was used to inhibit substrates of Complex II of the ETC, antimycin-A was used to inhibit substrates of Complex III of the ETC, and potassium cyanide was used to inhibit substrates of Complex IV of the ETC.

Results and Discussion

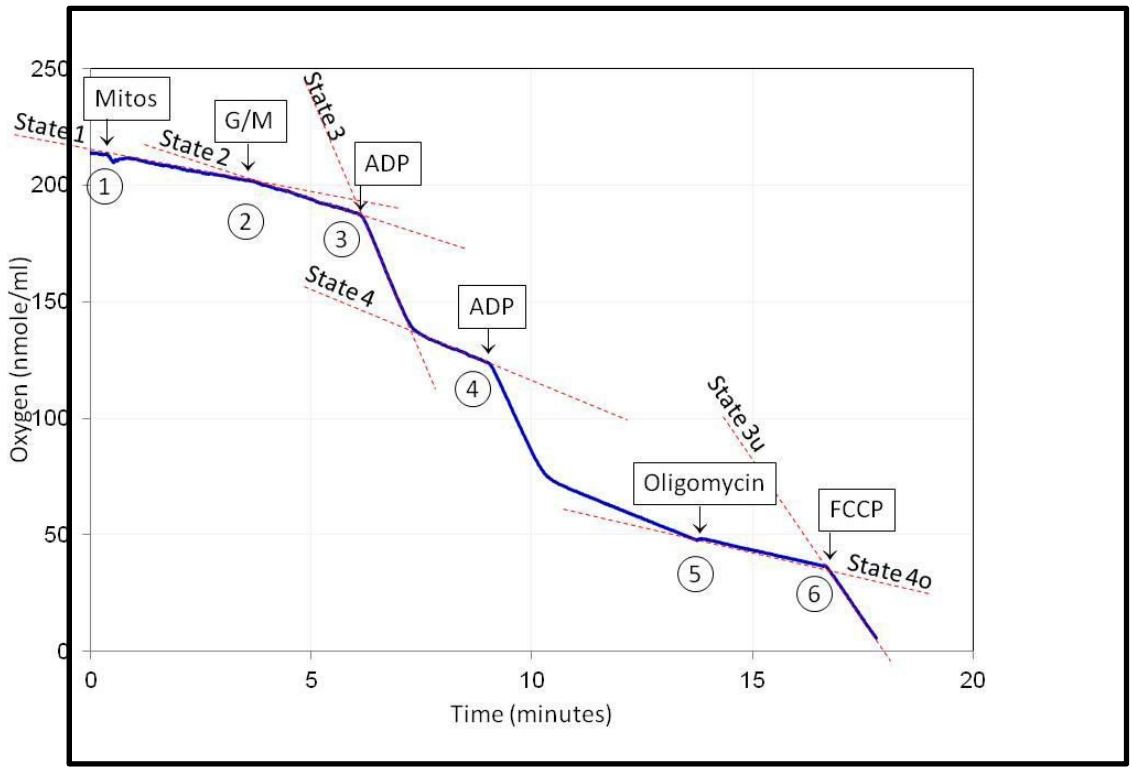

Figure 3. Glutamate/Malate fueled respiration in liver mitochondria.
Liver mitochondria are coupled and do not contain UCP1
Step 1: Liver mitochondria (mitos) added to a concentration of 1 mg mitochondria protein/ml
Step 2: Glutamate/Malate (G/M) added to a concentration of 5 mM each
Step 3 and 4: 200 nmoles ADP added
Step 5: 1 µg Oligomycin added, block ATP synthase
Step 6: 1 nmole FCCP added, uncouple mitochondria

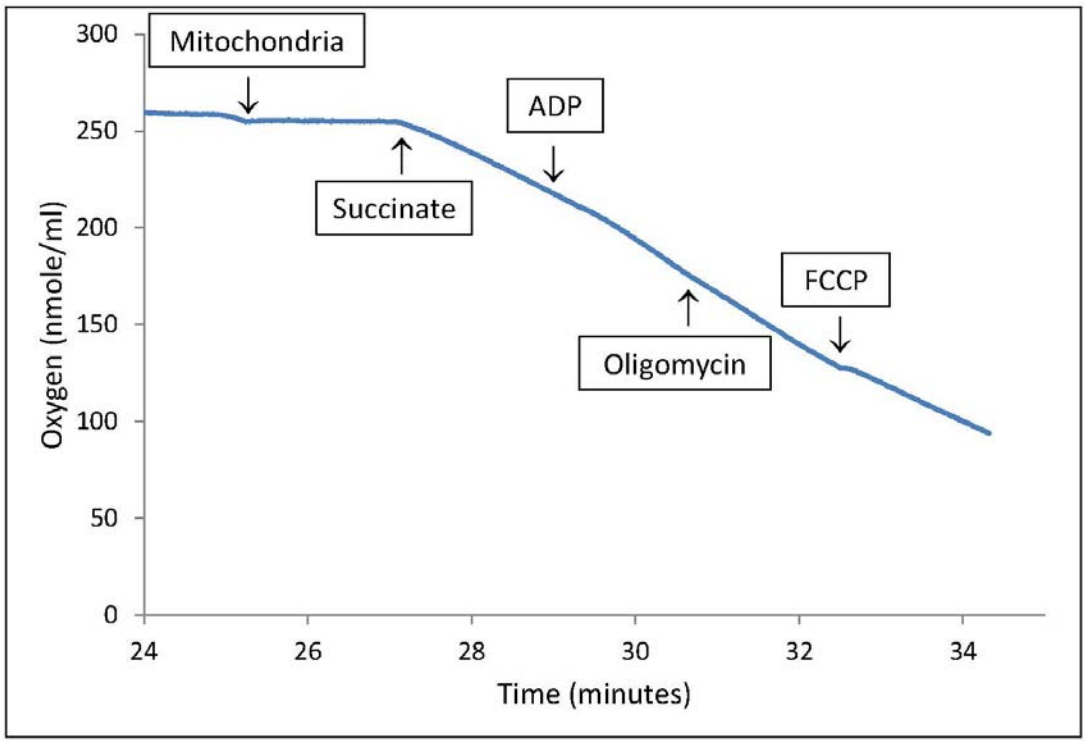

Figure 4. Succinate fueled respiration in BAT mitochondria.
BAT mitochondria are uncoupled due to the presence of UCP1
Step 1: BAT mitochondria added to a concentration of 1 mg mitochondria protein/ml
Step 2: Succinate added to a concentration of 5 mM
Step 3: 200 nmoles ADP added
Step 4: 1 µg Oligomycin added, block ATP synthase
Step 5: 1 nmole FCCP added, uncouple mitochondria

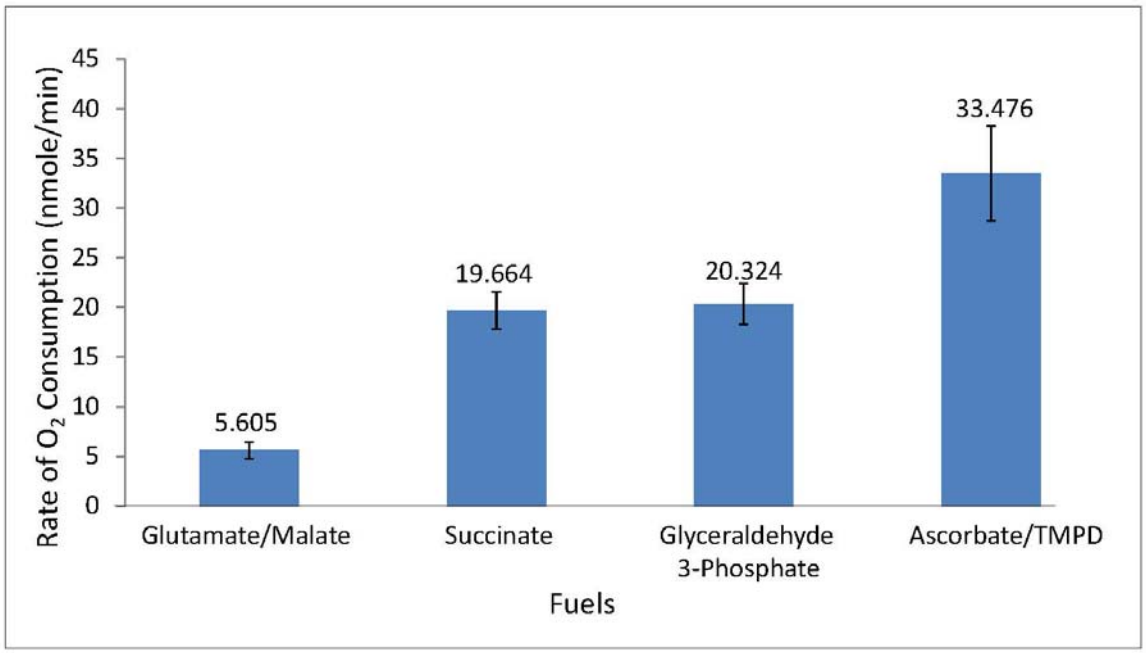

Figure 5. BAT mitochondria respiration rates of the13-lined ground squirrel during the fall time point (October) of the hibernation season.

Mitochondrial respiration was measured using six 13-lined ground squirrels
Glutamate/Malate donates electrons to Complex I of the ETC
Succinate donates electrons to Complex II of the ETC
Glyceraldehyde 3-Phosphate donates electrons to Complex III of the ETC
Ascorbate/TMPD donates electrons to Complex IV of the ETC

Conclusion

The parameters required to successfully isolate functioning mitochondria were optimized. The following protocol was developed to measure mitochondrial rates of respiration in the presence of several substrates.

1. Respiration fueled by glutamate/malate or pyruvate/malate
2. Inhibition of Complex I by rotenone
3. Respiration fueled by succinate
4. Inhibition of Complex II by malonate
5. Respiration fueled by glyceraldehyde 3-phosphate
6. Inhibition of Complex III by antimycin-A
7. Respiration fueled by ascorbate/TMPD
8. Inhibition of Complex IV by potassium cyanide
9. Inhibition of ATP synthase by oligomycin
10. Induce uncoupling by FCCP

•BAT mitochondria respiration rates of the13-lined ground squirrel during the fall time

point of the hibernation season were determined using four substrates
•BAT mitochondria respiration rates of the 13-lined ground squirrel using the same four substrates will be determined for the torpor, IBA, and spring time points
•Respiration analysis of BAT mitochondria will provide information on how mitochondrial capacity of BAT changes throughout the seasons.

Bibliography

Cypess, A.M.; Lehman, S.; Williams, G.; Tal, I.; Rodman, D.; Goldfine, A.B.; Kuo, F.C.; Palmer, E.L.; Tseng, Y.; Doria, A.; Kolodny, G.M.; Kahn, C.R., Identification and Importance of Brown Adipose Tissue in Adult Humans. *The New England Journal of Medicine* 2009, 360, 1509-1517.

Barrientos, A.; Fontanesi, F.; Diaz, F., Evaluation of the Mitochondrial Respiratory Chain and OxidativePhosphorylation System using Polarography and Spectrophotometric Enzyme Assays. *Curr Protoc Hum Genet.* 2009.