Energy Balance in the Cold in *Drosophila Melanogaster*

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Introduction

When ectotherms are exposed to cold temperatures they enter a cold-coma, whereby movement ceases. The time taken to recover from cold-coma varies widely both inter- and intra-specifically, but the physiological and biochemical mechanisms underlying this variation are largely unknown. We hypothesize that the speed of recovery from cold-coma is determined by the magnitude of cold-induced disruptions to intermediary metabolism, whereby more tolerant organisms are 1) better prepared for the onset of low temperatures; 2) better able to maintain metabolic processes at low temperatures; and 3) recover normal rates of metabolic processes more rapidly after returning to warm temperatures. Metabolomic experiments showed that hardy flies maintain metabolic homeostatis more effectively during cold exposure than susceptible flies[1].

High energy phosphates (e.g., adenosine triphosphate; ATP) are the primary molecular unit of energy currency for eukaryotes. Low temperatures induce ATP limitation in fish and other marine ectotherms which results from limitations to oxygen delivery[2], but there is no evidence to date that terrestrial ectotherms experience energy limitation in the cold[3]. We have used *Drosophila melanogaster*, selected in the laboratory for fast or slow recovery from cold-coma, as a model organism to test the hypothesis that ATP availability declines in the cold, and that cold-adaptation facilitates improved ATP homeostasis during cold exposure. This is the first study examining ATP homeostasis *in vivo* in a terrestrial ectotherm across a range of environmental temperatures, and thus represents an advance in our understanding of the impacts of low temperatures on energy homeostasis in terrestrial ectotherms.

Experimental

Flies were selected in the laboratory for fast or slow recovery (2 biological replicates of each selection regime) from cold-coma for 30 generations, then maintained at 25 °C. Four groups of 60 female flies from each line were aspirated into 5-mm NMR tubes and positioned between susceptibility plugs matched to deuterium oxide (Doty plugs, Wilmad-LabGlass, Wineland NJ). ^{31}P spectra were collected during cold exposure and 4h recovery on the Bruker Avance III-600 instrument. Spectra were phase- and baseline-corrected, and the β-phosphate of ATP integrated against the total phosphorus content of each spectrum.

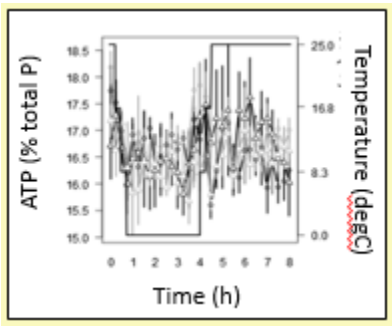

Figure 1- ATP levels during cold exposure and recovery in cold hardy (black) or cold susceptible (grey) *Drosophila melanogaster*. Temperature is the solid black line.

Results and Discussion

ATP does not change during cold exposure or recovery, suggesting that ATP limitation does not set lower thermal limits in Drosophila melanogaster[4]. An unidentified compound in the left region of the spectrum, possibly a glycerophospholipid, increases strongly during recovery in hardy but not susceptible flies.

Conclusions

Cold exposure does not induce energy limitation in D. melanogaster. An as yet unidentified phosphorus compound may be related to superior recovery of hardy flies.

Acknowledgements

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References

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[4] Williams, CM *et al*., Conference presentation American Physiological Society Intersociety meeting, Oct (2014)