Effects of Anoxia on Methionine Sulfoxide Reductase (Msr) Deficient *Drosophila*

Danielle Howard[1,2] and David Binninger[2]

1. National Science Foundation Undergraduate Research and Mentoring Program

2. Department of Biological Sciences, Charles E. Schmidt College of Science Florida Atlantic University, Boca Raton, Florida, 33431

Abstract

Reintroduction of oxygen following a period of oxygen deprivation (anoxia) leads to a rapid production of reactive oxygen species (ROS), which cause damage by oxidizing key cell constituents. The oxidation of methionine is particularly damaging due to its importance in protein function. This oxidation is reversible by the genes methionine sulfoxide reductase (Msr) A and B, which reduce the S and R enantiomers of methionine sulfoxide, respectively, to methionine. **In this study, flies lacking any known Msr activity were exposed to one hour of anoxic stress, and then their recovery times were recorded with the *Drosophila* Activity Monitoring (DAM) system**. Our preliminary studies showed that Msr deficient flies have significant increase in recovery time when compared to wildtype flies. Understanding the role Msr plays in anoxia could lead to further work on the stroke model in humans.

Introduction

Reactive Oxygen Species (ROS):

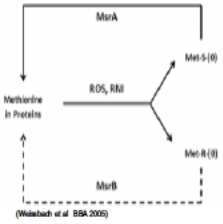
- Free radicals normally produced by the cell during metabolism [1]
- Production of ROS increased after anoxic stress
- Damage DNA, lipids, proteins, etc. via oxidation [1]
- Oxidation of protein-bound methionine to methionine sulfoxide (Met-SO) limits or ceases protein function

Methionine Sulfoxide Reductase (Msr):

- Reduces Met-SO back to functional methionine [2]
- Highly conserved
- Two forms: msrA (reduces Met-(S)-SO) and msrB (reduces Met-(R)-SO) [2]

Drosophila:

- Can withstand up to 4-5 hours of pure anoxia by going into spreading depression coma [3]
- Spreading depression is a state of reduced metabolism [3]
- Tolerance of anoxia makes *Drosophila* a good model organism for this study



Materials and Methods

Genotypes	
Wildtype:	Double LOF:
WT77	AB113
WT60	AB46

- Two genotypes of fly will be used: a wildtype and an *msrA / msrB* double Loss-Of-Function (LOF) fly
- Individual flies of a single genotype are placed into each of 32 tubes in the Drosophila Activity Monitor (DAM) holder
- The entire DAM holder is then placed into the anoxia chamber for one hour of anoxic stress
- The holder is put into the DAM monitor, which records, via an infrared beam, when the flies have recovered enough to walk

Results

Double LOF Flies (AB113) Take Significantly Longer to Recover than Wildtype Flies (WT77) for All Three Age Ranges

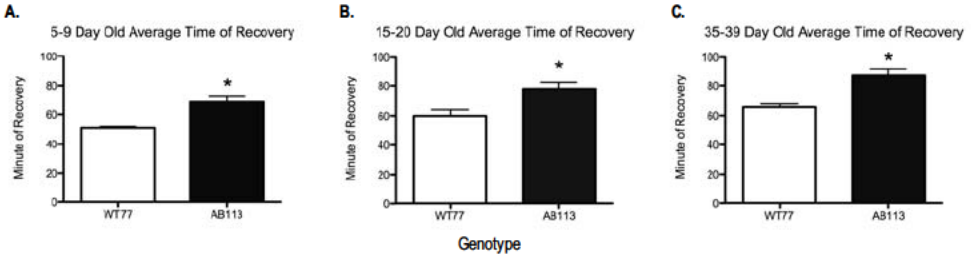


Figure 1: Although the average recovery times of both double LOF flies (AB113) and wildtype flies (WT77) increases as the flies age, double LOF flies recover, on average, 20 minutes later than wildtype flies for 5-9 day old flies ($p<0.0001$), 15-20 day old flies ($p = 0.0038$) and 35-39 day old flies ($p<0.0001$).

Double LOF Flies (AB113) Recover at a Significantly Reduced Rate Compared to Wildtype Flies (WT77) for All Three Age Ranges

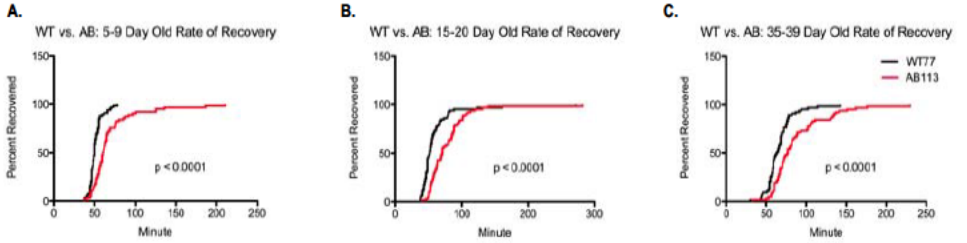


Figure 2: As both double LOF flies (AB113) and wildtype flies (WT77) age, they begin to recover at a reduced rate, most likely due to the accumulation of ROS in aged organisms. Additionally, the overall range of recovery times broadens for both genotypes, although the double LOF flies continually recover at a significantly slower rate than the wildtype flies.

Double LOF Flies (AB46) Have a Significantly Lower Survival Immediately Following Anoxic Stress Compared to Wildtype Flies (WT60)

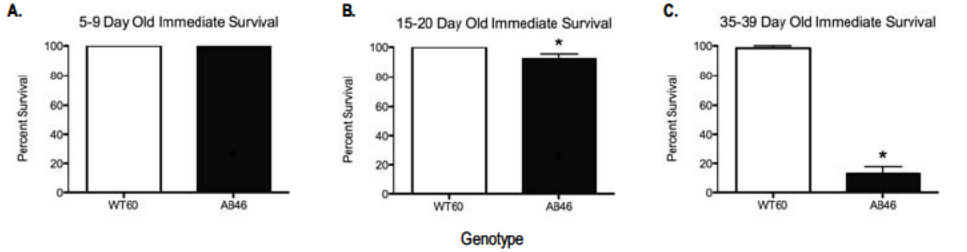
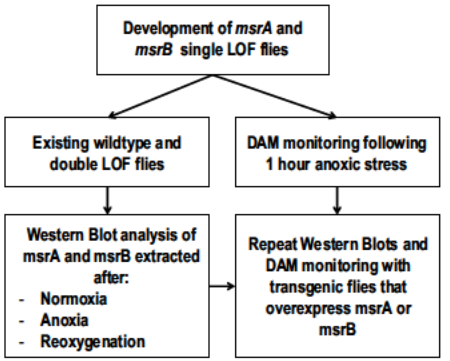


Figure 3: As both double LOF flies (AB46) and wildtype flies (WT60) age, they become more susceptible to long-term damage due to the anoxic stress, to the point where it can become fatal. Shown above, young flies for both genotypes generally have survival rates at 100%. However, as the flies age, the double LOF flies' survival rates begin to decrease significantly ($p = 0.0369$ for 15-20 day old flies and $p < 0.0001$ for 35-30 day old flies).

Discussion

- As flies age, ROS accumulates and they become more susceptible to anoxic stress.
- This can be seen by the increased times and rate of recovery, and decreased rate of survival following an hour of anoxic stress.
- Double LOF flies, on average, recover approximately 20 minutes later than wildtype flies. Likewise, double LOF flies recover at a significantly slower rate, and have a significantly reduced rate of survival.
- The reduced rate of recovery and average time of recovery in double LOF flies indicates that msrA and msrB play an important role in helping the fly recover from the effects of anoxic stress.
- The reduced survival rate for double LOF flies as they age indicates that not only do msrA and msrB help the flies recover following anoxic stress, but also that they have a protective role in the fly preventing anoxia from causing damage in addition to reversing damage done.

Future Work



Acknowledgements

- Lindsay Bruce, James Martin
- Ramon Garcia-Areas, as well as my lab mates and peers
- Dr. Sarah Milton and her students, for the use of their anoxia chamber
- Florida Atlantic University Undergraduate Research Grant
- National Science Foundation Undergraduate Research and Mentoring Program Grant (0829250)

References

1 Berlett, B S and E R Stadtman, *Protein oxidation in aging, disease, and oxidative stress* J Biol Chem, 1997 272 p 20313 20316

2 Ruan, H , et al , *High quality life extension by the enzyme peptide methionine sulfoxide reductase* Proc Natl Acad Sci , USA, 2002 99(5) p 2748 2753

3 Haddad, G G , et al , *Behavioral and Electrophysiologic Responses of Drosophila melanogaster to Prolonged Periods of Anoxia* J Insect Physiol, 1997 43(3) p 203 210