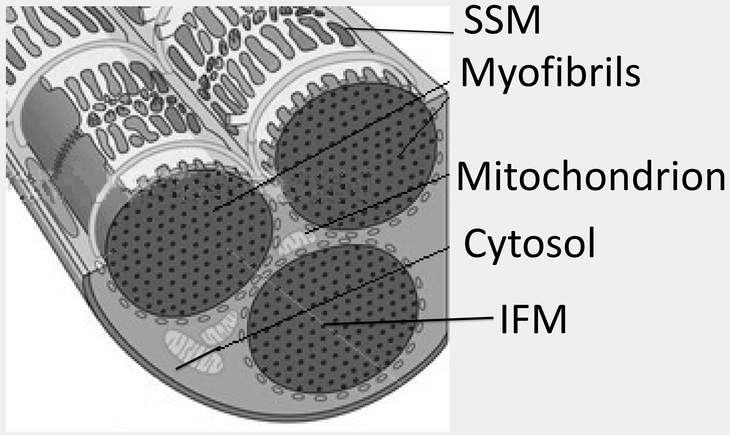
Comparison of carbonylation in young and old rat skeletal muscle

Isaac Willard[1], Vratislav Kostal[1], LaDora Thompson[2], Edgar Arriaga[1]
Department of Chemistry[1] *, Department of Physical Medicine and Rehabilitation*[2]
University of Minnesota, Minneapolis Minnesota

Introduction

Proteins, DNA, and lipids in most tissues become carbonylated due to oxidative stress. Skeletal muscles are particularly susceptible to such carbonylation because of the conversion from metabolic energy to mechanical energy during contraction. The morphology and function of muscle cells change in response to ageing and development. So the relative levels of carbonylation in intermyofibrillar mitochondria (IFM), the subsarcolemmal mitochondria (SSM), the cytoplasm, and the extracellular space of skeletal muscle may change with age. The carbonyl levels in the four areas above were measured in two muscle types of young and old animals; Soleus and Semimembranosus.



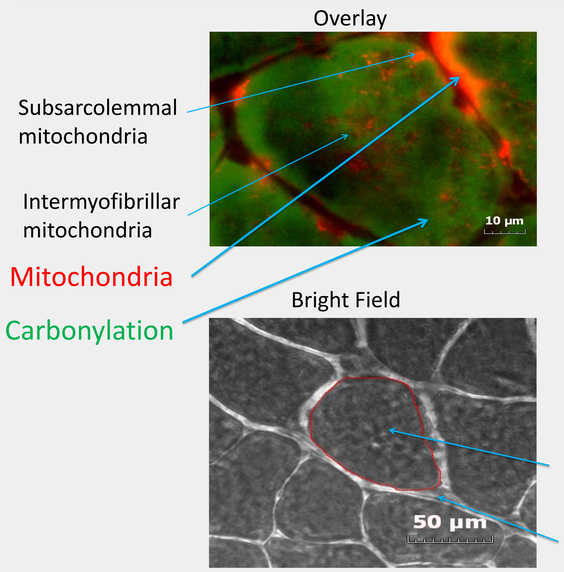
Hypothesis

Cellular carbonyl levels in skeletal muscle cells increase with age and vary with muscle type.

Objectives

- ❖ Compare carbonyl levels in each muscle type for young and old muscle cells
- ❖ Compare carbonyl levels in the Soleus versus the Semimembranosus
- ❖ Collect enough data to make the conclusions statistically significant

Experimental Strategy



- Mitochondria and carbonyls are selectively labeled with red and green respectively
- Red label intensity indicates the abundance of mitochondria in each cell
- Green label intensity indicates carbonylation
- The overlay of Red and Green intensities show the mitochondrial regions that have been carbonylated
- The Bright Field is the unlabeled cells
- The Bright Field allows the image analysis code to define the cell in terms of spatial orientation
- Results from the overlay images of young and old muscles from Soleus and Semimembranosus are compared in order to study carbonylation patterns

Image Analysis

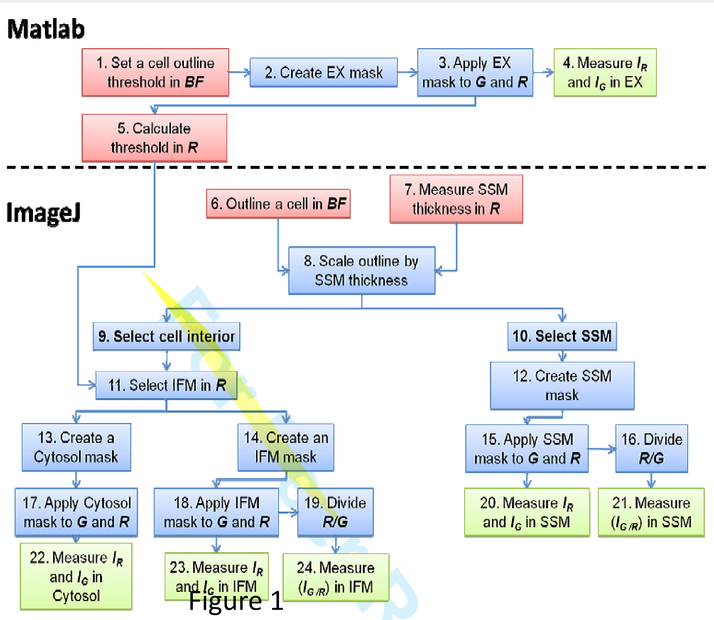
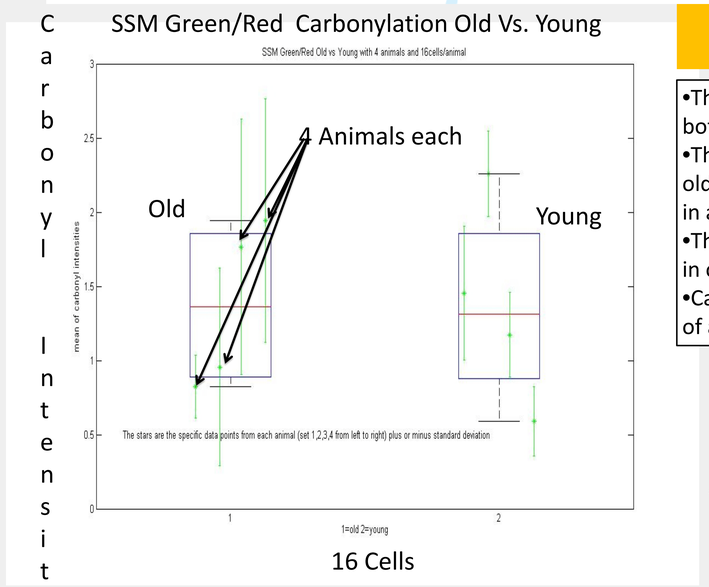
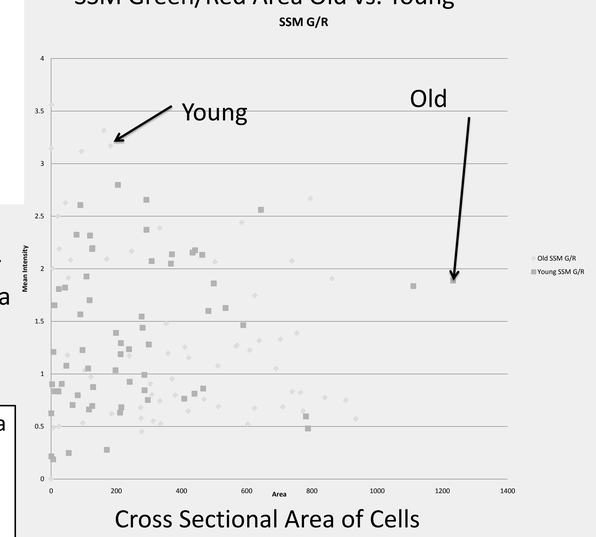


Figure 1



SSM Green/Red Carbonylation (above) shows that the average carbonyl levels in young and old muscles is similar and that the old muscle fibers have some cells with low carbonyl levels and other cells with much higher carbonyl levels.

SSM Green/Red Area (Right) plots carbonyl intensity against the area of the cell in an effort to determine the effect the size of the cell has on carbonylation in young and old muscles. The plot shows that carbonylation in both young and old muscles does not depend on area.



Future Work

1. Obtain data for the Soleus tissue similar to the data collected for the Semimembranosus tissue
2. Apply the automation used here to measure carbonyls to measurement of Giant Mitochondria

Acknowledgements

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References

•Bracel, K., Thompson, L., Arriaga, E., *Comparison of Carbonyl Levels in Young and Old Rats*, 2009, Poster.
•Arriaga *et al, Anal & Bio Chem,* "Semi Automated Image Analysis: Detecting Carbonylation in Subcellular Regions of Skeletal Muscle.", 2011, in press.

Conclusions

- •This analysis shows the variance in carbonylation between both cells and animals within one muscle fiber
- •The data suggests the carbonylation levels vary more in old muscles than in young muscles, so overall carbonyl level in a single cell could increase with age
- •The carbonylation in cells varies highly per cell, especially in old muscle tissue.
- •Carbonylation in both young and old cells is independent of area.