Comparison of carbonylation in young and old rat skeletal muscle
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Introduction
Reactive oxygen species (ROS) are a natural by-product of aerobic cellular metabolism. They are highly reactive molecules which cause oxidative damage, such as carbonylation, to proteins, lipids and DNA. Because of the high metabolism and energy demand of skeletal muscle, it is especially susceptible to the carbonylation. Thus, the level of carbonylation can be used as a quantitative estimate of the amount of oxidative damage to the tissue.

Objectives
• To compare levels of oxidative damage in the soleus muscle of young and old rats.
• To quantify the difference carbonylation between mitochondrial regions of the muscle cell, specifically subsarcolemmal mitochondria (SSM) and intermyofibrillar mitochondria (IFM).

Experimental Strategy
Rat soleus muscle
Muscle cross-sections are mounted on microscope slide
Label carbonyls and mitochondria with fluorescent antibodies, and image with a fluorescent microscope

Carbonyl Labeling

Microscopy imaging
Bright field image– 40x magnification
Fluorescent labeling

Image Analysis
Bright field 60x
RFP 60x– Mitochondria
GFP 60x– Carbonyls

Carbonyl Quantification
Subsarcolemmal mitochondria
Intermyofibrillar mitochondria

Preliminary Results
Preliminary analysis of young and old samples shows that when the level of carbonylation is normalized to mitochondrial density there is a greater ratio of carbonylation per mitochondria in the IFM region as compared to the SSM region. As expected, we also saw higher levels of carbonylation in the old muscle than in the young muscle.

Summary
Preliminary results suggest 1) older tissue will have a higher levels of carbonylation than younger tissue, and 2) the ratio of carbonylation per mitochondria in the IFM will be higher than the ratio in the SSM.

Future Work
After completion of the soleus analysis, the next step in the project is to use the same procedure to analyze carbonyl levels in the young and old rat semimembranosus in order to compare oxidative damage in slow-twitch (soleus) and fast twitch (semimembranosus) skeletal muscles.

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References

Carbonyls per Mitochondria

SSM
IFM

Green/Red Intensity Ratio

0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Young
Soleus
Old
Soleus

The ratio of green intensity to red intensity represents the level of carbonylation per mitochondria (or amount of oxidative damage per mitochondria) in the SSM and IFM regions.