Comparison of carbonylation in young and old rat skeletal muscle

Kiara Brancel, Edgar Arriaga, LaDora Thompson
Department of Chemistry, Department of Physical Medicine and Rehabilitation
University of Minnesota

Introduction

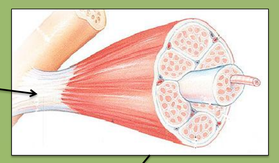
Reactive oxygen species (ROS) are a natural by-product of aerobic cellular metabolism. They are highly reactive molecules which cause oxidative damage, such as carbonylation, to proteins, lipids and DNA. Because of the high metabolism and energy demand of skeletal muscle, it is especially susceptible 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

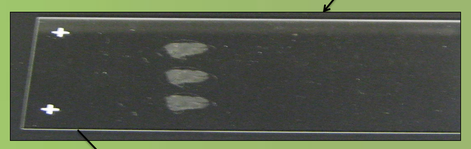
•To compare levels of oxidative damage in the soleus muscle of young and old rats.
•To quantify the difference carbonylation between mitochondrial regions of the muscle cell, specifically subsarcolemmal mitochondria (SSM) and intermyofibrillar mitochondria (IMF).

Experimental Strategy

Rat soleus muscle



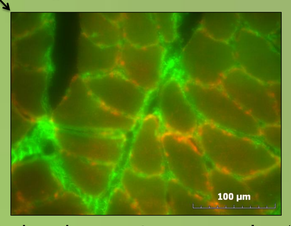
Muscle cross-sections are mounted on microscope slide



Label carbonyls and mitochondria with fluorescent antibodies, and image with a fluorescent microscope

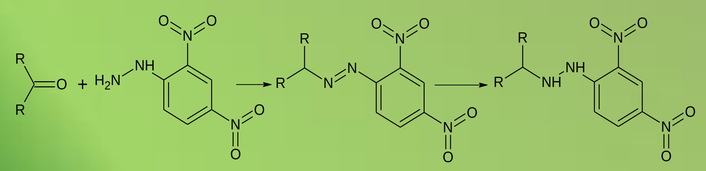
•Carbonyls labeled green.

•Mitochondria labeled red.



Red and green image overlay (40x)

Carbonyl Labeling

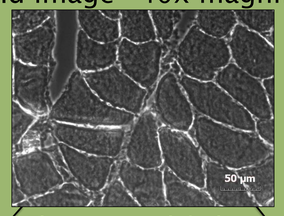


Carbonyl DNPH Carbonyl –DNPH product DNP

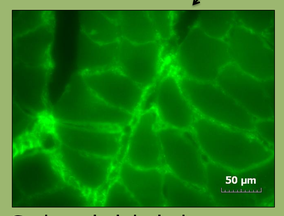
• Alexa-fluor488 rabbit anti-DNP primary antibody targets the stabilized DNP product.

Microscopy imaging

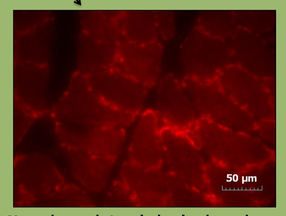
Bright field image- 40x magnification



Fluorescent labeling



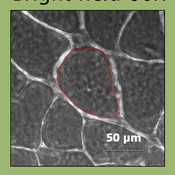
Carbonyls labeled green
Carbonyls react with DNPH to form DNP product, Alexa-fluor488 rabbit anti-DNP primary antibody



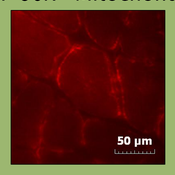
Mitochondria labeled red
Primary antibody- mouse anti-COXI targets cytochrome C oxidase I in the mitochondrial inner membrane. Secondary antibody- Alexa568-labeled goat anti-mouse

Image Analysis

Bright field 60x



RFP 60x- Mitochondria

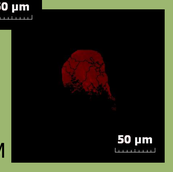


GFP 60x- Carbonyls

• Select a fiber (above)
• Use the threshold for mitochondrial detection on the red image to determine the location of the mitochondria in the SSM and IFM regions.
• Apply the region to the green image to determine carbonylation in the SSM and IFM regions.

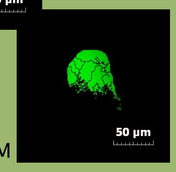
SSM

IFM



SSM

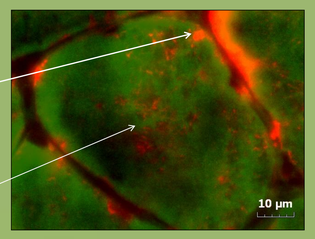
IFM



Carbonyl Quantification

Subsarcolemmal mitochondria

Intermyofibrillar mitochondria



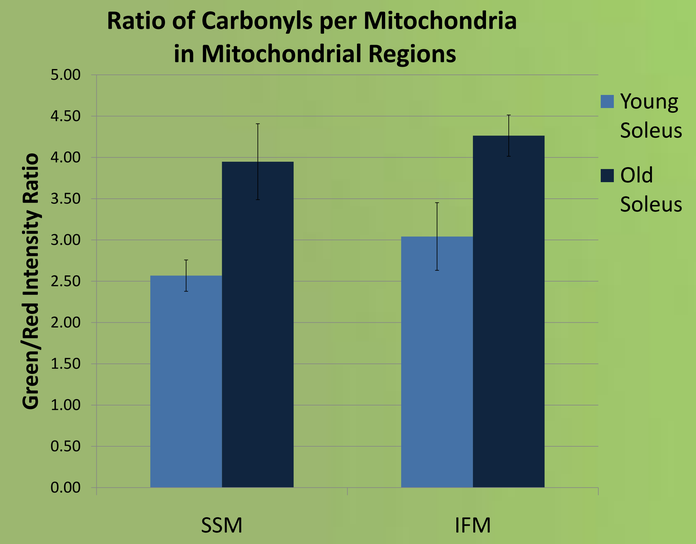
Red and green image overlay

• Green fluorescence corresponds to carbonyl levels.
• Red fluorescence is used to determine the location of the mitochondrial regions.

• Cells are analyzed individually to determine the location of the cellular regions of interest and the green fluorescent intensity of each region.
•A ratio of green intensity to red intensity shows the ratio of carbonyls per mitochondria and is used to compare carbonylation in the mitochondrial regions.

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.



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.

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.

Acknowledgments

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References

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Feng J, Navratil M, Thompson LV, Arriaga EA. (2008). Estimating relative carbonyl levels in muscle microstructures by fluorescence imaging. *Anal Bioanal Chem*. 391(7):2591-8. Erratum in: Anal Bioanal Chem. 2008 392(6):1249.