Comparison of carbonyl levels in young and old rats

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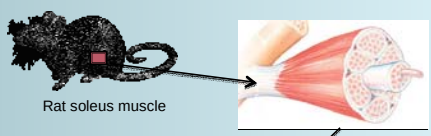
Introduction

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.

Goals

- To develop a photo-bleaching method to eliminate native fluorescence in muscle samples.
- To quantify the difference in carbonylation levels between mitochondrial regions of the muscle cell, specifically subsarcolemmal mitochondria (SSM) and intermyofibrillar mitochondria (IMF).
- To compare levels of oxidative damage in young and old animals.

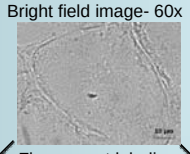
Experimental Strategy



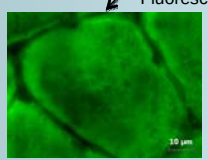
Rat soleus muscle

Muscle cross-sections are mounted on microscope slide

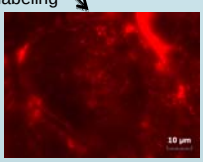
Bright field image- 60x



Fluorescent labeling



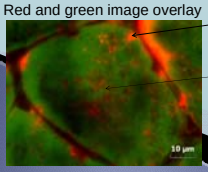
Carbonyls labeled green
Carbonyls react with DNPH to form DNP product, Alexa-fluor488 rabbit anti-DNP primary antibody



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

Microscopy imaging- 60x magnification

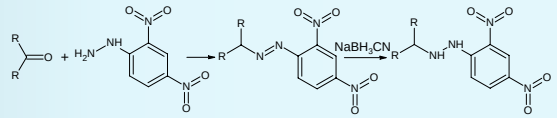
Red and green image overlay



Subsarcolemmal mitochondria

Intermyofibrillar mitochondria

Carbonyl Labeling



Carbonyl DNPH Carbonyl –DNPH product DNP

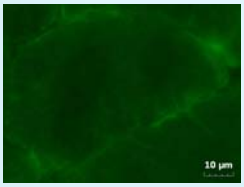
- Alexa-fluor488 rabbit anti-DNP primary antibody targets the stabilized DNP product.

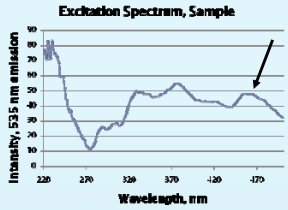
Photo-bleaching

Native fluorescence

Muscle tissue native fluorescence was causing high background and interfering with the quantification of carbonyls.

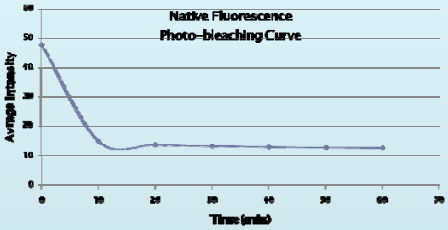
Green Native Fluorescence





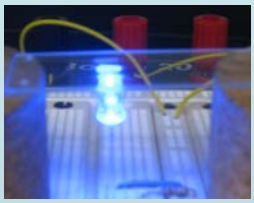
Microscope photo-bleaching - Small area

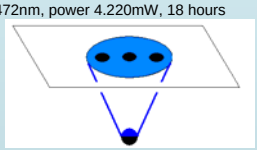
60x magnification only illuminates a small area



LED photo-bleaching - Large area

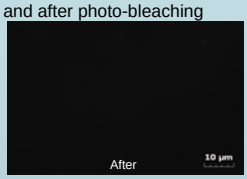
- Photo-bleaching apparatus using LEDs to photo-bleach the whole cross-section.
- Blue LED – B5515, nominal wavelength 472nm, power 4.220mW, 18 hours





Microscope slide with three cross-sections illuminated by a blue LED

Green native fluorescence before and after photo-bleaching



Carbonyls are more distinct after elimination of the native fluorescence in the background.

Images with carbonyls labeled green with Alexa488

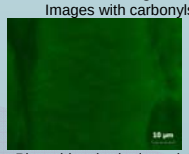
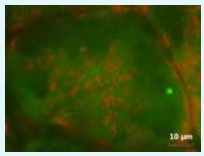


Photo-bleached prior to labeling

Without photo-bleaching

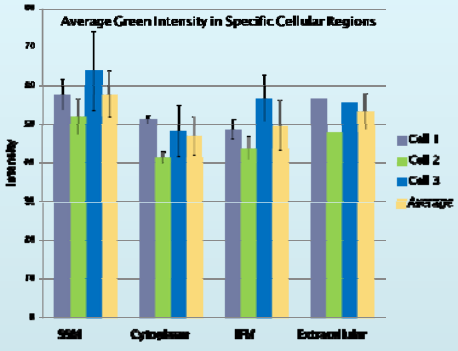
Carbonyl Quantification



- 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 fluorescent intensity of the cellular regions.

Cell	Area	SSM	Cytoplasm	IFM	Extracellular
1	178272	-	-	-	Area=21262
mean	-	57.63	51.25	48.92	56.6
stdev	-	3.88	1.14	2.59	-
total	-	16.632	6.150	9.233	1203376
stdev	-	1.181	0.211	3.478	-
2	190097	-	-	-	Area=17601
mean	-	52	41.36	43.87	48.15
stdev	-	4.63	1.63	3.07	-
total	-	8.622	6.984	2.044	847570
stdev	-	0.549	0.416	0.114	-
3	203739	-	-	-	Area=22743
mean	-	63.76	48.31	56.72	55.58
stdev	-	10.16	6.77	5.9	-
total	-	11.9250	4.6105	21.7559	1264022
stdev	-	0.6729	0.2579	5.6141	-



Summary

- A technique was developed using LEDs to photo-bleach native fluorescence in muscle samples.
- Preliminary results suggest 1) older tissue will have a higher levels of carbonylation than younger tissue, and 2) carbonyl levels in the SSM will be higher than the IFM.

Future Work

- Apply newly developed photo-bleaching technique to photo-bleach the native fluorescence in the samples before immuno-labeling the carbonyls and mitochondria.
- Compare levels of carbonylation in old and young animals
- Compare levels of carbonylation in cellular regions

Acknowledgments

Vratislav Kostal, Chad Satori, Joyce Chen, Janice Shoeman, Mark Chapman

Undergraduate Research Opportunity Program Grant

References

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.