GLOBAL CHANGES IN RIBOSOMAL PROTEIN EXPRESSION REGULATES MITOCHONDRIAL PROTEIN MASS AND FUNCTION IN DARK-CUTTING BEEF

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I. OBJECTIVES

Mitochondria remain active in postmortem muscles and can influence meat color via oxygen consumption. Previous studies have shown that dark-cutting beef has a high mitochondrial protein mass compared to normal-pH beef. However, the mechanisms that regulate mitochondrial proliferation in dark-cutting beef is still unknown.

II. MATERIALS AND METHODS

To evaluate how changes in mitochondrial protein expression may contribute to dark-cutting properties, mitochondria were isolated from 11 normal-pH and 11 dark-cutting loins. Oxygen consumption properties of isolated mitochondria from normal-pH and dark-cutting were determined using a Clark-oxygen electrode. Complex specific substrates were added to assess functions of complex I, II, and IV, membrane integrity (by addition of cytochrome c), and uncoupled oxidative phosphorylation using a Clark-oxygen electrode. Six ($n = 6$) out of the 11 samples from dark-cutting and normal-pH beef were subjected to liquid chromatography-tandem mass spectrometry-based proteomics analysis. A completely randomized block design was utilized to determine differences in mitochondrial functionality at $P < 0.05$, and the experiment was replicated 11 times ($n = 11$). The proteomics experiment was replicated 6 times ($n = 6$). The changes in mitochondrial protein expression were analyzed using multiple bioinformatics approaches and were considered significant at a false discovery rate < 0.05.

III. RESULTS

Liquid chromatography-tandem mass spectrometry-based proteomics analysis identified 1,863 proteins in the mitochondrial proteomes of dark-cutting and normal-pH beef. Of these, 162 proteins had significant changes in expression between dark-cutting versus normal-pH beef at false discovery rate < 0.05. In dark-cutting beef, 55 proteins were upregulated, while 37 were downregulated with a fold change >1.5. Functional annotation showed that these changes in protein expression comprised proteins involved in translation biological processes, structural constituent of ribosome, and large ribosomal subunit ribosomal RNA binding molecular functions. Pathway analysis showed that the majority of the upregulated DEP were involved in the ribosomal and mitochondrial electron transport pathways. The impact of these changes in mitochondrial protein expressions was further validated by assessing mitochondrial function in dark-cutting versus normal-pH beef. Dark-cutting beef had greater ($P < 0.05$) mitochondrial complex II respiration and uncoupled oxidative phosphorylation compared to normal-pH beef. However, evaluation of mitochondrial membrane integrity and respiration at complexes I, and IV, did not show significant differences ($P > 0.05$) between dark-cutting versus normal-pH beef.

IV. CONCLUSION

These results showed that changes in ribosomal proteins might regulate mitochondrial protein content and function in dark-cutting beef. Thus, a greater mitochondrial function could promote greater oxygen consumption, limiting myoglobin oxygenation in dark-cutting beef.

Keywords: dark-cutting beef, mitochondria, oxygen consumption, proteomics, ribosome