DISRUPTED MITOCHONDRIA INCREASE CALPAIN-1 ACTIVITY AND SUBSEQUENT PROTEOLYSIS *IN VITRO*

Chandler D. Stafford[1*], Jared F. Buhler[1], Mackenzie J. Taylor[1], David S. Dang[1], Kara J. Thornton[2], and Sulaiman K. Matarneh[*]

[1]Department of Nutrition, Dietetics and Food Sciences, Utah State University of Logan, Utah, USA

[2]Department of Animal, Dairy, and Veterinary Sciences, Utah State University of Logan, Utah, USA

*Corresponding author email: Sulaiman.Matarneh@usu.edu

I. INTRODUCTION

Tenderness is arguably the most important attribute of cooked meat that determines overall customer satisfaction [1]. Despite being influenced by several intrinsic and extrinsic factors, it is widely regarded that postmortem proteolysis is the primary contributor to end-product tenderness, with calpain-1 as the primary protease. A great deal of research has been devoted to understanding factors controlling calpain-1 activity. Yet, differences in tenderness still exist, suggesting that the mechanisms and modulators of calpain-1 are not fully understood. While mitochondrial contribution to meat quality are often disregarded, recent research has demonstrated that these organelles can modulate calpain-1 activity by regulating intracellular calcium levels [2]. However, a rise in mitochondrial calcium concentration is associated with generating reactive oxygen species, which trigger a cascade of events that eventually leads to mitochondrial dysfunction [3]. Hence, the aim of this research was to investigate how mitochondrial integrity influences calpain-1 activity and subsequent proteolysis *in vitro*.

II. MATERIALS AND METHODS

Freshly isolated mitochondria were disrupted and then added at different concentrations (0, 0.5, or 2.0 mg mitochondrial protein/ml) into an *in vitro* system (n = 6) that simulates postmortem glycolysis. This system consists of powdered muscle tissue and a reaction buffer containing metabolites needed for glycolysis. Calpain-1 autolysis and the degradation of protein markers were evaluated at 0, 2, 4, 24, 48, and 168 h. Data were analyzed using a mixed model for repeated measures. Differences were evaluated using a Tukey-Kramer Multiple Comparison Test, with $P \leq 0.05$ considered statistically significant.

III. RESULTS AND DISCUSSION

Our results indicate that samples with 2 mg mitochondrial protein/ml had a lower calpain-1 80 kDa subunit intensity at 4 and 24 h ($P \leq 0.05$; Fig. 1), indicating greater autolysis. However, at 48 and 168 h, the 80 kDa subunit was almost completely degraded in all treatments. The difference in calpain-1 autolysis among treatments may be attributed to calcium release from the mitochondria upon disruption, with potentially greater calcium release in the 2 mg mitochondrial protein/ml treatment. Enhanced desmin and troponin-T degradation was elicited as a consequence of increased calpain-1 autolysis in reactions containing 2 mg mitochondrial protein/ml. Increased degradation was observed at 2, 4, 24, 48, and 168 h ($P \leq 0.05$; Fig. 2 and 3, respectively). The 0.5 mg mitochondrial protein/ml treatment also had greater troponin-T degradation than the control at 24-168 h ($P \leq 0.05$). These results suggest that the disruption of mitochondria increases calpain-1 activity and proteolysis. However, disruption of the mitochondria may also release apoptotic factors (i.e., cytochrome c) that could activate caspase-3. Hence, further research is warranted to investigate the effect of mitochondrial disruption on caspase activity and subsequent proteolysis.

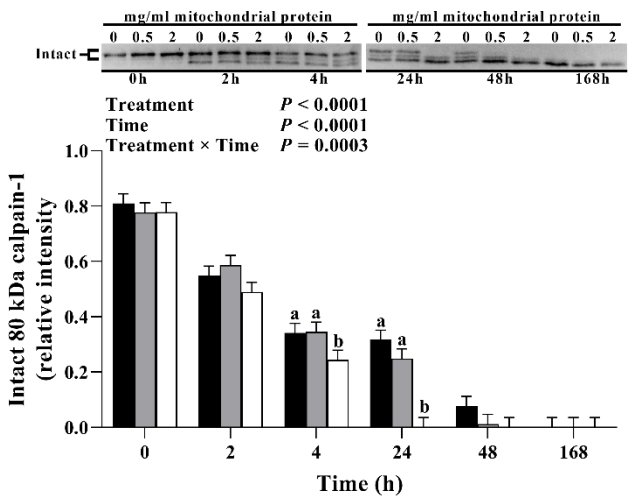

Figure 1. Calpain-1 autolysis over time

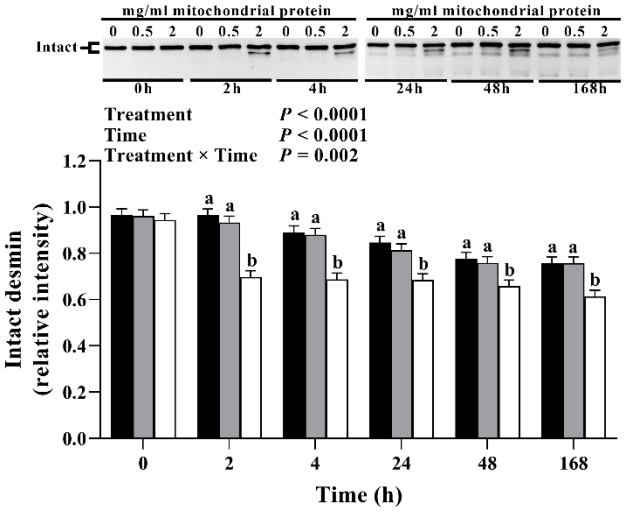

Figure 2. Desmin degradation over time

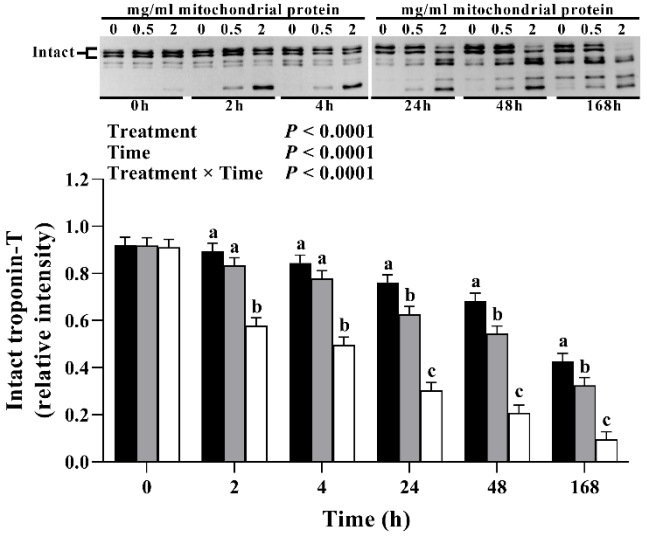

Figure 3. troponin-T degradation over time

IV. CONCLUSION

These findings suggest that mitochondria have the propensity to influence postmortem proteolysis by increasing calpain-1 autolysis. However, this effect may depend on mitochondrial integrity throughout the postmortem period. Future research is warranted to further elucidate the mitochondrial role in meat tenderization.

REFERENCES

Paper:

1. Destefanis, G., Brugiapaglia, A., Barge, M.T., Dal Molin, E., (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. Meat Science. 78: 153-156.
2. Dang, D.S., Buhler, J.F., Davis, H.T., Thornton, K.J., Scheffler, T.L., Matarneh, S.K., (2020). Inhibition of mitochondrial calcium uniporter enhances postmortem proteolysis and tenderness in beef cattle. Meat Science.162: 108039.
3. Kemp, C.M., Parr, T. (2012). Advances in apoptotic mediated proteolysis in meat tenderisation. Meat Science. 92: 252-259.