16 Sarcomere length influences μ-calpain mediated proteolysis of troponin-T.

A. D. Weaver*[1], B. C. Bowker[2], and D. E. Gerrard[1], [1]*Purdue University*, *West Lafayette, IN*, [2]*USDA-ARS, Food Technology and Safety Laboratory*, *Beltsville, MD*.

Muscle shortening and postmortem proteolysis are well established as mechanisms controlling beef tenderness. Inherent myofibril structure and the extent of overlap between myosin and actin filaments are hypothesized to affect the availability of substrates for degradation by calpains. The objective of this study was to determine the influence of sarcomere length on the extent of calpain-induced proteolysis of bovine myofibrils in vitro. Bovine semitendinosus (ST) muscles were excised within 20 min postmortem and dissected into strips which were stretched and attached to applicator sticks or allowed slack to generate samples with different sarcomere lengths upon rigor completion. Samples were allowed to undergo rigor in a buffer containing a protease inhibitor. Myofibrils were isolated and incubated at room temperature with exogenous μ-calpain at pH 6.8 for 0, 2, 60, 1440 or 2880 min. Purified troponin was also subjected to the same digestion conditions. Proteolysis of troponin T (TnT) was monitored using SDS-PAGE and western blotting. Sarcomere length was greater ($P < 0.0001$) in stretched versus shortened samples (2.99 μm ± 0.03 vs. 2.12 μm ± 0.03 respectively, means ± SE). The abundance of intact TnT decreased ($P < 0.0001$) with incubation time across both treatments. At 1440 and 2880 min, less ($P < 0.05$) intact TnT was detected in samples with long sarcomeres. These data indicate proteolysis of TnT occurs to a greater extent in samples with longer sarcomeres possibly due to easier access of proteases to their targeted substrates. Degradation patterns of TnT were similar between myofibrils and purified troponin following incubation with μ-calpain. Therefore it is unlikely that the mechanism by which proteolysis is limited in short sarcomeres involves an actomyosin-mediated interference of TnT.