EFFECTS OF ELECTRICAL STIMULATION ON μ-CALPAIN AND CATHEPSIN B+L ACTIVITIES IN POSTMORTEM BOVINE *LONGISSIMUS* MUSCLE

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Key Words: beef, calpain, cathepsin B+L, electrical stimulation, Warner-Bratzler shear force

Introduction

Consumers are aware of variability in beef product quality. According to one US study, 50% of consumers rated tenderness to be the single most important attribute of beef eating quality (Miller et al., 1995). High-voltage electrical stimulation (HV-ES) has been found to improve beef tenderness (Elgasim et al., 1981; Marsh et al., 1987), while the effect of low-voltage electrical stimulation (LV-ES) seems to be more variable (Hildrum et al., 1999). Despite concerns over its effectiveness, LV-ES of carcasses is in widespread use to enhance meat tenderness.

In a recent study we found a significant positive effect of LV-ES on Warner-Bratzler shear force (WBSF) in bovine *longissimus* (LD) muscle at 2 and 8 days *postmortem*, however large variation was observed in individual animals with regard to pH decline and WBSF (Hollung et al., 2007). This variation was not always correlated for the two responses; specifically some animals showed no response of LV-ES on pH decline but still displayed improved WBSF, and visa versa. Collectively, these results indicate that mechanisms other than accelerated pH decline and prevention of cold-shortening are affecting meat tenderness. Using samples from the Hollung study (Hollung et al., 2007), we sought to investigate the effects of LV-ES on proteases (μ-calpain and cathepsin B+L) in order to reveal whether altered activity of these enzymes could be a mechanism through which LV-ES improves meat tenderness.

Materials and methods

The ten Norwegian Red bulls (12-14 months) used in this study were slaughtered and treated as describe by Hollung et al. (2007). At 1, 3, 6, 10, and 24 h post-ES, samples for μ-calpain analysis were frozen in liquid nitrogen and stored at -80ºC for later analysis. Casein zymography (Raser et al. 1995) was used for analysis of μ-calpain activity. Regions of transparent gel (indicating μ-calpain activity) were measured (Quantity One, Version 4.5.0, Bio-Rad Laboratories, Inc., Hercules, CA), and quantities were expressed as a percentage of the 1-h sample activity measured in the NES side of each animal. Analyses of cathepsin B+L activities were performed on fresh samples at 2 and 8 days *postmortem* from 4 animals. Extractions and subcellular fractionation was performed according to Ertbjerg et al. (1999). Fractions were stored at -80ºC until measured using the fluorometric procedure described by Kirschke et al. (1983). Total activity of cathepsin B+L was calculated as the sum of the activity in the different subcellular fractions.

Analysis of variance in μ-calpain and cathepsin B+L data was performed using MINITAB's general linear model (Minitab, version 14.2). The model for cathepsin B+L included the following factors: animal (fixed), level of ES treatment, ageing period, and all two- and three-factor interactions. The model for μ-calpain included the following factors: animal (fixed), level of ES treatment, time post-ES, and the interaction of ES and time post-ES.

Results and discussion

We observed significant effects of LV-ES on μ-calpain activity at 1 and 24 h post-ES (Figure 1A). Taken together, the increased μ-calpain activity at 1 h and reduced activity at 24 h post-ES indicate accelerated μ-calpain activity and autolysis. It is likely that these changes would lead to an accelerated and increased *postmortem* proteolysis of myofibrillar and cytoskeletal proteins, and thus improved meat tenderness. Indeed, several groups have previously found that ES can accelerate *postmortem* proteolysis through changes in the calpain system (Ferguson et al., 2000; Geesink et al., 2001).

Release of cathepsins from the lysosomes has previously been suggested to accelerate proteolysis in muscle (Dutson et al., 1980; Pommier, 1992). No significant changes in cathepsin B+L activities resulted from the LV-ES treatment in this study (Figure 1B), making it less likely that these proteases play a crucial role in the observed improvement of meat tenderness. However, there was a strong tendency ($P = 0.052$) towards an increase in their activity in the soluble fraction as a result of LV-ES, suggesting that their influence cannot be excluded altogether.

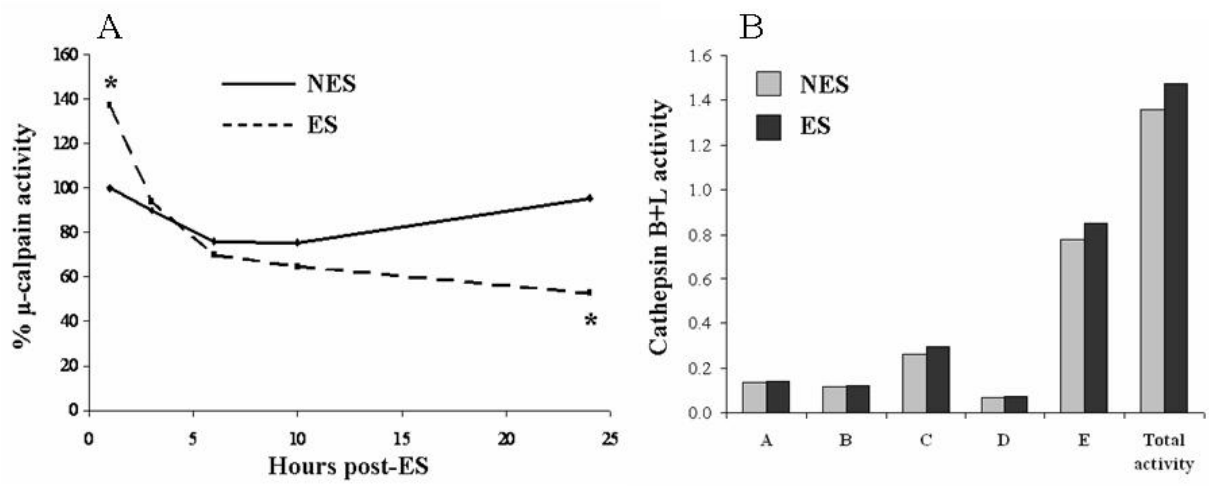

Figure 1. Protease activities in bovine LD muscle during *postmortem* storage. A. Activity of μ-calpain over time as a percentage of 1h-NES activity (n=10). B. Cathepsin B+L activities in different subcellular fractions (A=myofibrillar, B=heavy mitochondrial, C=lysosomal, D=microsomal, E=soluble) and total activity (n=4). *Significant ($P < 0.05$) differences between NES and ES

Conclusions

Hollung (et al. 2007) found a significant effect of LV-ES on pH at 1, 3, 6 and 10 h post-ES, but not at 24 and 48 h. Moreover, a significant positive effect of LV-ES on WBSF at both 2 and 8 days of ageing was observed. Since sarcomere lengths were unaltered following LV-ES, the reduced WBSF was not caused by a prevention of cold-shortening and must result from a different mechanism. The results of this study indicate that this mechanism is most likely increased μ-calpain activity. The accelerated rate of μ-calpain autolysis in the LV-ES samples indicates that the tenderisation process in these samples would be accelerated, and can explain the reduced WBSF seen as early as 2 days *postmortem*.

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