4-P24

EFFECTS OF ELECTRICAL STIMULATION & CHILLING RATE ON LYSOSOMAL ENZYME ACTIVITIES IN BEEF

J.M. O'Halloran[1], D.M. Ferguson[2], A.F. Egan[1] and I.H. Hwang[1]

[1] Cooperative Research Centre for the Cattle and Beef Industry (Meat Quality), University of New England, Armidale, NSW 2351
[2] Food Science Australia, PO Box 3312, Tingalpa DC, Qld 4173

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Background

Commercial interest in mechanisms, which accelerate postmortem tenderisation, has intensified recently, as these will lead to significant economic benefits through reduced storage costs. The exact contribution of all proteolytic enzyme systems involved in postmortem tenderisation has not been fully elucidated. Evidence suggests that the degradation of cytoskeletal proteins via the calcium activated calpain system plays a prominent role during ageing (Dransfield, 1993; Goll, 1991; Koohmaraie, 1994). The contribution of the lysosomal cathepsins is one issue, which continues to invoke considerable debate (Goll *et al.*, 1983; Ouali, 1992). The involvement of cathepsins in tenderisation is dependent on their release from the lysosomal vesicle (Goll *et al.*, 1983). Increased lysosomal lability and the release of cathepsins have been associated with postmortem ageing (Chambers *et al.*, 1994; Kas *et al.*, 1983; Pommier, 1992). Disruption of the lysosomal membrane has also been associated with storage at high temperatures (Moeller *et al.*, 1977), conditions of rapid glycolysis and low pH (Ertbjerg, 1996) and electrical stimulation (Pommier, 1992).

Objectives

The objective of this study was to determine the effects of electrical stimulation and chilling rate on the release of the lysosomal enzymes; cathepsin B+L. Variation in both pH and temperature decline was achieved using different stimulation durations and chilling regimes.

Methods

Animals: Twenty grass-fed Angus x Hereford steers approximately 22 months of age were slaughtered at the Food Science Australia research facility. Groups of four steers were slaughtered each day over a five-day period. Mean live and carcass weights were 353 and 182kg, respectively, with an average 12/13th fat depth of 2.9 mm.

Treatments: Carcasses were randomly allocated to two low voltage electrical stimulation (LVES) treatments (10 seconds or 40 seconds duration) and the sides of each carcass were either chilled rapidly or slowly. The electrical stimulation (45 V peak voltage, 36 pulses/second, 500 mA current) was applied within five minutes post-slaughter via a nostril/rectal probe configuration. Rapid chilling was achieved by placing the right sides in a 1°C chiller equipped with a blast fan (1.25 m/s air speed) and spraying them with an ice slurry every 5 – 10 minutes for the first hour in the chiller. Slow chilling of the left sides was achieved by holding in a room at 18°C for two hours before transfer to a chiller (6°C, 0.45 m/s air speed) for 7 – 11 hours. Thereafter the sides were relocated to the fast chiller.

Measurements: Temperature decline in the *m.longissimus* was monitored using data loggers (Cox Tracer, USA). pH was measured every 15 minutes for first two hours post-slaughter and every hour thereafter for the next five hours. The procedure involved taking muscle cores (approx. 0.5 g) from the caudal end of the *m.longissimus* and freezing in liquid nitrogen. The pH was then measured in iodoacetate homogenate (Bendall, 1973). Muscle samples (2 g) were collected at 0.5, 24 and 72 hours post-mortem for determination of lysosomal cathepsin activity according to the procedures of Ertbjerg (1996) and Kirschke *et al.* (1983). Briefly this involved homogenising the sample prior to differential centrifugation to obtain the various sub-cellular fractions (ie. myofibrillar, heavy mitochondrial, lysosomal, microsomal and soluble fractions). The combined cathepsin B+L activities in each fraction were then determined fluorimetrically using Z-Phe-Arg-NMec as the substrate.

Statistical Analysis: The pH decline was modeled using the exponential function $pH(t) = pH_{\mu} + (pH_0 - pH_{\mu})e^{-kt}$, where pH(t) was the pH at time t, pH_0 and pH_{μ} were the pH at t = 0 and t= μ, respectively and k was the rate constant of pH decay. This equation was fitted using a non-linear statistical package and the coefficients were used to calculate the time to pH 6.0. The effects of the electrical stimulation and chilling treatments were analysed using a SAS's GLM procedure. Subcellular fraction was also included in the model as a fixed effect. The cathepsin B+L activities of the heavy mitachondrial, lysosomal and microsomal fractions were pooled together and defined as the membrane fraction for graphical presentation of the results.

Results and Discussion

The duration of electrical stimulation and chilling rate resulted in significant changes in the rate of release of cathepsin B+L from the lysosomes. In general, there was increased catheptic activity in the soluble fraction in those treatments where the rate of pH decline was accelerated (ie. 40 seconds LVES and slow chilling). In earlier studies (Kas *et al.*, 1983; Chambers *et al.*, 1994), it was shown that with increasing ageing time there is a gradual increase in catheptic activity within the soluble fraction due to slow deterioration of the lysosomal membranes. The results here indicate a similar pattern for those carcasses receiving 10 seconds where cathepsin B+L activity in the soluble fraction increased linearly with postmortem time (Figure 1). However, this contrasts with the trend observed in the 40 second LVES group (Figure 2). Cathepsin B+L activity in the soluble fraction increased rapidly to a peak value by 24 hours postmortem. These contrasting trends are largely the result of the accelerated rate of glycolysis in the 40 seconds LVES group relative to 10 seconds LVES. Rapid rates of glycolysis (O'Halloran *et al.*, 1997) and conditions of low pH (Ertbjerg, 1996) or high temperature (Moeller *et al.*, 1977) have been shown to enhance the rate of cathepsin release from lysosomes. Electrical stimulation, in addition to its influence on glycolytic rate, may also cause physical disruption of lysosomal membranes and therefore accelerated release of lysosomal enzymes (Pommier *et al.*, 1987).

Membrane cathepsin B+L activity would be expected to inversely reflect changes in the enzyme activity of the soluble fraction (Pommier, 1992; Chambers *et al.*, 1994) as enzymes are released from the lysosomal vesicle into the soluble fraction. This is

supported by the data obtained for the 40 second LVES group which shows a concomittant decline in membrane cathepsin B+L activity as enzyme activity in the soluble fraction increases at 24 hours postmortem. Membrane cathepsin B+L activity for the 10 second LVES group declined with postmortem time until 24 hours postslaughter.

Given the effect of pH decline, the 24 hour post-mortem data was reanalysed using time to pH 6.0 as a covariate in the model. As expected, a significant interaction ($P<0.001$) was observed between time to pH 6.0 and fraction activities. The faster the pH decline, the higher the cathepsin B+L activities within the soluble fraction. There was a tendency for the soluble fraction activities to decline exponentially as time to attain pH 6.0 increased (Figure 3). Cathepsin B+L activity in both the myofibrillar and membrane fractions tended to increase as time to reach pH 6.0 increased. These results suggest that the rapid rate of glycolysis induced by the application of electrical stimulation promoted release of lysosomal enzymes with enhanced release after 40 seconds LVES.

Conclusions

Post-slaughter carcass treatments designed to accelerate the rate of pH decline resulted in increased lysosomal lability with concomitant increases in cathepsin B+L activity within the soluble fraction. This was particularly evident for carcasses receiving the recommended 40 seconds of LVES. It can be concluded that cathepsins are released, however, the question of their activity remains. What myofibrillar or cytoskeletal proteins do they target during ageing?

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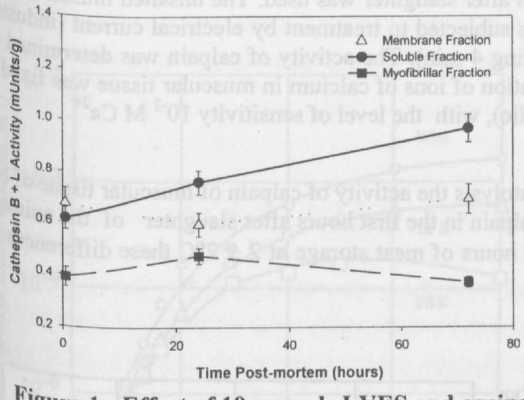

Figure 1: Effect of 10 seconds LVES and ageing on the subcellular distribution cathepsin B+L activities

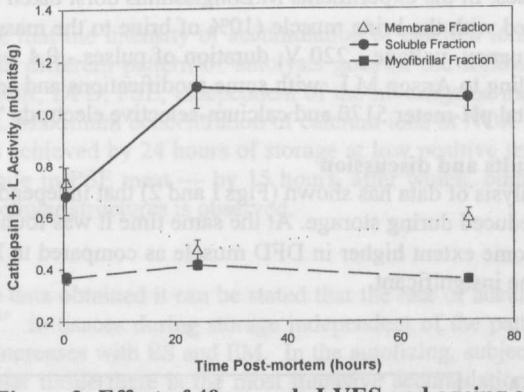

Figure 2: Effect of 40 seconds LVES and ageing on the subcellular distribution cathepsin B+L activities

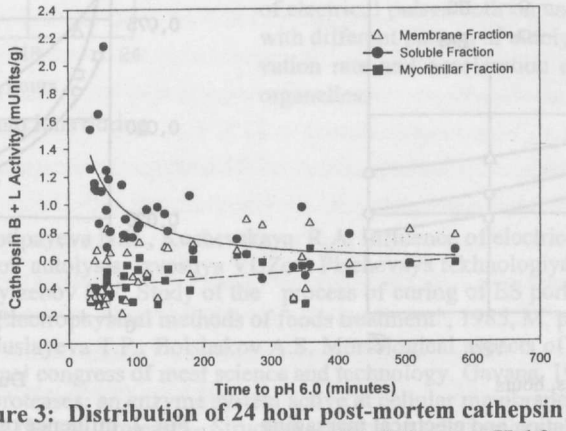

Figure 3: Distribution of 24 hour post-mortem cathepsin B+L activities as a function of predicted time to pH 6.0