Seasonal changes in the growth and calpain system of red deer neck muscles

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Introduction

Secondary sexual characteristics of male deer, such as antler growth and neck muscle hypertrophy, are associated with seasonal changes in photoperiod which control the annual androgen sexual cycle (Fennessey et al., 1988, 1991; Barrel et al., 1985). The hypertrophy found in the neck muscle, the M. *splenius*, results from increased muscle fibre size. This change in muscle fibre size may be due to changes in either the circulating concentrations of androgens or with the levels of circulating insulin-like growth factors. These hormones also change with daylight length and have been shown to be involved in muscle growth (Suttie et al., 1983,1985, Asher et al., 1987, 1989). The growth of the M. *splenius* and other muscles during the rut, the period of sexual activity, is dependent on the accretion of protein and this has been associated with protein turnover. The calcium dependent enzymes, μ- and m-calpains and their inhibitor calpastatin have been implicated in protein turnover and the calpain system is also involved in postmortem tenderisation.

The present study examined the relationship of the calpain system with the M. *splenius* during its seasonal growth cycle in both young and old stags and builds on our previous study on muscle changes in and out of the rut (Dobbie et al., 1994). The relationship between the calpain system and meat tenderness was also examined.

Materials and Methods

Samples were obtained throughout the year, from late June to following April, from 210 pasture fed red deer stags. The animals ranged from 18 months to 5 years of age and were slaughtered in a commercial abattoir. The sampling regime covered the time periods of "late rut" through "out of rut" to "early rut."

As a routine practise electrical stimulation was applied to the carcass immediately post slaughter. The animals were slaughtered, dressed, hot carcass weights were recorded and within 30 minutes of slaughter the M. *splenius* was dissected out and processed for later analysis. Calpain and calpastatin analyses were performed using the method of Wheeler and Koohmaraie (1991) with slight modifications (Sainz et al., 1992). In brief, 5 gram muscle samples were homogenised in Tris[hydroxymethyl]amino-methane (Tris) buffer (40 mM Tris., 10 mM ethylenediaminetetra-acetic acid (EDTA), 2% Triton X-100, 10 mM ß-mercaptoethanol (MCE); pH 7.5) containing protease inhibitors (2.5 μM E-64, Boehringer, Germany; 100 mg/litre Ovomucoid, Sigma Type III-O Trypsin Inhibitor, U.S.A; and 2.0 mM Phenylmethanesulfonyl fluoride, Boehringer). The soluble extract was applied to a DEAE Sephacel ion exchange column (10 mm x 100 mm) and equilibrated with Buffer A (40 mM Tris, 0.5 mM EDTA, 10 mM MCE; pH 7.5). Using a stepwise salt gradient calpastatin was eluted with Buffer A + 100 mM NaCl, μ-calpain with Buffer A + 200 mM NaCl and m-calpain with Buffer A + 300 mM NaCl. Calpain enzyme activities were assessed using casein (Hammarsten, Merck, Germany) as the substrate. One unit of calpain activity is defined as the amount of enzyme that catalyses an increase of one absorbance unit at 278 nm in 60 minutes at 25°C. Calpastatin activity was assayed as the inhibition of m-calpain activity (Wheeler and Koohmaraie 1991). Ageing rate was determined using an accelerated ageing regime in a 15 °C waterbath and after 1,2,4 and 7 days sub-samples were cooked to an internal temperature of 75°C, allowed to cool to 2 °C (Graafhuis et al., 1991) and peak shear-force assessments made using the MIRINZ pneumatic tenderometer (Frazerhurst and MacFarlane, 1983). The data was analysed by ANOVA.

Results

Regardless of age, both the calpastatin ($p<0.001$) and μ-calpain ($p<0.01$) activity had a significant quadratic association with time (Fig. 1). Both the activities were low during the spring and early summer (August-December) and then increased during the summer, reaching their peaks in mid winter. The μ-calpain and calpastatin activities increase as the M. *splenius* size increases and muscle hypertrophy is occurring. There were no differences in the seasonal trends between old (>2 years) and young (≤2 years) stags, although the young growing stags had consistently lower levels of calpastatin ($p<0.05$) and μ-calpain ($p<0.05$) activity than the older stags. There was no similar relationship for m-calpain activity although there was a linear trend of increased activity with age ($p<0.05$). Shear force after 7 days at 15°C for M. *splenius* was related to time through the season ($p<0.01$) with the highest shear force being associated with the lowest μ-calpain and calpastatin levels of activity (Fig. 2). In young deer there was a significant relationship between the shear force measured at day 7 post mortem ($p<0.01$) and the rate of ageing ($p<0.01$) with time of year. Out of rut deer had the lowest rate of ageing and highest ultimate shear force which again correlates with the lowest levels of μ-calpain and calpastatin activity (Fig. 2).

Discussion

The results from this study clearly show that as the M. *splenius* decreases after the rut the levels of μ-calpain and calpastatin activity also decrease. The calpain proteolytic system has been related to protein degradation in muscle therefore a decrease in the activity of the calpain system would be expected to be associated with increased muscle mass. This was not the case in this study nor in an earlier report (Dobbie et al., 1995). An explanation of the observations are that the calpain system plays a positive house keeping role (Illian and Forsberg., 1992) in that protein accretion associated with muscle growth is a balance between protein degradation controlled by the interaction of μ-calpain and its inhibitor calpastatin and protein synthesis. In this study the calpain / calpastatin ratio did not show a significant correlation with muscle growth. Meat tenderness and ageing rate were lowest when the muscle size was at a minimum and the levels of μ-calpain and calpastatin activity were at their lowest. These results indicate as previously shown that the calpain system is negatively related to meat tenderness and rate of ageing.

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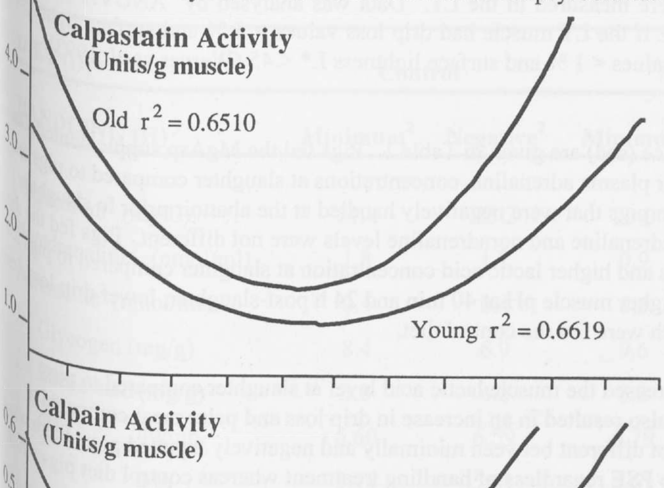

Figure 1. Quadratic lines of best fit for activities of calpastatin and μ-calpain showing levels of activity throughout the year for young and old male Red Deer in and out of rut.

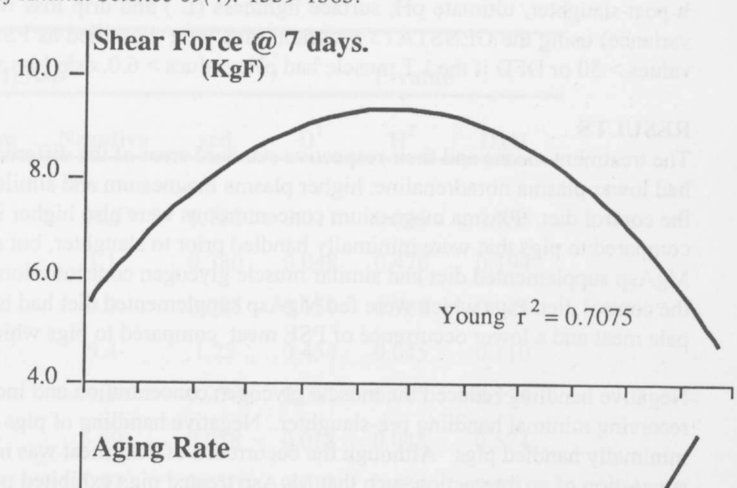

Figure 2. Quadratic lines of best fit for shear force (KgF) and aging rate of meat from M.*splenius* of young male Red Deer throughout the year.