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Inter-muscle variation in the calpain system of red deer:- implications for meat tenderness

P.M. DOBBIE, K.K. SINGH, B.C. THOMSON, G.K. MERCER, J.J. BASS AND P.A. SPECK.

AgResearch, Ruakura Research Centre, Private Bag 3123., Hamilton, New Zealand.

ABSTRACT

There is little information available on the muscle biology of deer and how this affects meat quality. The reported effects of the calcium dependent protease system (calpains and their inhibitor calpastatin) on protein turnover *in vivo* and myofibrillar structure post mortem suggest it may have a pivotal role in regulating muscle growth and meat tenderisation. This study examines inter-muscle variation in the calpain system under two physiological conditions in red deer and its relation to meat tenderness. Nine muscle samples from 3 stags in rut (IR) and 3 out of rut (OR), were processed for calpain and calpastatin analysis within 20 minutes of slaughter. Tenderness of aged muscles was determined by measurement of ultimate shear force. The majority of the inter-muscle differences in the calpain system were associated with calpastatin activity. A positive relationship between muscle calpastatin levels and meat ultimate shear force was found.

Keywords: deer; rut; calpain; calpastatin; muscle; hypertrophy.

INTRODUCTION

Seasonal fluctuations of photoperiod give rise to the annual cycle of androgen induced secondary sex characteristics such as the antler and pronounced neck muscle growth exhibited by adult male deer during the rut (Fennessy *et al.*, 1988., 1988., Suttie *et al.*, 1989). The increase in neck girth and concomitant increased growth of *M. splenius* is the result of increased fibre size (Field *et al.*, 1985). The rates of protein biosynthesis and degradation largely determine the extent of protein content and mass of skeletal muscle. Studies have indicated that the calpain proteolytic system (ie. μ - and m-calpains, active at μ M and mM Ca^{2+} ion concentrations respectively and their endogenous inhibitor, calpastatin) is involved in the control of muscle growth, protein turnover and meat tenderisation (Koochmaraie, 1992). Post mortem these enzymes continue to be active and affect the textural and tenderness properties of meat.

The present study examines the biochemical basis of neck muscle hypertrophy observed during the rut compared with other muscles and assesses the impact on subsequent meat tenderness.

MATERIALS AND METHODS.

Aged red deer stags were grazed on pasture then slaughtered in late rut (mid June) (n=3) and out of rut (November) (n=3). On the day of slaughter the stags were penned, loaded and trucked 1 km to the Ruakura experimental abattoir with the minimum of stress. Following dressing hot carcass weights were recorded and samples collected from *M. biceps femoris*, *M. gastrocnemius*, *M. infraspinatus*, *M. longissimus dorsi*, *M. psoas major*, *M. semitendinosus*, *M. splenius*, *M. supraspinatus*, *M. vastus intermedius*. Calpain and calpastatin analyses were performed using the method of Wheeler and Koochmaraie (1991) with slight modifications (Sainz *et al.*, 1992). In brief, 5 gram muscle samples were homogenised within 20 minutes of slaughter in Tris[hydroxymethyl]amino-methane (Tris)

buffer (40 mM Tris., 10 mM ethylenediaminetetra-acetic acid (EDTA), 2% Triton X-100, 10 mM β -mercaptoethanol (MCE); pH 7.5) containing protease inhibitors (2.5 μ M 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 (Sainz *et al.* (1992). 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 Koochmaraie, 1991).

Carcasses were aged at 4°C for 96 hours then muscles were dissected, weighed and stored frozen at -20 °C until required for shear force measurements. The samples were thawed at 4 °C, cooked in a 100°C water bath to an internal temperature of 75°C, allowed to cool to 2 °C (Graafhuis *et al.*, 1991) and peak shear-force measurements made using the MIRINZ pneumatic tenderometer (Frazerhurst and MacFarlane, 1983). Statistical analysis was performed using the Minitab Statistical analysis package (Version 7.2; Minitab Inc., State College, PA, USA) using an analysis of variance routine.

RESULTS

In agreement with an earlier study (Stevenson *et al.*, 1992) stags in rut (IR) had lighter carcass weights (85 ± 1.0 kg) than those out of rut (OR) (106 ± 2.4 kg) ($p < 0.05$). The *M. splenius*, *M. longissimus dorsi* and *M. psoas major* were heavier IR ($p < 0.05$), however there were no significant differences in weight when comparing other IR and OR muscles (Table 1). The results indicate that in general, muscle mass is

maintained despite a significant decline in live weight. A change in fat cover occurred, possibly the result of voluntary food deprivation for a period during the rut (Suttie and Simpson, 1983).

Calpastatin levels were elevated ($p < 0.05$) in all muscles IR compared to all muscle in the OR period. (Figure 1). The μ - and m-calpain activities were not significantly different ($p > 0.10$) between muscles or between IR and OR animals (Table 1). The ultimate shear force was significantly different ($p < 0.05$) between some muscle types (Figure 2). The rutting period appears to have no significant effect on ultimate meat tenderness. There was a significant ($p < 0.05$) positive relationship between calpastatin inhibitory activity and shear force (Figure 3), (Calpastatin activity (U/g fresh muscle) = $0.969 \text{ shear-force (kgF)} + 0.053$, $r^2 = 0.45$, $p < 0.05$), the slope and the intercept of the relationship did not differ significantly between IR or OR periods (data not shown).

DISCUSSION

The focus of this preliminary study was to elucidate the biochemical basis of muscle hypertrophy in male red deer during the rut and to determine the effects this may have on meat tenderness.

The calpain proteolytic system is an important factor in protein degradation *in vivo* and it was expected that a reduction in the activity of the calpain system would result in increased muscle size. In this study the μ - and m-calpain activities were similar among the different muscles, did not change markedly IR and OR (Table 1), and were similar to those measured in sheep and cattle by our laboratory. The lack of change in μ - and m-calpain activity with animals IR and OR is consistent with earlier studies that suggest the calpains play a house keeping role *in vivo* (Ilian and Forsberg, 1992).

Calpastatin activities increase during the rut, although this elevated calpastatin activity was not always associated with muscle hypertrophy, those muscles which did not undergo hypertrophy included *M. infraspinatus*, *M. semitendinosus* and the *M. vastus intermedius* (Table 1). The varied response in muscle growth during the rut may be the result of a marked increase of calpastatin inhibitory activity, allowing protein synthesis to exceed protein degradation, resulting in hypertrophy in some muscles. In the other muscles smaller increases in calpastatin activity were observed which may have been sufficient to simply reduce protein degradation to balance the reduced protein synthesis that may be expected during periods of nutritional challenge. The possibility exists that differences in response to the increased

FIGURE 1: Muscle calpastatin activities expressed as units per gram of tissue for out of rut animals (□) and in rut animals (■). (Means \pm SEMs.) * denotes a significant difference of $p < 0.05$ between OR and IR.

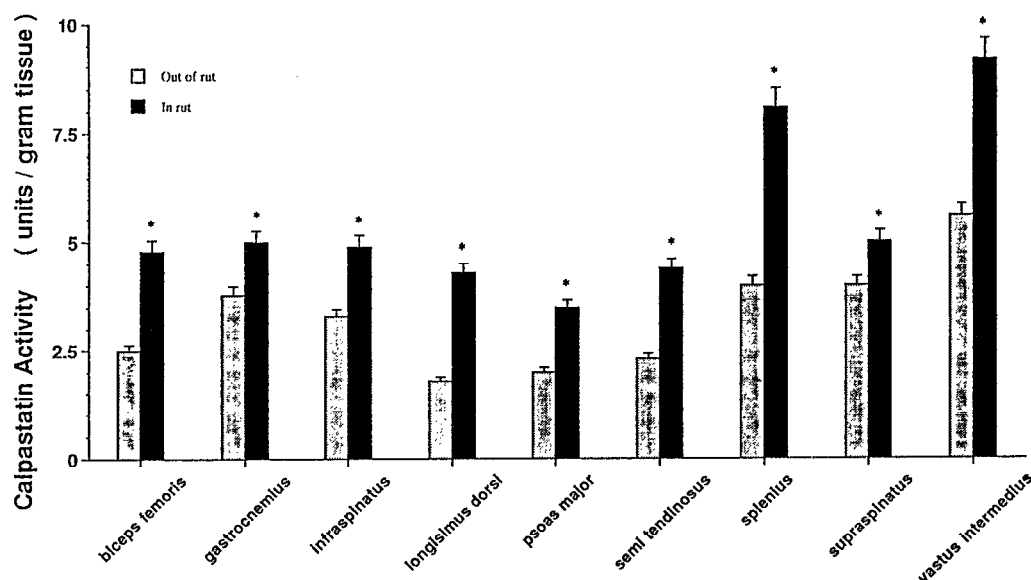
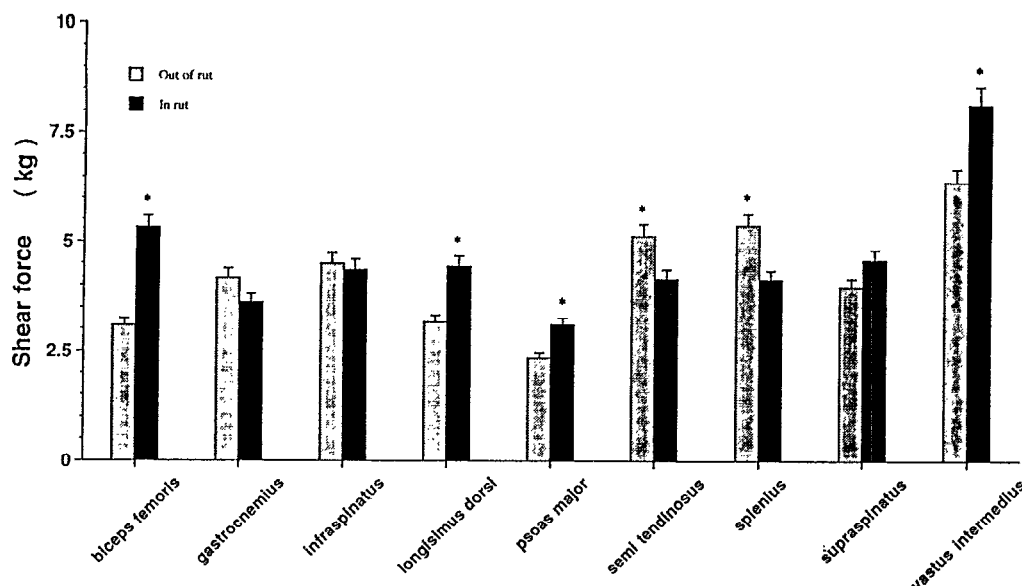


TABLE 1: Activity levels of μ - and m-calpain (Units of activity / gram of muscle) \pm SEM and the muscle weights (kg) \pm SEM, out of rut (OR) and in rut (IR).

Muscle	μ -calpain		m-calpain		Muscle weight	
	OR	IR	OR	IR	OR	IR
biceps femoris	0.8 \pm .05	1.0 \pm .30	0.7 \pm .06	0.7 \pm .09	1.74 \pm .12	1.81 \pm .07
gastrocnemius	0.8 \pm .20	1.0 \pm .23	0.8 \pm .10	0.7 \pm .09	0.17 \pm .01	0.18 \pm .02
infraspinatus	0.7 \pm .06	0.9 \pm .10	0.7 \pm .01	0.7 \pm .05	0.57 \pm .05	0.61 \pm .02
longissimus dorsi	0.7 \pm .11	0.8 \pm .04	0.6 \pm .05	0.7 \pm .15	2.24 \pm .13	2.81 \pm .06*
psoas major	0.8 \pm .04	1.2 \pm .27	0.6 \pm .05	0.7 \pm .09	0.45 \pm .02	0.55 \pm .05*
semi tendinosus	0.8 \pm .03	1.0 \pm .18	0.7 \pm .04	0.6 \pm .07	0.66 \pm .08	0.61 \pm .05
splenius	1.1 \pm .08	1.1 \pm .09	0.8 \pm .07	1.0 \pm .12	0.36 \pm .01	0.52 \pm .02*
supraspinatus	0.8 \pm .04	0.8 \pm .25	0.7 \pm .03	0.8 \pm .15	0.75 \pm .04	0.81 \pm .02
vastus intermedius	1.1 \pm .37	0.7 \pm .02	0.8 \pm .02	0.7 \pm .09	0.18 \pm .02	0.15 \pm .03

* denotes a significant difference between OR and IR ($p < 0.05$)

FIGURE 2: Individual muscle shear force in kilograms as measured using the MIRINZ tenderometer for out of rut animals (□) and in rut animals (■). (Means ± SEMs.) *denotes a significant difference of $p < 0.05$ between OR and IR.



calpastatin activity may in part be explained by the multiple isoforms of calpastatin although these measurements were not carried out in the present experiment (Asada *et al.*, 1989 and Lee *et al.*, 1992).

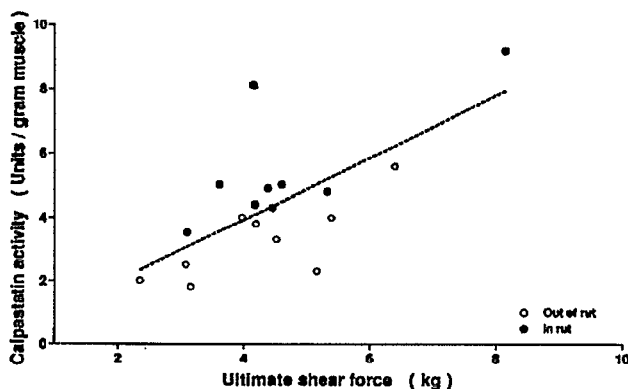
It has been suggested that the calpastatin inhibitory activity post-mortem is a key regulator of post mortem proteolysis and, ultimately, tenderness (Koochmaraie, 1992). The present study agrees with those results reported in sheep and cattle (Whipple and Koochmaraie, 1992) and supports a generalised relationship between increased calpastatin activity and decrease in tenderness. However, despite all muscles having elevated levels of calpastatin IR, a range of responses with respect to shear force values between different muscles was observed (Figure 2). Muscles in which shear force was elevated IR included *M. biceps femoris*, *M. longissimus dorsi*, *M. psoas major* and *M. vastus intermedius*. Other muscles showed no significant change in shear force despite an elevated level of calpastatin IR, these included the *M. gastrocnemius*, *M. infraspinatus* and the *M. supraspinatus*. Whilst other muscles showed a significant decline in shear force, despite elevated levels of calpastatin IR, these included *M. splenius* and *M. semitendinosus*. This preliminary study suggests that for some muscles in the rutting stag the effects of the calpain system on muscle size and meat tenderness may be varied. Therefore this model offers the opportunity to examine these differences and determine the pre and post mortem effects on muscle. Further work is required to fully evaluate the temporal nature of the calpain/calpastatin system and its impact on muscle growth and post mortem proteolysis.

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FIGURE 3: Calpastatin expressed as units of activity per gram of muscle vs ultimate shear force in kilograms as measured by the MIRINZ tenderometer.

Calpastatin activity = $0.969 \text{ ultimate shear force} + 0.053$, $r^2 = 0.458$ ($p < 0.05$).



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