The calpain proteolytic system in different types of ovine skeletal muscles and relationship to meat tenderness

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ABSTRACT

The calpain system is involved in postmortem tenderisation of meat. Muscle type also influences tenderness and aging rate. This study investigated the extent to which differences in the calpain system may account for differences in tenderness. Calpain and calpastatin activities were measured from 12 different ovine muscles. Shear force was determined on muscle aged at 15°C for 1, 2, 3 and 4 days postmortem and ultimate pH measured. Calpastatin, μ- and m-calpain, shear force and pH measures were different between muscles. Calpastatin activity had a positive association and both μ-calpain:calpastatin ratio and μ-calpain had a negative association with shear force of aged muscle, within muscles. These results suggest that greater calpastatin activity and less μ-calpain activity results in tougher meat. This may result from increased inhibitory actions of calpastatin on the proteolytic actions of calpain, resulting in less proteolysis of myofibrillar proteins and tougher meat.

Keywords: calpains; calpastatin; tenderness; muscle type; ovine.

INTRODUCTION

Meat tenderness differs between different muscles from within one animal (Olson *et al.*, 1976). Several factors have been implicated in influencing tenderness, such as postmortem proteolysis of myofibrillar proteins (Goll *et al.*, 1989), muscle fibre composition (Dransfield et al., 1981) and size (Crouse *et al.*, 1991), total collagen, type and quantity of reducible crosslink of collagen (Light et al., 1985). The proteolytic enzymes μ- and m-calpain and their inhibitor calpastatin are considered important in postmortem tenderisation of meat by degrading myofibrillar proteins thus causing a weakening of myofibrillar structure (Koohmaraie *et al.*, 1988). The aim of this study was to determine the extent to which differences in calpain and calpastatin activities may account for differences in tenderness between muscles. This will provide a further understanding of mechanisms involved in tenderisation of meat.

MATERIALS AND METHODS

Coopworth x Dorset ewe lambs, 4 months old (n=17), were fed a pelleted diet (lucerne 60% and barley 30%) and water ad libitum for 3 weeks. Muscles were collected within 30 min of slaughter and ranged in fibre type composition. Muscles composed of predominantly type I (slow-twitch oxidative) fibres; *M. masseter* (M), *M. supraspinatus* (SS), *M. vastus intermedius* (VI): type IIA *(fast-twitch oxidative-glycolytic) fibres; M. biceps femoris* (BF), *M. gastrocnemius* (G), *M. longissimus dorsi* (LD), *M. semimembranosus* (SM): type IIB (fast-twitch glycolytic) fibres; *M. gracillus* (GR), *M. semitendinosus* (ST), *M. tensor fascia latae* (TFL): type II fibres; *M. cutaneous trunci* (CT) *and M. psoas major* (PM). Calpastatin, μ- and m-calpain activities were extracted from fresh 5g samples and separated on a DEAE Sephacel column using a stepwise NaCl gradient (Wheeler and Koohmaraie, 1991; Sainz et al., 1992). Calpain activities were determined against casein (Hammarsten, Merck, Germany). One unit of calpain activity is defined as the amount of enzyme that catalyses an increase of 1 absorbance unit at 278nm in 60min at 25°C. Calpastatin was assayed as the inhibition of m-calpain activity. Ultimate pH was measured using an Ingold stab probe in muscle aged at 15°C for 24h postmortem. Shear force analysis was carried out on muscles aged for 1 and 3 days or 1, 2 and 4 days postmortem at 15°C. Samples were cooked and analysed for peak shear force using a MIRINZ pneumatic tenderometer (Frazerhurst and MacFarlane, 1983). Restricted maximum likelihood (REML), using the Genestat statistical package, was used to obtain means for the muscles. This also provided likelihood ratio (LR) tests (asymptotically chi-squared) for this effect and the relationships between tenderness and activities of calpastatin and calpains on a between and within muscle mean basis.

RESULTS

There were significant differences between muscles in calpastatin ($p<0.001$), μ-calpain ($0.05<p<0.06$) and m-calpain activities ($p<0.001$, Table 1). Calpastatin activity in LD was lower ($p<0.05$) than TFL, GR, CT, PM, SS, G and VI. The LD, BF, M, SM and ST had lower ($p<0.05$) activity than CT, PM, SS, G and VI. The VI had higher ($p<0.05$) calpastatin activity than all other muscles. The μ-calpain activity was lower ($p<0.05$) in CT and PM than G, VI and BF. The m-calpain activity was lower ($p<0.05$) in CT, ST, PM, SM, LD, TFL, SS, BF and GR than VI and M. The G had lower ($p<0.05$) activity than M.

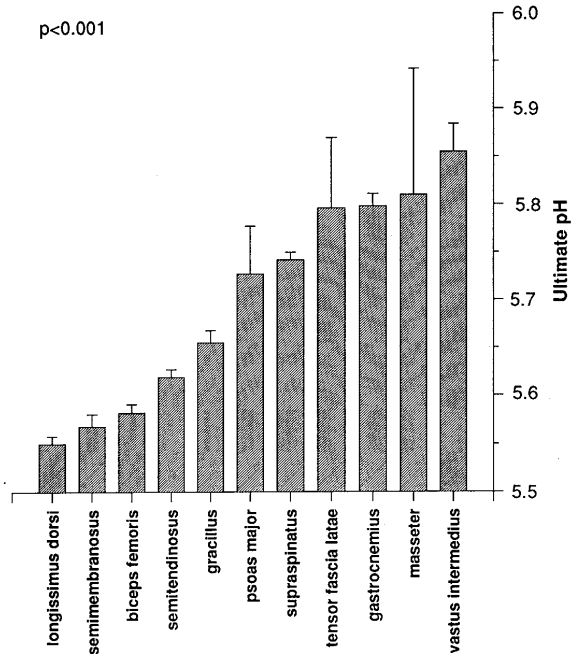
The ultimate pH values were significantly different between muscles ($p<0.001$, Figure 1). The LD, SM and BF

TABLE 1. The levels of calpastatin, μ- and m-calpain activities in different ovine muscles expressed as U/g muscle (mean ± SEM).

Muscle	calpastatin	μ-calpain	m-calpain
LD	2.37±0.136[a]	0.906±0.0437[ab]	1.353±0.0614[a]
BF	2.57±0.152[ab]	1.030±0.0478[b]	1.397±0.0676[a]
M	2.62±0.152[ab]	0.894±0.0491[ab]	1.870±0.0695[bc]
SM	2.68±0.220[ab]	0.946±0.0677[ab]	1.324±0.0976[a]
ST	2.68±0.136[bc]	0.898±0.0429[ab]	1.283±0.0602[a]
TFL	2.82±0.152[bc]	0.950±0.0478[ab]	1.392±0.0676[a]
GR	2.84±0.220[bc]	0.936±0.0677[ab]	1.470±0.0976[a]
CT	3.04±0.210[c]	0.815±0.0685[a]	1.199±0.0986[a]
PM	3.07±0.152[c]	0.833±0.0491[a]	1.288±0.0695[a]
SS	3.21±0.136[c]	0.952±0.0437[ab]	1.396±0.0614[a]
G	3.22±0.210[c]	1.001±0.0685[b]	1.536±0.0986[b]
VI	4.92±0.136[d]	1.004±0.0429[b]	1.730±0.0602[bc]

Superscript letters group muscles not significantly different at p<0.05 for each column.

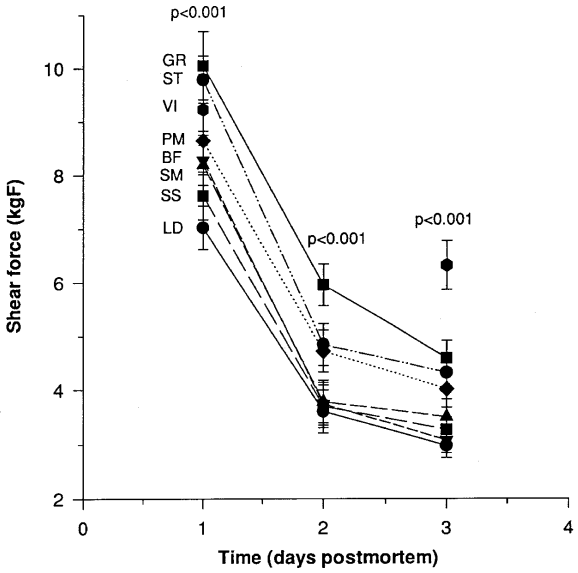
FIGURE 1: Ultimate pH values in different ovine muscles (mean ± SEM).



had lower (p<0.05) ultimate pH values than the other muscles. The LD also had lower (p<0.05) ultimate pH than BF. The GR had a lower (p<0.05) value than SS, TFL, G, M and VI. The PM had lower (p<0.05) ultimate pH than VI, and SS had lower value than G, M and VI.

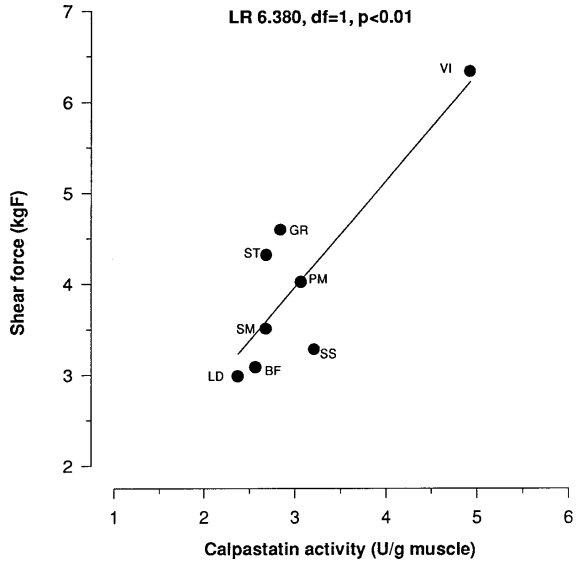
There was a significant difference between muscles for shear force, at each time point (day 1, p<0.001; day 2, p<0.001; day 3, p<0.001; and day 4, p<0.001, Figure 2). Shear force was not determined in M, CT and G due to the size of muscle. For shear force analysis on muscles aged for 4 days postmortem, SS had lower (p<0.05) shear force than all muscles except ST. The TFL and VI had greater (p<0.05) shear forces than SS, ST, LD and BF. The VI had higher (p<0.05) shear force than TFL.

FIGURE 2. Shear force during postmortem aging in different ovine muscles (mean ± SEM).



Calpastatin activity (LR 6.380, df=1, p<0.01) had a positive association (Figure 3), μ-calpain:calpastatin ratio had a negative association (LR 5.036, df=1, p<0.01) and ultimate pH had a positive association (LR 4.062, df=1, p<0.01) with shear force analysed on day 3 postmortem, between muscle means. However, these effects were mainly due to the VI. After removal of the between muscle variation, the within muscle variation showed increased calpastatin activity (LR 5.259, df=1, p<0.01) was associated with increased shear force, suggesting that higher calpastatin activity resulted in tougher meat. In agreement, the μ-calpain:calpastatin ratio had a negative association (LR 5.154, df=1, p<0.01) with shear force analysed on day 3 postmortem, within muscles. In contrast, there was no relationship of m-calpain:calpastatin ratio and shear force analysed on day 3 postmortem. Within muscles, there was

FIGURE 3. Association between calpastatin activity and shear force at day 3 postmortem. LR 6.380, df=1, p<0.01.



a negative association of μ-calpain (LR 5.374, df=1, $p<0.01$) and an indication of a negative association of m-calpain (LR 3.482, df=1, $p<0.1$) and shear force analysed on day 4, suggesting that higher calpain activity results in more tender meat.

DISCUSSION

In the present study muscles differing in fibre type composition were analysed for calpain and calpastatin activities to determine their relationship with meat tenderness, as indicated by shear force. The results demonstrated that different muscles differ in their levels of calpastatin inhibitory activity and μ- and m-calpain proteolytic activity. In contrast to earlier reports (Ouali and Talmant, 1990; Whipple and Koohmaraie, 1992), muscle fibre type did not influence the level of activity of the calpain system. However, these earlier reports only analysed one muscle characteristic of each fibre type group.

The pH of meat influences meat quality traits such as water-holding capacity, texture and tenderness (Dutson, 1983). In the present study, the difference between muscles in ultimate pH had no influence on meat tenderness due to the small range of pH values and these not being in the range considered tough (Purchas, 1990).

In the present study, muscles differed in their shear force values during the aging process. There was a positive association between calpastatin activity and a negative association between μ-calpain:calpastatin ratio and shear force of aged meat. This suggests that the increased inhibitory actions of calpastatin on the calpains results in tougher meat. In agreement, earlier reports investigating different ovine muscles and the calpain system report a relationship between calpastatin 24-h activity and the amount of postmortem proteolysis (Whipple and Koohmaraie, 1992) and a relationship between meat aging and calpain efficiency as indicated by the calpain:calpastatin ratio (Ouali and Talmant, 1990).

In the present study there was a negative association between μ-calpain and a tendency for a negative association between m-calpain and shear force of aged meat. This suggests that increased proteolytic activity of the calpains results in more tender meat. It has been suggested that m-calpain may not be involved in postmortem proteolysis since it is not active postmortem (Koohmaraie, 1988), however μ-calpain is active postmortem and plays a role in postmortem myofibrillar proteolysis. Shackelford et al. (1995) report that the shear force of the LD is not strongly related to shear force of other muscles. This may explain the lack of association between the calpain system and tenderness between aged muscles in the present trial. However, there are associations within muscles between the calpain system and tenderness of aged muscles. This suggests that the calpain system may not be a useful tool for prediction of tenderness between different muscles, but may be useful within the same muscles from different carcasses.

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