The effects of the calpain proteolytic system on meat tenderisation rates in different ovine skeletal muscles

K. Singh[1], P. M. Dobbie[1], N. J. Simmons[2], D. Duganzich[1] and J. J. Bass[1].
[1]AgResearch, Ruakura Agricultural Research Centre, PO Box 3123, Hamilton, New Zealand
[2]Meat Industry Research Institute of New Zealand, P.O. Box 167, Hamilton, New Zealand

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Introduction

Muscle fibre composition has an influence on postmortem meat tenderisation of ruminants (Olson et al., 1976, Dransfield *et al.*, 1981, Koohmaraie et al., 1988a, Ouali, 1990). The proteolytic enzymes μ- and m-calpain, which are involved in cellular events mediated by calcium and their specific inhibitor calpastatin are involved in postmortem tenderisation of meat by degrading myofibrillar proteins thus causing a weakening of myofibrillar structure (Koohmaraie *et al.*, 1988b). Differences in activity of the ovine calpain system may also be influenced by muscle fibre composition (Ouali and Talmant 1990; Whipple and Koohmaraie 1992). Earlier studies suggest a relationship between calpastatin 24-h activity and the amount of postmortem proteolysis (Whipple and Koohmaraie, 1992) and a relationship between meat tenderisation aging rates and calpain efficiency as indicated by the calpain:calpastatin ratio (Ouali and Talmant, 1990). These earlier studies report a limited number of muscles representative of different fibre typing groups. The aim of this study was to determine the extent to which differences in calpain and calpastatin activities may account for differences in ultimate tenderness and rates of meat tenderisation in a wide range of ovine muscles which differ in muscle fibre composition.

Materials and Methods

Lambs at 4 months of age were fed a pelleted diet (lucerne 60% and barley 30%) and water *ad libitum* for the 3 weeks. Muscles were collected within 30 min of slaughter and ranged in fibre type composition. Trial 1 (n=9): 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. psoas major* (PM): type IIB (fast-twitch glycolytic) fibres; *M. semitendinosus* (ST), *M. tensor fascia latae* (TFL): type II fibres; *M. cutaneous trunci* (CT). Trial 2 (n=8): the same muscles were used except CT and G were replaced by the type IIA muscle *M. semimembranosus* (SM) and type IIB muscle *M. gracillus* (GR) (Briand et al., 1981; Sumi and Tamate, 1988). Calpastatin, μ- and m-calpain were extracted from fresh 5 g 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 using casein as the substrate (Hammarsten, Merck, Germany). One unit of calpain activity was defined as the amount of enzyme required to increase 1.0 absorbance unit 278 nm in 60 min at 25 °C. One unit of calpastatin activity was defined as the amount of calpastatin required to inhibit one unit of m-calpain. Shear force analysis was carried out on muscles aged at 15°C for 1 and 3 days postmortem (trial 1) or 1, 2 and 4 days postmortem (trial 2). Samples were cooked to an internal temperature of 75°C and analysed for peak shear force by shearing perpendicular to the fibre axis using a MIRINZ pneumatic tenderometer. There were no shear force measures for the CT, G and M due to muscle size. Tenderisation aging rates (k) were determined using an exponential decay equation as described by Dransfield et al. (1981). Restricted maximum likelihood (REML), using Genstat statistical package, was used to obtain means for the muscles. This also provided likelihood ratio 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 among muscles in calpastatin ($p<0.001$), μ-calpain ($p<0.05$) and m-calpain ($p<0.001$) activities (Table 1). μ-calpain:calpastatin ($p<0.001$) and m-calpain:calpastatin ($p<0.001$) ratios were different among the muscles. There was also a significant difference among muscles for shear force measures at each time point ($p<0.001$) and aging rates ($p<0.01$, table 2). The fibre typing groups of muscles does not account for all the muscle variation and this residual variation was used as the error for testing between type differences. Calpastatin ($p<0.05$) and m-calpain activities ($p<0.05$) were higher in type I muscles in comparison to type II muscles. There were no significant differences in calpastatin and m-calpain activities between type IIA and type IIB muscle. There was also no significant difference in μ-calpain activity between the different fibre types. The aging rates were greater ($p<0.05$) in type IIA muscles in comparison to type IIB muscles. There was no significant difference in aging rate between type I muscles in comparison to type II muscles. These data suggest that the fibre type composition of a muscle has an influence on the calpain system and the rate of meat aging.

Calpastatin activity had a positive association ($p<0.01$) and μ-calpain:calpastatin ratio had a negative association ($p<0.05$) with ultimate shear force, between muscle means. However, these effects were mainly due to the VI. After removal of the between muscle variation, the within muscle variation showed calpastatin activity had a positive association ($p<0.05$), μ-calpain had a negative association ($p<0.05$) and μ-calpain:calpastatin ratio had a negative association ($p<0.05$) with ultimate shear force, There was also an indication of a negative association of m-calpain ($p<0.1$) with ultimate shear force. These data suggest that higher calpastatin activity and lower calpain activity results in tougher meat. A significant correlation with aging rate was only found with m-calpain:calpastatin ratio ($p<0.05$). There was a tendency for a positive association between μ-calpain:calpastatin ratio ($p<0.1$). These data suggest that higher calpastatin activity and lower calpain activity influences ultimate tenderness and the rate of meat aging.

Discussion

In the present study results demonstrated that different ovine muscles differ in their levels of calpastatin inhibitory activity and μ- and m-calpain proteolytic activity. Calpastatin and m-calpain activities were influenced by metabolic and contractile type of a muscle, with the activities being higher in slow-twitch muscles with higher oxidative activity compared to fast-twitch muscles with oxidative-glycolytic activity and greater

glycolytic activity. Previous reports also suggest that calpastatin activity may be influenced by metabolic and contractile muscle types (Ouali and Talmant, 1990; Whipple and Koohmaraie, 1992). The m-calpain activity was higher in type I muscles, although the μ-calpain activity was not influenced by muscle fibre composition in the present study. Whipple and Koohmaraie (1992) report that both μ- and m-calpain activities were greater in type I muscles compared to type II muscles. In contrast, Ouali and Talmant (1990) report the m-calpain is influenced only by metabolic type of a muscle, with the activity being higher in type IIB whiter muscles compared to type I and IIA redder muscles.

The meat aging rates were greater in type IIA fast-twitch oxidative-glycolytic muscles than type IIB fast-twitch glycolytic muscles. There was no difference in aging rates between type I muscles in comparison to type II muscles. In contrast a previous report suggests aging rates were greater in fast-twitch than in slow-twitch muscles (Ouali and Talmant, 1990).

In the present study the positive association between calpastatin activity and the negative association among the calpains and shear force of aged meat, and the positive association among the calpain:calpastatin ratios and aging rates within muscles suggests the increased inhibitory actions of calpastatin on the calpains results in decreased proteolytic activity of calpain resulting in tougher meat and differences in the rate at which meat ages. Earlier studies investigating ovine muscles report a relationship between calpastatin activity and the amount of postmortem proteolysis (Whipple and Koohmaraie, 1992) and a relationship between meat tenderisation aging rates and calpain efficiency as indicated by the calpain:calpastatin ratio (Ouali and Talmant, 1990). There was no relationship between calpastatin and aging rate in this study which agrees with Ouali and Talmant (1990). This study has clearly shown that the fibre composition of a muscle influences the activity of the calpain system and the rate of meat aging. The differences in calpastatin and calpain activities within muscles influence the degree of ultimate tenderness and the rate of aging in ovine muscles.

References

Briand, M., Talmant, A., Briand, Y., Monin, G., Durand R. (1981) Eur. J. Appl. Physiol. 46, 347
Dransfield, E., Jones, R.C.D., MacFie, H.J.H. (1981) Meat Sci., 5, 131
Koohmaraie, M., Seidman, S.C., Schollmeyer, J.E., Dutson, T.R., Babiker,A.S. (1988a) J. Food Sci., 53, 407
Koohmaraie, M., Babiker, A.S., Schroeder, A.L., Merkel, R.A., Dutson, T.R. (1988b) J. Food Sci., 53, 1638
Olson, D.G., Parrish, F.C. Jr., Stromer, M.H. (1976) J. Food Sci., 41, 1036
Ouali, A., Talmant, A. (1990) Meat Sci., 28, 331
Sainz, R.D., Thomson, B.C., Macsood F.N. (1991) Fed. Am. Soc. Exp. Biol., 6(5), A1968
Suzuki, A., Tamate, H. (1988) The Anatomical Record 221, 494
Wheeler, T.L., Koohmaraie, M. (1991) J. Anim. Sci., 69, 1559
Whipple, G., Koohmaraie M. (1992) J. Anim. Sci., 70, 798

Table 1. Effects of muscle on calpastatin, μ- and m-calpain activities (U/g muscle)

Muscle	calpastatin	μ-calpain	m-calpain
[1] Longissimus dorsi	2.37	0.906	1.353
[1] Biceps femoris	2.57	1.030	1.397
[1] Masseter	2.62	0.894	1.870
[2] Semimembranosus	2.68	0.946	1.324
[1] Semitendinosus	2.68	0.898	1.283
[1] Tensor fasciae latae	2.82	0.950	1.392
[2] Gracillus	2.84	0.936	1.470
[2] Cutaneous Trunci	3.04	0.815	1.199
[1] Psoas major	3.07	0.833	1.288
[1] Supraspinatus	3.21	0.952	1.396
[2] Gastrocnemius	3.22	1.001	1.536
[1] Vastus intermedius	4.92	1.004	1.730
sed			
within group 1	0.192	0.06	0.09
between groups 1 and 2	0.250	0.08	0.11
within group 2	0.299	0.09	0.14
df	157	151	170
Effect of muscle	***	*	***

subscript letters represent groupings for comparisons.
Data are expressed as means.
*, 0.05
***, p<0.001

Table 2. Effects of muscle on the rates (*k*) of postmortem aging

Muscle	rates (*k*)
[1] Biceps femoris	.0343
[2] Gracillus	.0181
[1] Longissimus dorsi	.0426
[2] Psoas major	.0325
[2] Semimembranosus	.0353
[1] Semitendinosus	.0183
[1] Supraspinatus	.0387
[2] Tensor fasciae latae	.0453
[2] Vastus intermedius	.0387
sed	
within group 1	0.00648
between groups 1 and 2	0.00807
within group 2	0.00896
TFL vs groups 1 and 2	0.0102
df	84
Effect of muscle	**

subscript letters represent groupings for comparisons
Data are expressed as means.
**, p<0.01