# Indicators of milk and beef quality


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# Beef tenderness: significance of the calpain proteolytic system

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## Summary

The purpose of this paper is to review the role of the calpain proteolytic system in beef tenderization during *post-mortem* cooler storage. Ultimate beef tenderness is dependent on three factors: the background toughness, the toughening phase, and the tenderization phase. However, the large variation in beef tenderness at the consumer level is mainly a result of variation in the tenderization phase. Proteolysis of key myofibrillar and myofibril-associated proteins leads to a weakening of the rigid structure of the myofibrils, and meat becomes more tender. Although several proteolytic systems have been suggested to play a role in *post-mortem* proteolysis in skeletal muscle, ample evidence shows that the calpain proteolytic system is responsible for this process. Moreover, μ-calpain activity regulated by the calpain-specific inhibitor calpastatin starts shortly after slaughter, and proceeds throughout the storage period. Towards the end of this paper, different *in vitro* and *in vivo* models used to study the calpain proteolytic system in *post-mortem* skeletal muscle are discussed.

**Keywords**: calpain, calpastatin, muscle, *post-mortem*, proteolysis

## Introduction

Overall beef meat quality is determined by multiple factors, including water-holding capacity, colour, palatability, nutritional value and safety. The importance of these traits varies depending on both the end product as well as the consumer profile. The eating quality, or palatability, of beef is influenced by flavour, juiciness and tenderness. Consumers in the USA ranked tenderness to be the most important attribute of beef eating quality (Miller *et al.*, 1995). Further, it has been shown that consumers can distinguish between tough and tender meat (Huffman *et al.*, 1996), and that they are willing to pay a premium for tender meat (Boleman *et al.*, 1997; Lusk *et al.*, 2001). One particular cut of meat that demonstrates the importance of beef tenderness is the tenderloin (i.e. *psoas major*). Although the tenderloin is found to be one of the least flavourful and juicy cuts of meat, it is the most highly valued retail cut due to its supreme tenderness (Savell and Shackelford, 1992).

The objective of this review paper is to discuss the role of the calpain proteolytic system in *post-mortem* tenderization of beef meat. A brief overview of other factors affecting ultimate beef tenderness will also be given.

## Factors influencing ultimate beef tenderness

In this paper, ultimate beef tenderness is defined as the tenderness level found after cooler storage of meat, at a time when further cooler storage would not lead to additional tenderization. Significant variations in ultimate beef tenderness can be found when comparing different muscles within an animal (Ramsbottom *et al.*, 1945; Strandine *et al.*, 1949). Further, variation in ultimate beef tenderness is also seen when comparing the same muscle from different breeds (Shackelford *et al.*, 1991). Variation in ultimate beef tenderness can exist at the time of slaughter, can be created

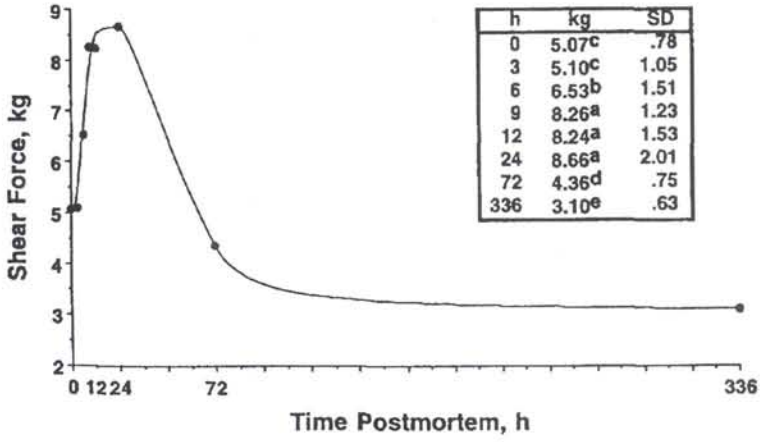
during the *post-mortem* storage period, or can be a combination of both. The three factors that determine ultimate beef tenderness is the background toughness, the toughening phase, and the tenderization phase. While the toughening and tenderization phases take place during the *post-mortem* storage period, the background toughness exists at the time of slaughter and does not change during the storage period. The effect of the opposing toughening and tenderization phase on meat tenderness is illustrated in Figure 1.

**Background toughness**

Background toughness of meat is defined as "the resistance to shearing of the unshortened muscle" (Marsh and Leet, 1966), and variation in the background toughness results from the connective tissue component of muscle. In particular, it seems as if the organization of the perimysium affects this toughness, since a general correlation between perimysium organization and the tenderness of muscles has been found in both chicken and beef (Strandine *et al.*, 1949). Moreover, double muscled cattle contain less perimysium and have larger fasciculi than normal cattle (Boccard, 1981), and these factors seem to be related to the improved tenderness of these animals. Nevertheless, extensive evidence shows that the variability in toughness found between similar muscles from individual animals is independent of connective tissue properties (Whipple *et al.*, 1990b; Silva *et al.*, 1999; Ngapo *et al.*, 2002).

**Toughening phase**

The toughening phase is caused by sarcomere shortening during *rigor* development. This process usually occurs within the first 24 h *post-mortem*, after which sarcomere lengths do not change (Wheeler and Koohmaraie, 1994; 1999). The relationship between sarcomere shortening and meat toughness was first reported in 1960 (Locker, 1960). Later it was shown that there is a strong negative relationship between sarcomere length and meat toughness when sarcomeres are shorter than 2 μm, and that the relationship is poorer at longer sarcomere lengths (Herring *et al.*, 1967; Bouton *et al.*, 1973). A theory relating the tightly bound actin-myosin complex to the increased meat toughness during the first 24 h *post-mortem* has also been suggested (Goll *et al.*, 1995). However, this theory has been weakened by a report showing that muscles prevented from contraction during *rigor* development does not go through the normal toughening phase even




| h | kg | SD |
|---|---|---|
| 0 | 5.07$^{c}$ | .78 |
| 3 | 5.10$^{c}$ | 1.05 |
| 6 | 6.53$^{b}$ | 1.51 |
| 9 | 8.26$^{a}$ | 1.23 |
| 12 | 8.24$^{a}$ | 1.53 |
| 24 | 8.66$^{a}$ | 2.01 |
| 72 | 4.36$^{d}$ | .75 |
| 336 | 3.10$^{e}$ | .63 |

*Figure 1. Tenderness of ovine longissimus, measured as Warner-Bratzler shear force, at various times* post-mortem. *Means without a common superscript differ ($P < 0.05$; from Wheeler and Koohmaraie, 1994).*

though these muscles also form the tightly bound actin-myosin complex at the onset of *rigor mortis* (Koohmaraie *et al.*, 1996). Thus, it is not the formation of the tightly bound actin-myosin complex at *rigor mortis* that results in the increased toughness within the first 24 h *post-mortem*, but rather the contraction of sarcomeres immediately prior to the onset of *rigor mortis*.

### Tenderization phase

Is has been known for almost a century that meat tenderness improves during cooler storage, and it was suggested that the changes taking place in meat during storage were largely due to enzymatic activity (Hoagland *et al.*, 1917). It is now well established that *post-mortem* proteolysis of myofibrillar and myofibril-associated proteins is responsible for this process. While the toughening phase is similar in all carcasses, the tenderization phase is highly variable. There is a large variation in both the rate and extent of *post-mortem* tenderization of meat, and this results in the inconsistency in meat tenderness found at the consumer level.

## Post-mortem proteolysis and meat tenderization

Multiple studies have been performed to identify which proteins are degraded during *post-mortem* storage of meat and what specific changes are responsible for tenderization. Initially researchers suggested that degradation of the Z-discs was related to meat tenderization (Davey and Gilbert, 1969), however a major component of Z-discs, α-actinin, showed no sign of degradation until after 2 weeks of *post-mortem* storage at 4°C (Hwan and Bandman, 1989). Degradation of costameres (e.g. desmin, dystrophin, vinculin), intermediate filaments (e.g. desmin, vimentin), titin, and nebulin are now thought to be responsible for *post-mortem* tenderization of meat (Taylor *et al.*, 1995). The rigid structure of the myofibrils is weakened once some of these proteins are degraded, and meat becomes more tender.

The strong association between *post-mortem* proteolysis and tenderization of meat during cooler storage has been demonstrated in several studies. Differences in meat tenderness found between *Bos taurus* (tender) and *Bos indicus* (tough) cattle are related to the reduced rate of *post-mortem* protein degradation in the tougher breed (Whipple *et al.*, 1990b; Shackelford *et al.*, 1991). Treatment of sheep and cattle with a β-adrenergic agonist (BAA) gave similar results, where meat from treated animals showed no *post-mortem* proteolysis and no tenderization (Fiems *et al.*, 1990; Kretchmar *et al.*, 1990). Ultimately, species differences in *post-mortem* tenderization rate in pigs, sheep, and cattle were directly related to the rate of myofibrillar protein degradation in the three species (Ouali and Talmant, 1990; Koohmaraie *et al.*, 1991b).

In skeletal muscle there are three endogenous proteolytic systems that can be responsible for *post-mortem* proteolysis during cooler storage of meat: the calpain system, the lysosomal cathepsins, and the multicatalytic proteinase complex (MCP). In addition to being endogenous in skeletal muscle, these proteolytic systems would have to fulfil two other requirements before they could be considered responsible for *post-mortem* proteolysis in meat (Koohmaraie, 1994; Goll *et al.*, 1983). Firstly, the proteases must have access to the substrates, and secondly, they must be able to reproduce the substrate fragmentation pattern observed after *post-mortem* storage of meat. Cathepsins are located in lysosomes, and would therefore not have access to the myofibrils unless they are released from the lysosomes. However, evidence for the release of cathepsins from lysosomes during *post-mortem* storage of meat is conflicting. A shift in cathepsin activity from the lysosomal to the soluble fraction of bovine muscle indicates release of cathepsins from lysosomes (O'Halloran *et al.*, 1997). In contrast, it has been shown that cathepsins still were located in lysosomes after electrical stimulation and 28 days of storage of meat at 4°C (LaCourt *et al.*, 1986). Regardless of their release or not, the major reason why a role of cathepsins in meat

tenderization is doubtful is their proteolytic abilities. Actin and myosin are very good substrates for cathepsin, and these two proteins are not degraded during *post-mortem* storage of meat (Koohmaraie, 1994). Additionally, studies where different protease inhibitors were injected into lamb *longissimus* have shown that inhibition of cathepsins does not affect *post-mortem* proteolysis and meat tenderization (Hopkins and Thompson, 2001ab). A significant role of MCP could also be excluded, since myofibrils are very poor substrates for this protease (Koohmaraie, 1992a). Furthermore, several publications have shown that increased $Ca^{2+}$ concentrations in *post-mortem* muscle greatly improves meat tenderness through increased proteolysis (Koohmaraie *et al.*, 1988; Polidori *et al.*, 2000), however neither cathepsins nor MCP are activated by $Ca^{2+}$ (Koohmaraie, 1992b). Based on this, the calpain proteolytic system is the only proteolytic system to fulfil all the requirements and is thought to be the system responsible for *post-mortem* proteolysis and tenderization of meat.

Although most reports investigating the mechanism behind meat tenderization support the importance of *post-mortem* proteolysis of myofibrillar and myofibril-associated proteins, a calcium theory of tenderization has also been suggested (Takahashi, 1992; 1996). According to this theory, meat tenderization during *post-mortem* storage is a result of fragmentation of structural proteins, weakening of the Z-discs, and lengthening of *rigor*-shortened muscles caused directly by calcium through non-enzymatic processes. However, several groups have now refuted this theory. *Post rigor* lengthening of sarcomeres was not detected in lamb *longissimus* even though a considerable variation was seen in tenderization of the muscles (Geesink *et al.*, 2001). Moreover, it was shown that degradation of troponin-T correlated well with meat tenderization, although this particular protein, according to Hattori and Takahashi (1982), was not directly affected by calcium. Additionally, incubations of single bovine muscle fibres in 1 or 10 mM $Ca^{2+}$ decreased the strength of the fibres. However this weakening was prevented by addition of protease inhibitors, suggesting that the weakening was a result of proteolysis and not a direct effect of calcium (Christensen *et al.*, 2004).

## The calpain proteolytic system

The first report of the calpain proteolytic system came 40 years ago, when a $Ca^{2+}$-activated proteolytic enzyme was purified from rat brain (Guroff, 1964). A similar $Ca^{2+}$-activated proteolytic enzyme was later purified from pork skeletal muscle (Dayton *et al.*, 1976ab). Some years later, the existence of a second $Ca^{2+}$-activated protease with a lower $Ca^{2+}$-requirement was purified from canine cardiac muscle (Mellgren, 1980). These proteases were named calpains (Murachi, 1989), and are now referred to as μ-calpain and m-calpain according to their $Ca^{2+}$-requirement (Cong *et al.*, 1989). An inhibitor of calpain, calpastatin, was later isolated from human erythrocytes and bovine cardiac muscle (Takano and Murachi, 1982; Otsuka and Goll, 1987). Over the last decade multiple calpain-like genes have been identified in different tissues and species, however little or nothing is known about the proteins encoded by these genetic variants (Goll *et al.*, 2003).

Both μ-calpain and m-calpain are composed of two subunits with molecular weights of 28 and 80 kDa (Dayton *et al.*, 1976ab; Dayton *et al.*, 1981; Emori *et al.*, 1986). While μ-calpain and m-calpain have identical small subunits encoded by a single gene, they possess genetically different large subunits (Ohno *et al.*, 1990). An important characteristic of μ-calpain and m-calpain is their ability to undergo autolysis in the presence of calcium. Autolysis reduces the $Ca^{2+}$-requirements for half maximal activity of μ-calpain and m-calpain (Suzuki *et al.*, 1981ab; Dayton, 1982; Nagainis *et al.*, 1983), however the specific activity of the enzymes does not change (Edmunds *et al.*, 1991). Autolysis of the large subunit of μ-calpain produces a 78-kDa fragment followed by a 76-kDa fragment (Inomata *et al.*, 1988). However, autolysis of the large subunit of m-calpain produces a 78-kDa fragment only (Brown and Crawford, 1993). The small subunit of calpain

produces a fragment of 18 kDa upon autolysis (McClelland *et al.*, 1989). It seems as if autolysis of the large subunit gives rise to the reduced $Ca^{2+}$-requirements of autolyzed calpains (Brown and Crawford, 1993; Elce *et al.*, 1997).

Calpastatin is the endogenous specific inhibitor of μ-calpain and m-calpain (Maki *et al.*, 1988). Several isoforms exist of this protein, however the predominant form found in skeletal muscle consists of four repetitive calpain-inhibiting domains in series with an N-terminal leader region (Lee *et al.*, 1992). A 125-kDa isoform of calpastatin is found in ovine, porcine, and bovine skeletal muscle (Geesink *et al.*, 1998). Calpastatin requires calcium to bind and inhibit calpains (Cottin *et al.*, 1981; Imajoh and Suzuki, 1985). However since calpastatin does not bind calcium itself, the $Ca^{2+}$-requirement of calpain-calpastatin interaction must originate from the protease. Calpastatin is also a substrate for calpains and can be degraded in the presence of calcium (Mellgren *et al.*, 1986; Doumit and Koohmaraie, 1999). Degradation of calpastatin by calpains does not lead to loss of inhibitory activity, and even after extensive proteolysis it retains inhibitory function (DeMartino *et al.*, 1988; Nakamura *et al.*, 1989).

For more detailed information regarding the calpain proteolytic system, see review by Goll *et al.* (2003).

## Calpains and meat tenderization

Much of the evidence for the involvement of calpains in meat tenderization has come from studies showing an involvement of calcium in *post-mortem* tenderization of meat. As early as 1969 it was demonstrated that $Ca^{2+}$-treatment resulted in the disappearance of Z-discs within the myofibrils (Davey and Gilbert, 1969). Moreover, it was shown that $Ca^{2+}$ ions provoked myofibrillar fragmentation, and that the chelating agent ethylenediaminetetraacetic acid (EDTA) inhibited this fragmentation (Busch *et al.*, 1972). However, more direct evidence for the importance of calpains in meat tenderization was demonstrated when whole carcasses or cuts of meat were infused with calcium chloride. Infusion of whole lamb carcasses with calcium chloride immediately after death greatly accelerated the *post-mortem* tenderization process (Koohmaraie *et al.*, 1988; Polidori *et al.*, 2000), as did calcium chloride injections into *longissimus* from *Bos indicus* cattle (Koohmaraie *et al.*, 1990). Moreover, shear force values of beef round muscles at 1, 8, and 14 days *post-mortem* were greatly reduced as a result of calcium chloride injection shortly after slaughter, and it has been shown that injections of calcium chloride 24 h *post-mortem* are just as effective as infusions performed at 0 h *post-mortem* (Wheeler *et al.*, 1991; 1992). Another report, however, found that calcium injection at 0 h *post-mortem* was more efficient at reducing shear force values measured at 10 days *post-mortem* than injections performed at 12 or 24 h *post-mortem* (Boleman *et al.*, 1995). By comparing carcasses infused with solutions of calcium chloride and sodium chloride of equal ionic strengths, it was proven that the tenderization was caused by calcium and not by an increase in ionic strength (Koohmaraie *et al.*, 1989). Since calpains are $Ca^{2+}$-dependent, all of the above is compelling evidence for the calpains involvement in *post-mortem* meat tenderization. Stronger evidence of their role in meat tenderization was established when infusions of zinc chloride (an inhibitor of calpains) into lamb carcasses prevented the *post-mortem* tenderization process (Koohmaraie, 1990). Injections of zinc chloride into beef strip loins have also been proven to drastically inhibit meat tenderization (Lawrence *et al.*, 2003).

Some of the changes seen in meat during *post-mortem* cooler storage include the disappearance of desmin and troponin-T and the appearance of 28-32-kDa fragments, while both actin and myosin are not affected (Koohmaraie, 1994). Incubations of purified myofibrils with calpain *in vitro* have produced the exact proteolytic pattern found in *post-mortem* meat (Koohmaraie *et al.*, 1986; Huff-Lonergan *et al.*, 1996; Geesink and Koohmaraie, 1999a). Moreover, incubations of bovine single

muscle fibres with μ-calpain resulted in thinner Z-lines and reduced fibre strength, demonstrating that μ-calpain is capable of reducing the mechanical strength of muscle fibres (Christensen *et al.*, 2003).

Recently a role of calpain 3 (i.e. p94) has been suggested in *post-mortem* proteolysis leading to meat tenderization (Ilian *et al.*, 2001; 2004ab). A good correlation was reported between calpain 3 mRNA level and ultimate tenderness of bovine and ovine muscles (Ilian *et al.*, 2001). However, no relationship was found between calpain 3 levels, determined by western blot using a specific calpain 3 antibody, and meat tenderness of porcine *longissimus* (Parr *et al.*, 1999). Mutations in the calpain 3 gene in humans are linked with limb-girdle muscular dystrophy type 2a (LGMD 2a), a disease associated with excessive protein catabolism (Richard *et al.*, 1995). These mutations result in loss of catalytic activity of calpain 3 (Ono *et al.*, 1998). The suggestion that elevated levels of calpain 3 are associated with increased tenderization seems contradictory to the enzymes behaviour in LGMD 2a. Thus, based on these data alone, calpain 3 is not likely to play a role in meat tenderization. Furthermore, calpastatin which has been clearly documented to play a key regulatory role in *post-mortem* proteolysis and meat tenderness does not inhibit calpain 3 (Sorimachi *et al.*, 1993). Geesink *et al.*, (2004) using calpain 3 knockout mice, demonstrated that absence of calpain 3 had not effect on *post-mortem* proteolysis. Therefore, it is clear that calpain 3 does not play a relevant role in *post-mortem* proteolysis. The hypothesis of Ilian and co-workers with respect for the role of calpain 3 in *post-mortem* proteolysis and meat tenderization (Ilian *et al.*, 2001; 2004a,b) can essentially be summarized as (1) unlike with the ubiquitous form of the calpain there are no methods for purification and quantification of calpain 3, (2) native calpain 3 has never been isolated from skeletal muscle, hence neither the proteolytic capacity nor substrates are known, (3) the only method available are mRNA quantification and autolysis of calpain 3 and their correlation to *post-mortem* proteolysis and meat tenderization, and (4) because mRNA level and pattern of calpain 3 is correlated with *post-mortem* proteolysis, calpain 3 must be involved in this process. Correlations and cause and effect are very different phenomena. The data presented by Geesink *et al.* (2004) indicated that while calpain 3 autolysis occurred as reported repeatedly by Ilian and co-workers, such autolysis is independent of degradation of proteins that are involved in *post-mortem* meat tenderization. Therefore because: (1) absence of calpain 3 does not affect *post-mortem* proteolysis, (2) overexpression of calpastatin which shuts down *post-mortem* proteolysis has no effect of the pattern of autolysis of calpain 3 (Kent *et al.*, 2004), and (3) autolysis of calpain 3 in *post-mortem* muscle is the sole basis for suggesting a role for calpain 3 in *post-mortem* muscle proteolysis (Ilian *et al.*, 2001a, b, 2004a, b), it appears that calpain 3 plays a minor, if any, role in *post-mortem* proteolysis of the proteins analyzed.

**Calpain activity in *post-mortem* muscle**

A body of evidence has been gathered which indicates that the calpain proteolytic system is responsible for meat tenderization during cooler storage, and numerous studies have been performed to determine the activity of μ-calpain, m-calpain, and calpastatin in *post-mortem* muscle. In general, reports show that μ-calpain and calpastatin activities rapidly decline during *post-mortem* storage of meat, while m-calpain activity seems to be stable (Ducastaing *et al.*, 1985; Geesink and Koohmaraie, 1999b; Kretchmar *et al.*, 1990). However, some reports have shown that m-calpain activity also declines *post-mortem* (Sensky *et al.*, 1996; Beltrán *et al.*, 1997). These declines in m-calpain activity have later been shown to be artefacts, caused by the use of inappropriate extraction buffers, rather than an actual decline in activity (Veiseth and Koohmaraie, 2001). Since exposure of calcium leads to inactivation of both μ-calpain and m-alpain through autolysis, the fact that m-calpain activity is stable in *post-mortem* muscle while μ-calpain activity is not has been used to suggest that μ-calpain rather than m-calpain is responsible for *post-mortem* proteolysis in meat (Koohmaraie *et al.*, 1987). This assertion was more recently confirmed when

analysis using casein zymography showed that m-calpain did not undergo autolysis during a 15-day *post-mortem* storage period, and that only μ-calpain is active during the ageing period (Veiseth *et al.*, 2001). Typical changes in μ-calpain, m-calpain, and calpastatin activities in *post-mortem* muscle are shown in Figure 2.

Calpain activity in *post-mortem* muscle is affected by factors such as temperature and pH. Both temperature and pH decline in muscle during the first 24 h *post-mortem*, and this has raised questions whether μ-calpain is active at the typical pH and temperature of *post-mortem* muscle. However, it has been shown that μ-calpain retained 24 to 28% of its maximum activity, found at pH 7.5 and 25°C, under conditions simulating *post-mortem* muscle (Koohmaraie *et al.*, 1986). Additionally, incubation of bovine myofibrils with μ-calpain at pH 5.6 and 4°C produced the same pattern of proteolysis as observed in bovine *longissimus* during *post-mortem* storage (Koohmaraie *et al.*, 1986; Huff-Lonergan *et al.*, 1996).

As mentioned earlier, the large variation in meat tenderness found at the consumer level is caused by a large variation in both the rate and the extent of *post-mortem* tenderization. Although pH and temperature affect μ-calpain activity in *post-mortem* muscle, this would not introduce variation in meat tenderness, since their declines in *post-mortem* muscle do not vary significantly between carcasses subjected to the same pre- and post-slaughter conditions. However, it is possible to manipulate the pH and temperature declines in carcasses by using different *post-mortem* cooling regimes or electrical stimulation. Post-mortem cooling rate of carcasses influences meat tenderness through its effect on sarcomere shortening during *rigor* mortis development (Redmond *et al.*, 2001; Van Moeseke *et al.*, 2001; Devine *et al.*, 2002), and also through its effect on proteolysis (Devine *et al.*, 1999; Geesink *et al.*, 2000). Electrical stimulation of carcasses accelerates *post-mortem* pH decline, which again affects *rigor mortis* development due to its effect on the intracellular $Ca^{2+}$ concentration in muscle (Whiting, 1980). Although μ-calpain activity is affected by pH, the effect of electrical stimulation on *post-mortem* proteolysis is less clear, and conflicting results have been reported (Hwang *et al.*, 2003).

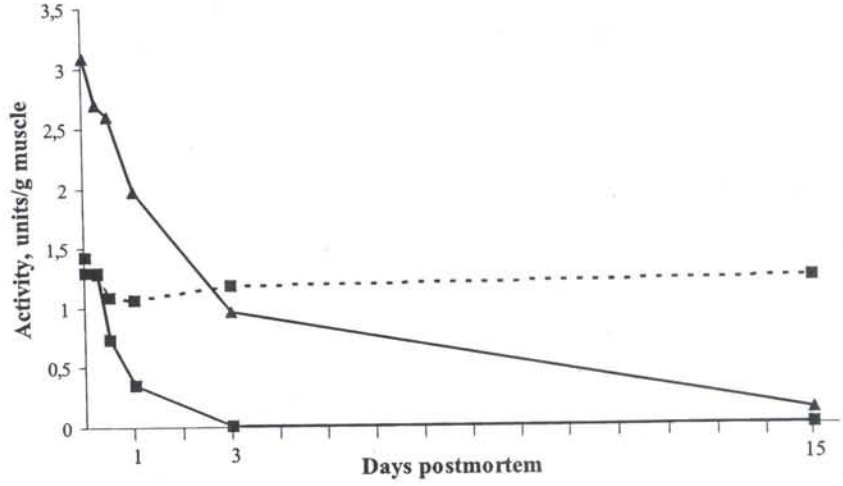



*Figure 2. Changes in μ-calpain (–■–), m-calpain (- -■- -), and calpastatin (–▲–) activities during* post-mortem *cooler storage of ovine longissimus (graphs based on data reported by Veiseth et al., 2004b).*

The principal regulator of calpain activity in *post-mortem* muscle is calpastatin, and a negative relationship exists between calpastatin activity and *post-mortem* proteolysis in muscle. A concern that has been raised regarding μ-calpain activity in *post-mortem* muscle is the excess of calpastatin found in muscle. Myofibril incubations with μ-calpain and calpastatin have shown that calpastatin limits the rate and the extent of proteolysis and autolysis of μ-calpain (Geesink and Koohmaraie, 1999a). Importantly however, it was also shown that a 1:4 ratio of μ-calpain to calpastatin was not sufficient to completely inhibit μ-calpain activity. In another study, co-eluted μ-calpain and calpastatin extracted from ovine *longissimus* muscle at different times *post-mortem* showed a net proteolytic activity at all times *post-mortem* (Veiseth *et al.*, 2004b). This not only indicated that μ-calpain could be active in the presence of excess calpastatin, but also that μ-calpain was still active at 15 days *post-mortem*.

Autolysis of μ-calpain has been detected as early as 3 h *post-mortem* in ovine *longissimus*, indicating that μ-calpain has sufficient calcium levels for proteolytic activity shortly after slaughter (Veiseth *et al.*, 2001). Activity of μ-calpain has also been demonstrated in ovine *longissimus* after 56 days of cooler storage (Geesink and Koohmaraie, 1999b). These results indicate that μ-calpain can be active within a short time after slaughter and throughout an extended storage period. Significant amount of *post-mortem* proteolysis has been observed as early as 9 h in ovine *longissimus*, resulting in a significant improvement in myofibril fragmentation index (MFI) at 12 h *post-mortem* (Veiseth *et al.*, 2004b). A significant increase in MFI of bovine *longissimus* has also been detected at 12 h *post-mortem* (Koohmaraie *et al.*, 1987). Although MFI is an indirect measure of meat tenderness, it has been shown to correlate well with both Warner-Bratzler shear force (WBSF) and trained sensory panel tenderness ratings in bovine *longissimus* (Whipple *et al.*, 1990a; Crouse *et al.*, 1991). Improvements in MFI at 12 h *post-mortem* in both ovine and bovine *longissimus* therefore indicate that the tenderization process starts early *post-mortem*.

**Model systems for the involvement of calpain activity in meat tenderization**

Multiple animal models have been used to investigate the role of calpains and calpastatin in meat tenderization. A muscle hypertrophy condition in lambs, referred to as callipyge, leads to increased weights of all major leg and loin muscles (Jackson *et al.*, 1993). Meat from callipyge lamb was found to remain tough throughout the *post-mortem* storage period, while WBSF of meat from normal lamb decreased during the same storage period (Koohmaraie *et al.*, 1995). This lack of tenderization in meat from callipyge lambs is a result of a greatly reduced rate and extent of *post-mortem* proteolysis due to elevated levels of calpastatin in these animals (Geesink and Koohmaraie, 1999b; Duckett *et al.*, 2000). Muscles from animals fed BAA also do not undergo *post-mortem* proteolysis, and results in tough meat. BAA-administration has given comparable results in both lamb and cattle, and the reduced *post-mortem* proteolysis has been attributed to the reduced activity of calpains caused by increased calpastatin levels (Fiems *et al.*, 1990; Koohmaraie *et al.*, 1991a). Animal age also affects the calpain proteolytic system in skeletal muscles. In general, these studies have revealed that calpain and calpastatin activities decline with increasing animal age (Ou and Forsberg, 1991; Shackelford *et al.*, 1995). Recently, it was reported that calpastatin activity in *longissimus* from lambs ranging 2 to 10 months of age decreased, and that this resulted in increased *post-mortem* proteolysis and meat tenderization in the older animals (Veiseth *et al.*, 2004a).

Comparison of species has also been used as a model to study the calpain system in relation to meat tenderization. Meat tenderization occurred faster in pigs than in lambs, while beef was the slowest, and this difference in meat tenderization was a reflection of the rate of *post-mortem* proteolysis in the different species. Analysis of the calpain proteolytic system in muscles from these three species revealed that calpastatin levels were low in pork, intermediate in lamb, and

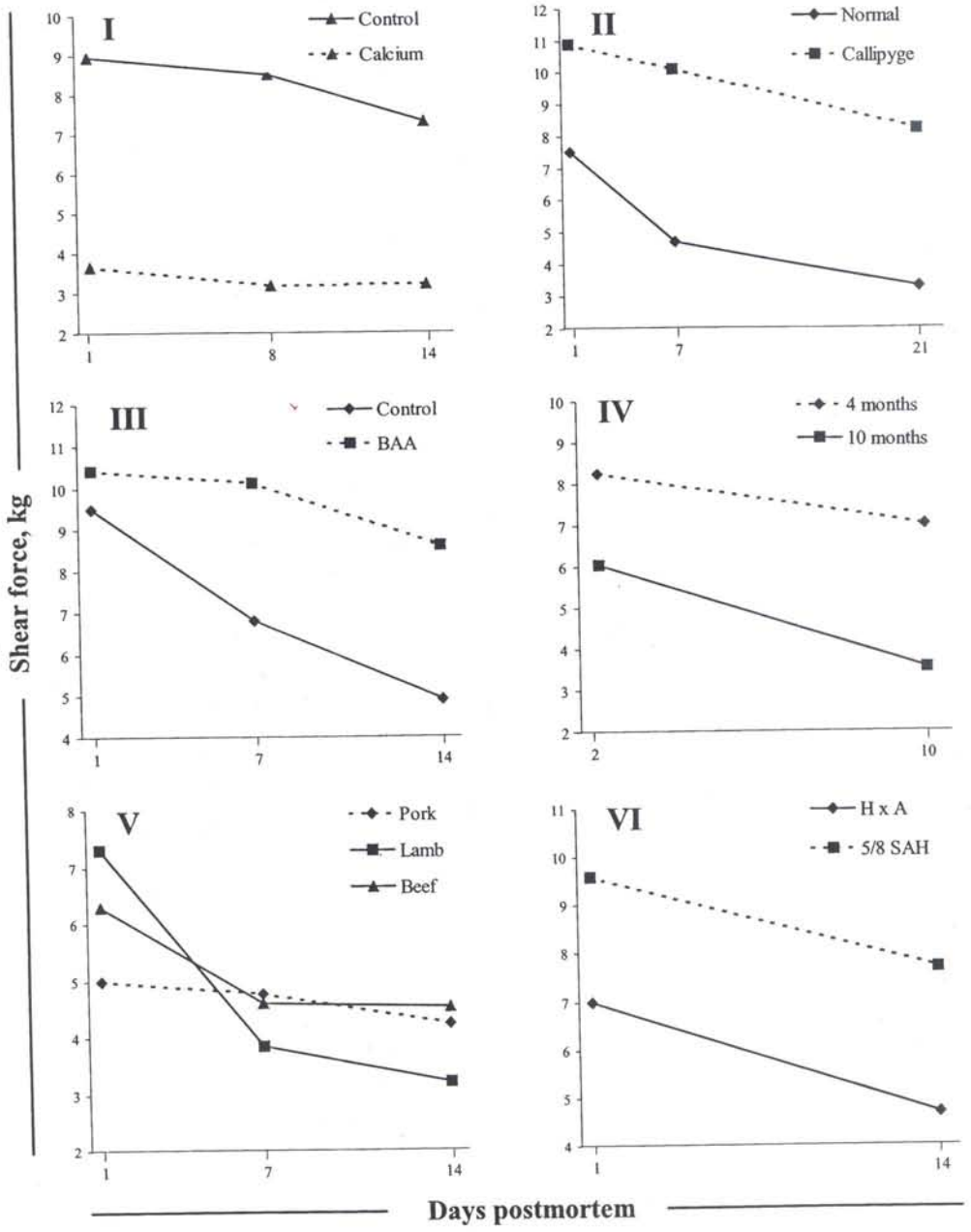



*Figure 3. Rate and extent of* post-mortem *meat tenderization affected by: I) $CaCl_2$ injection in cattle (data from Wheeler et al., 1991), II) callipyge phenotype in lambs (data from Koohmaraie et al., 1995), III) β-adrenergic agonist (BAA) treatment in lambs (data from Koohmaraie et al., 1991a), IV) animal age in lambs (data from Veiseth et al., 2004a), V) species (data from Koohmaraie et al., 1991b), and VI) breeds in cattle (data from Whipple et al., 1990b).*

high in beef. Thus, the difference in the rate of *post-mortem* meat tenderization between these three species is caused by a variation in calpastatin activity in their muscles at the time of slaughter (Ouali and Talmant, 1990; Koohmaraie *et al.*, 1991b). Meat obtained from *Bos indicus* carcasses has been demonstrated to be tougher meat than that obtained from *Bos taurus* carcasses. *Bos indicus* cattle have reduced *post-mortem* proteolysis compared to the more tender *Bos taurus* cattle, and this reduction is associated with a higher content of calpastatin in those animals (Whipple *et al.*, 1990b; Ferguson *et al.*, 2000). In fact, calpastatin activity at 24 h *post-mortem* in *longissimus* from crossbred cattle explained 44% of the variation in WBSF and trained sensory panel tenderness ratings (Whipple *et al.*, 1990a). The effects of some *in vitro* and *in vivo* models on meat tenderization are illustrated in Figure 3.

The key finding, independent of model, has been that muscles with elevated levels of calpastatin compared to calpain have reduced *post-mortem* proteolysis and produce tough meat. However, the most direct evidence thus far for calpains involvement in *post-mortem* proteolysis in skeletal muscle, comes from a study of transgenic mice overexpressing calpastatin (Kent *et al.*, 2004). In these mice, calpastatin activity in hind limb muscles was 370-fold greater than in control mice, while μ-calpain and m-calpain was unaffected by the transgene. Calpastatin overexpression resulted in a slower autolysis of μ-calpain, and *post-mortem* proteolysis measured by degradation of desmin and troponin-T was inhibited. Based on their results, the authors concluded that calpain activity alone is responsible for *post-mortem* proteolysis in skeletal muscle.

## Conclusion

Tenderness is a very important quality trait for consumer acceptance of whole meat products, and the estimated cost of inadequate beef tenderness in the USA is 200-300 million $US annually. It is therefore crucial to reduce the variation in beef tenderness at the consumer level. Substantial evidence exists that μ-calpain activity regulated by calpastatin is responsible for *post-mortem* proteolysis of key myofibrillar and myofibril-associated proteins in skeletal muscle leading to meat tenderization. Further, *post-mortem* proteolysis mediated by μ-calpain starts shortly after slaughter and proceeds throughout the storage period. Information on the activity and regulation of this proteolytic system can be used to improve the rate and extent of *post-mortem* proteolysis, and thereby reduce the amount of tough beef reaching the consumers. Additionally, approaches to predict ultimate tenderness levels should be further explored.

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