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Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system

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Abstract

Tenderness has been repeatedly reported as the most important quality aspect of meat. However, a number of studies have shown that a significant portion of retail meat can be considered tough. As a consequence, a significant consumer segment is willing to pay a premium for guaranteed tender meat. However, apart from measuring the shear force, there is no reliable method to predict tenderness. Most of the branded meat programs therefore attempt to ensure eating quality by controlling some of the factors that affect tenderness.

Meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and postmortem proteolysis of myofibrillar and myofibrillar-associated proteins. Given the effect of postmortem proteolysis on the muscle ultrastructure, titin and desmin are likely key substrates that determine meat tenderness.

A large number of studies have shown that the calpain proteolytic system plays a central role in postmortem proteolysis and tenderization. In skeletal muscle, the calpain system consists of at least three proteases, μ-calpain, m-calpain and calpain 3, and an inhibitor of μ- and m-calpain, calpastatin. When activated by calcium, the calpains not only degrade subtrates, but also autolyze, leading to loss of activity. m-Calpain does not autolyze in postmortem muscle and is therefore not involved in postmortem tenderization. Results from a number of studies, including a study on calpain 3 knockout mice, have shown that calpain 3 is also not involved in postmortem proteolysis. However, a large number of studies, including a study on μ-calpain knockout mice, have shown that μ-calpain is largely, if not solely, responsible for postmortem tenderization. Research efforts in this area should, therefore, focus on elucidation of regulation of μ-calpain activity in postmortem muscle. Discovering the mechanisms of μ-calpain activity regulation and methods to promote μ-calpain activity should have a dramatic effect on the ability of researchers to develop reliable methods to predict meat tenderness and on the meat industry to produce a consistently tender product.
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Keywords: Meat; Tenderness; Postmortem; Calpain

1. Introduction

Multiple factors, including palatability, water-holding capacity, color, nutritional value and safety, determine meat quality. The importance of these traits varies depending on both the end product and the consumer profile. Flavor, juiciness and tenderness influence the palatability of meat. Among these traits, tenderness is ranked as most important (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). An example of the importance of tenderness over flavor or juiciness is the tenderloin (*psoas major*). Although this cut is one of the least flavorful and least juicy cuts of meat, it is the most highly valued retail cut due to its supreme tenderness (Savell & Shackelford, 1992). 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 guaranteed tender meat (Boleman et al., 1997; Lusk, Fox, Schroeder, Mintert, & Koohmaraie, 2001; Shackelford et al., 2001).

The objective of this review paper is to discuss (1) the importance and value of meat tenderness, (2) the effect of

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postmortem storage on muscle structure and tenderness, and (3) the role of the calpain proteolytic system in postmortem tenderization.

2. The importance and value of meat tenderness

Tenderness has been repeatedly reported as the most important quality attribute of meat (Huffman et al., 1996; Miller et al., 2001). However, surveys of beef packers, purveyors, restaurateurs and retailers indicate that tenderness is among the highest ranked quality concerns (Smith et al., 1995). This concern is warranted because a number of studies have shown that a significant proportion of retail meat cuts can be considered tough. In a large beef tenderness survey in 14 cities in the United States it was found that 10.7–61.8% of samples, depending on the retail cut, had a 68% chance of receiving panel tenderness scores of "slightly tough" or worse (Morgan et al., 1991). In a similar study it was found that 24% of US retail beef loin steaks were rated lower than "slightly tender" (George, Tatum, Belk, & Smith, 1999). Results from a large study in New Zealand indicated that toughness problems are not limited to beef (Bickerstaffe, Bekhit, Robertson, Roberts, & Geesink, 2001). For retail beef, lamb and pork midloin cuts 9.8%, 3.8% and 10.7%, respectively, could be classified as "tough" or "very tough" (Bickerstaffe et al., 2001).

Traditional carcass grading systems are not effective at identifying meat tenderness variation. For instance, the US grading system uses marbling as a measure of quality. However, marbling explains at most 5% of the variation in beef tenderness (Wheeler, Cundiff, & Koch, 1994). A grading system aimed at meat quality was developed by Meat Standards Australia (Polkinghorne et al., 1999). Based on critical control points for tenderness, juiciness, flavor and overall satisfaction, from production to processing and consumption, different cuts are graded "unsatisfactory" (no grade), "good everyday" (3 star), "better than everyday" (4 star) or "premium quality" (5 star). However, although this system works to reduce the probability that steaks are of unsatisfactory quality, 71% of striploins of "no grade" carcasses were judged acceptable by consumers, whereas 11% of striploins of graded carcasses were deemed unacceptable (Thompson, Polkinghorne, Watson, Gee, & Murrison, 1999).

A large amount of effort has been devoted to the development of systems to classify carcasses according to tenderness. These include visible and near-infrared spectroscopy (Byrne, Downey, Troy, & Buckley, 1998; Park, Chen, Hruschka, Shackelford, & Koohmaraie, 1998; Shackelford, Wheeler, & Koohmaraie, 2004, 2005), image texture analysis (Li & Shatadal, 2001), image analysis using BeefCam (Belk et al., 2000; Vote, Belk, Tatum, Scanga, & Smith, 2003), a combination of colorimeter, marbling and hump height traits (Wulf & Page, 2000) or measurement of *longissimus* slice shear force (Shackelford, Wheeler, & Koohmaraie, 1999). A direct comparison between the latter three methods indicated that only slice shear force accurately identified tender beef (Wheeler et al., 2002). Furthermore, accurately segregating carcasses based on *longissimus* tenderness also sorts other muscles for tenderness (Wheeler, Shackelford, & Koohmaraie, 2000a; Wheeler et al., 2002). However, it appears that the industry is reluctant to implement slice shear force measurement because it is perceived as too costly (Wheeler et al., 2002).

The costs of automated classification using slice shear force were estimated at $4.35 per carcass (Wheeler, Shackelford, & Koohmaraie, 1999). The premium consumers are willing to pay should outweigh these costs. Boleman et al. (1997) reported that 94.6% of families ($n = 42$) chose steaks that were tender over intermediate and tough steaks, even though a $1.10/kg price difference was placed between each category. Shackelford et al. (2001) reported that 50% of consumers ($n = 1{,}036$) were willing to pay $1.10/kg premium for the assurance of tenderness with the Tender Select concept. Lusk et al. (2001) found that 51% of participants ($n = 86$) in a survey were willing to pay an average premium of $4.05/kg for guaranteed tender beef. Recently, Feldkamp, Schroeder, and Lusk (2005) conducted a consumer evaluation study where participants were given a generic 12-oz. steak and asked to place bids to exchange it for a "guaranteed tender" steak. Consumers were willing to pay a $2.79/kg premium for "guaranteed tender" steak. From these numbers it appears that the benefits of a guaranteed tender beef concept may outweigh the costs. However, as mentioned by Shackelford et al. (2001), a number of factors need to be considered by retailers before implementing a guaranteed tender concept. These factors are: (1) how large does the premium for this product have to be to offset the costs of identifying, branding, and marketing the product line; (2) is there a sufficiently large enough market at the aforementioned premium price; (3) will sales of this branded product increase or decrease the sales of other higher-profit items; and (4) will there be a steady supply of the product line. From the studies mentioned above it appears that additional research is needed to determine the benefits and costs of a tenderness-certified program.

Approaches using spectroscopy, although not as accurate as slice shear force, are accurate enough to be useful to the industry and should be available commercially in the near future. Implementation of non-invasive approaches such as those using spectroscopy will be much less costly than slice shear force and should make it easier to develop a profitable marketing strategy for products with more consistent and/or superior tenderness.

3. Growth of branded products and guaranteed tenderness products

Because of the importance of eating quality to consumer satisfaction, there has been an explosion of branded meat products in the United States and probably worldwide. The first branded beef program in the United States was

Certified Angus Beef (CAB) which was introduced in the late 1970s. To date, CAB is a very successful beef promotion program. It is interesting to note that CAB does not include processes that are known to affect beef tenderness (e.g., 14 days' postmortem storage specification). Most of the branded beef programs that were introduced in the 1990s and 2000s attempt to ensure eating quality by controlling some of the factors that are known to affect beef tenderness (e.g., length of postmortem storage, electrical stimulation, tender-stretch, breed of cattle, etc.). We know of only one branded beef program in the United States that is based on actual measurement of tenderness. This level of interest by the industry to provide a highly acceptable product to consumers gives the researcher a unique opportunity to develop useful technology for the industry with a very high likelihood of implementation.

4. The effect of postmortem storage on tenderness

The three factors that determine meat tenderness are background toughness, the toughening phase and the tenderization phase. While the toughening and tenderization phases take place during the postmortem storage period, background toughness exists at the time of slaughter and does not change during the storage period. The effect of postmortem storage on tenderness is illustrated in Fig. 1.

The background toughness of meat is defined as "the resistance to shearing of the unshortened muscle" (Marsh & Leet, 1966), and variation in the background toughness is due to the connective tissue component of muscle. In particular, the organization of the perimysium appears to affect the background toughness, since a general correlation between the perimysium and the tenderness of muscles has been found for both chicken and beef (Strandine, Koonz, & Ramsbottom, 1949).

The toughening phase is caused by sarcomere shortening during rigor development (Koohmaraie, Doumit, & Wheeler, 1996; Wheeler & Koohmaraie, 1994). For beef, this process usually occurs within the first 24 h postmortem (Wheeler & Koohmaraie, 1999). The relationship between sarcomere shortening and meat toughness was first reported by 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 (Bouton, Harris, Shorthose, & Baxter, 1973; Herring, Cassens, Suess, Brungardt, & Briskey, 1967; Wheeler, Shackelford, & Koohmaraie, 2000b).

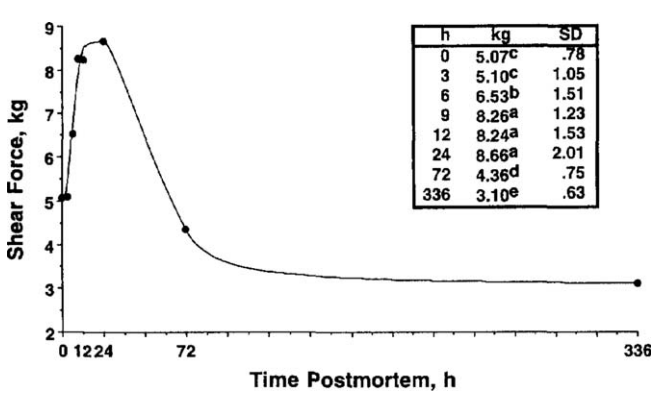

Fig. 1. Tenderness of ovine *longissimus*, measured by Warner–Bratzler shear force, at various times postmortem. Means without a common superscript differ ($P < 0.05$; from Wheeler and Koohmaraie, 1994).

While the toughening phase is similar in all carcasses under similar processing conditions, the tenderization phase is highly variable. There is a large variation in both the rate and extent of postmortem tenderization of meat, and this results in the inconsistency of meat tenderness found at the consumer level. It has been known for a long time that meat tenderness improves during cooler storage, and it was suggested almost a century ago that this is due to enzymatic activity (Hoagland, McBryde, & Powick, 1917). It is now well established that postmortem proteolysis of myofibrillar and myofibrillar-associated proteins is responsible for this process.

5. The effect of postmortem storage on muscle ultrastructure

A variety of studies have shown that weakening of the myofibers is the key event in tenderization. The most consistently reported ultrastructural change associated with tenderization is breaks at the junction of the I band and Z-disk (Abbot, Pearson, Price, & Hooper, 1977; Davey & Dickson, 1970; Dutson, Pearson, & Merkel, 1974; Ho, Stromer, Rouse, & Robson, 1996; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995a). Because of the weakening of the myofibers, aged meat yields a higher proportion of smaller fragments upon homogenization than unaged meat. The myofibril fragmentation index (MFI), which is based on the fragmentation concept, has been used as an index for meat tenderness, as well as for postmortem tenderization (Davey & Gilbert, 1969). Since then the MFI has been shown to be a predictor of meat tenderness in numerous studies (Olson, Parrish, Dayton, & Goll, 1977; Taylor et al., 1995a; Whipple, Koohmaraie, Dikeman, & Crouse, 1990a).

The proteins that are degraded during myofiber degradation are myofibrillar and cytoskeletal proteins, which include troponin-I, troponin-T, desmin, vinculin, *meta*-vinculin, dystrophin, nebulin and titin (see Robson et al., 1997 & Taylor et al., 1995a, for reviews). Three major cytoskeletal structures are degraded when meat is tender: Z- to Z-line attachments by intermediate filaments, Z- and M-line attachments to the sarcolemma by costameric proteins and the elastic filament protein titin (Taylor et al., 1995a). Z- to Z-line attachments are mostly composed of desmin. The importance of these attachments for meat tenderness is illustrated by the model of callipyge sheep, which show little postmortem tenderization (Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995). For several weeks postmortem little degradation of desmin occurs (Geesink & Koohmaraie, 1999b; Koohmaraie et al.,

1995), and the Z- to Z-line attachments remain largely intact (Fig. 2; Taylor & Koohmaraie, 1998). Similarly, titin also remains largely intact for several weeks postmortem in muscles from callipyge sheep (Geesink & Koohmaraie, 1999b). Detachment of the Z- and M-lines from the sarcolemma is probably not a limiting factor for tenderization. The detachment of these structures at 14 days postmortem was almost complete in both callipyge and control sheep, whereas a large difference in tenderness was observed (Taylor & Koohmaraie, 1998). Thus, titin and desmin are likely key substrates that determine meat tenderness.

6. What characteristics should a protease have to be considered as a candidate for causing postmortem tenderization?

Three proteolytic systems present in muscle have been investigated for their possible role in postmortem proteolysis and tenderization: the calpain system, the lysosomal cathepsins and the multicatalytic proteinase complex (MCP). In addition to being endogenous in skeletal muscle, these proteolytic systems must fulfill two other requirements to consider them involved in postmortem proteolysis in meat (Goll et al., 1983; Koohmaraie, 1988). First, the proteases must have access to the substrates, and secondly, they must be able to reproduce the proteolysis pattern observed after postmortem storage of meat. Incubation of myofibrillar proteins with cathepsins results in different degradation patterns than those that occur during postmortem storage of muscle, and it is doubtful that cathepsins are released from the lysosomes in postmortem muscle (Koohmaraie, 1988). A significant role for MCP can be excluded, since myofibrils are very poor substrates for this protease system (Koohmaraie, 1992). Moreover, the degradation pattern of myofibrillar proteins by MCP does not mimic the degradation pattern observed in postmortem muscle (Taylor et al., 1995b). This leaves the calpain system or potentially another, not yet investigated, proteolytic system responsible for postmortem proteolysis of key myofibrillar proteins and the resultant meat tenderization.

7. μ-Calpain is largely, if not solely, responsible for postmortem tenderization

Calpains are calcium-activated proteases with an optimum activity at neutral pH. In skeletal muscle, the calpain system consists of at least three proteases, μ-calpain, m-calpain and skeletal muscle-specific calpain, p94 or calpain 3, and an inhibitor of μ- and m-calpain, calpastatin. Over the last decade multiple calpain-like genes have been identified, however little is known about the proteins encoded by these genes (Goll, Thompson, Li, Wei, & Cong, 2003).

Both μ- and m-calpain are composed of two subunits with molecular weights of 28 and 80 kDa (Dayton, Goll, Zeece, Robson, & Reville, 1976; Dayton, Reville, Goll, & Stromer, 1976; Dayton, Schollmeyer, Lepley, & Cortés, 1981; Emori, Kawasaki, Imajoh, Kawashima, & Suzuki, 1986). An important characteristic of μ- and m-calpain is that they undergo autolysis in the presence of calcium.

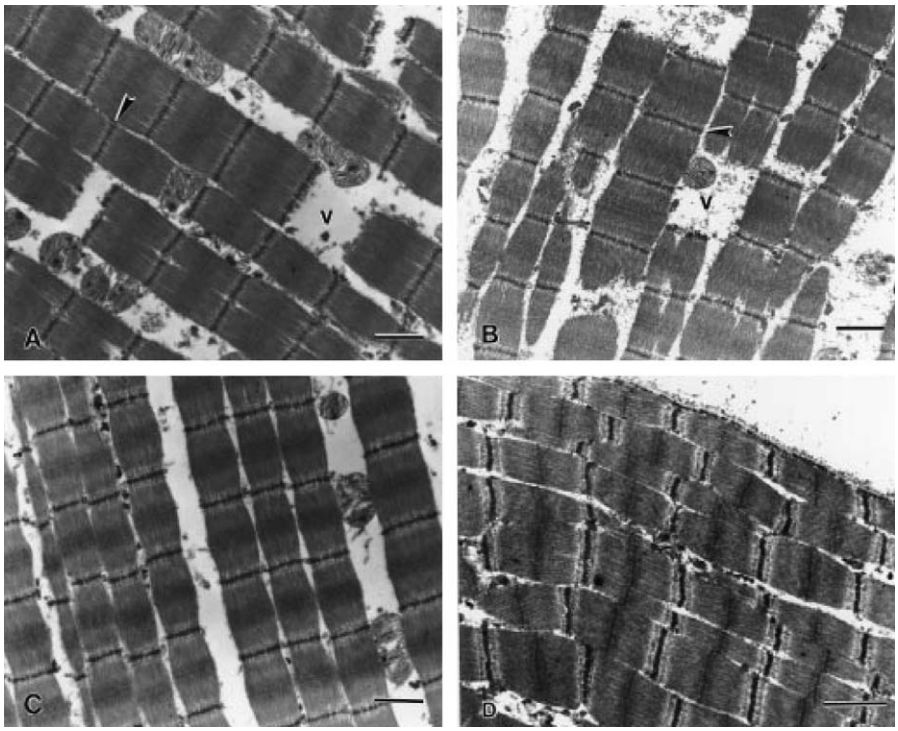

Fig. 2. Electron micrograph of myofibrils at 14 days after death. (A) and (B) show postmortem changes in normal lamb *longissimus* with I-band breaks (v), and loss of Z-line alignment. (C) and (D) show callipyge *longissimus* without I-band breaks. Bars represent 1 μm (from Taylor and Koohmaraie, 1998).

Autolysis reduces the Ca^{2+}-requirement for half maximal activity of μ- and m-calpain (Dayton, 1982; Nagainis, Sathe, Goll, & Edmunds, 1983; Suzuki et al., 1981; Suzuki, Tsuji, Kubota, Kimura, & Imahori, 1981). Initial autolysis of the large subunit of μ-calpain produces a 78-kDa fragment followed by a 76-kDa fragment (Inomata, Kasai, Nakamura, & Kawashima, 1988). Initial autolysis of the large subunit of m-calpain produces a 78-kDa fragment only (Brown & Crawford, 1993). Further autolysis of μ- and m-calpain leads to lower molecular weight fragments of the large subunit and loss of activity.

Calpain 3 is a single polypeptide of 94 kDa with sequence homology to the large subunits of μ- and m-calpain (Sorimachi, Ishiura, & Suzuki, 1989). Purification and characterization of calpain 3 has been extremely difficult for several reasons. Unlike μ- and m-calpain, calpain 3 can not be easily extracted from skeletal muscle due to its association with the myofibrillar protein, titin (Sorimachi et al., 1995). Expression of p94 in vitro is hampered by rapid autolysis of the enzyme at physiological levels of calcium, and furthermore, the autolysis is not affected by calpain inhibitors (Sorimachi et al., 1993).

Calpastatin is the endogenous specific inhibitor of μ- and m-calpain (Maki et al., 1988). Several isoforms of this protein exist, but the predominant form in skeletal muscle contains four calpain-inhibiting domains (Lee et al., 1992). Calpastatin requires calcium to bind and inhibit calpains (Cottin, Vidalenc, & Ducastaing, 1981; Imajoh & Suzuki, 1985). Calpastatin is also a substrate for the calpains and can be degraded in the presence of calcium (Doumit & Koohmaraie, 1999; Mellgren, Mericle, & Lane, 1986). Degradation of calpastatin does not lead to complete loss of inhibitory activity, and even after extensive proteolysis some inhibitory activity remains (DeMartino, Wachendorfer, McGuire, & Croall, 1988; Nakamura, Inomata, Imajoh, Suzuki, & Kawashima, 1989).

For more detailed information regarding the calpain system, the reader is referred to the extensive review by Goll et al. (2003).

The evidence for the involvement of the calpain system in postmortem proteolysis and tenderization comes from a variety of studies (Fig. 3):

1. Incubation of myofibrils with calpains produces the same proteolytic pattern as observed in postmortem muscle (Geesink & Koohmaraie, 1999a; Huff-Lonergan et al., 1996; Koohmaraie, Schollmeyer, & Dutson, 1986).
2. Infusion or injection of muscles with calcium accelerates postmortem proteolysis and tenderization (Koohmaraie, Babiker, Schroeder, Merkel, & Dutson, 1988; Koohmaraie, Whipple, & Crouse, 1990; Wheeler, Crouse, & Koohmaraie, 1992; Wheeler, Koohmaraie, & Crouse, 1991), whereas infusion or injection of muscles with calpain inhibitors inhibits postmortem proteolysis and tenderization (Koohmaraie, 1990; Uytterhaegen, Claeys, & Demeyer, 1994).
3. Differences in the rate of proteolysis and tenderization between species can be explained by the variation in calpastatin activity (Koohmaraie, Whipple, Kretchmar, Crouse, & Mersmann, 1991a; Ouali & Talmant, 1990).
4. Differences in the rate of postmortem proteolysis and tenderization between *Bos taurus* and *Bos indicus* cattle can be explained by the variation in calpastatin activity (Shackelford, Koohmaraie, Miller, Crouse, & Reagan, 1991; Whipple et al., 1990b).
5. The toughening effect of treatment with β-agonists can be explained by an increase in calpastatin activity (Garssen, Geesink, Hoving-Bolink, & Verplanke, 1995; Koohmaraie, Shackelford, Muggli-Cockett, & Stone, 1991b).
6. The greatly reduced rate and extent of postmortem proteolysis and tenderization in callipyge lamb can be attributed to elevated levels of calpastatin in these animals (Geesink & Koohmaraie, 1999b; Koohmaraie et al., 1995).
7. Overexpression of calpastatin in transgenic mice results in a large reduction in postmortem proteolysis of muscle proteins (Kent, Spencer, & Koohmaraie, 2004).

From the above-cited studies and others, it is clear that the calpain system plays an important role in postmortem proteolysis and tenderization. The remaining important question is, which of the calpains is responsible for postmortem proteolysis and tenderization?

An important characteristic of the calpains is that they autolyze once activated, ultimately leading to loss of activity (Dayton, 1982; Nagainis et al., 1983; Suzuki, Tsuji, Kubota, et al., 1981). In bovine and ovine postmortem muscle, the extractable activity of μ-calpain declines, but the activity of m-calpain is remarkably stable (Ducastaing, Valin, Schollmeyer, & Cross, 1985; Geesink & Koohmaraie, 1999b; Kretchmar, Hathaway, Epley, & Dayton, 1990; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). This observation led Koohmaraie, Seideman, Schollmeyer, Dutson, and Crouse (1987) to conclude that μ-calpain, but not m-calpain, is responsible for postmortem tenderization.

Using Western blotting, it has been established that calpain 3 does autolyze in postmortem muscle (Anderson et al., 1998; Ilian, Bekhit, & Bickerstaffe, 2004; Parr et al., 1999). In contrast with μ-calpain and m-calpain, calpain 3 is not inhibited by calpastatin (Sorimachi et al., 1993). This observation excludes a major role of calpain 3 on postmortem proteolysis and tenderization, given the great influence of calpastatin activity on these events. This conclusion was further corroborated by results of a recent study showing that postmortem proteolysis is not affected in calpain 3 knockout mice (Geesink, Taylor, & Koohmaraie, 2005).

The conclusion that μ-calpain is responsible for postmortem proteolysis (Koohmaraie et al., 1987) was recently confirmed using μ-calpain knockout mice (Geesink, Kuchay, Chishti, & Koohmaraie, 2006). The results of this

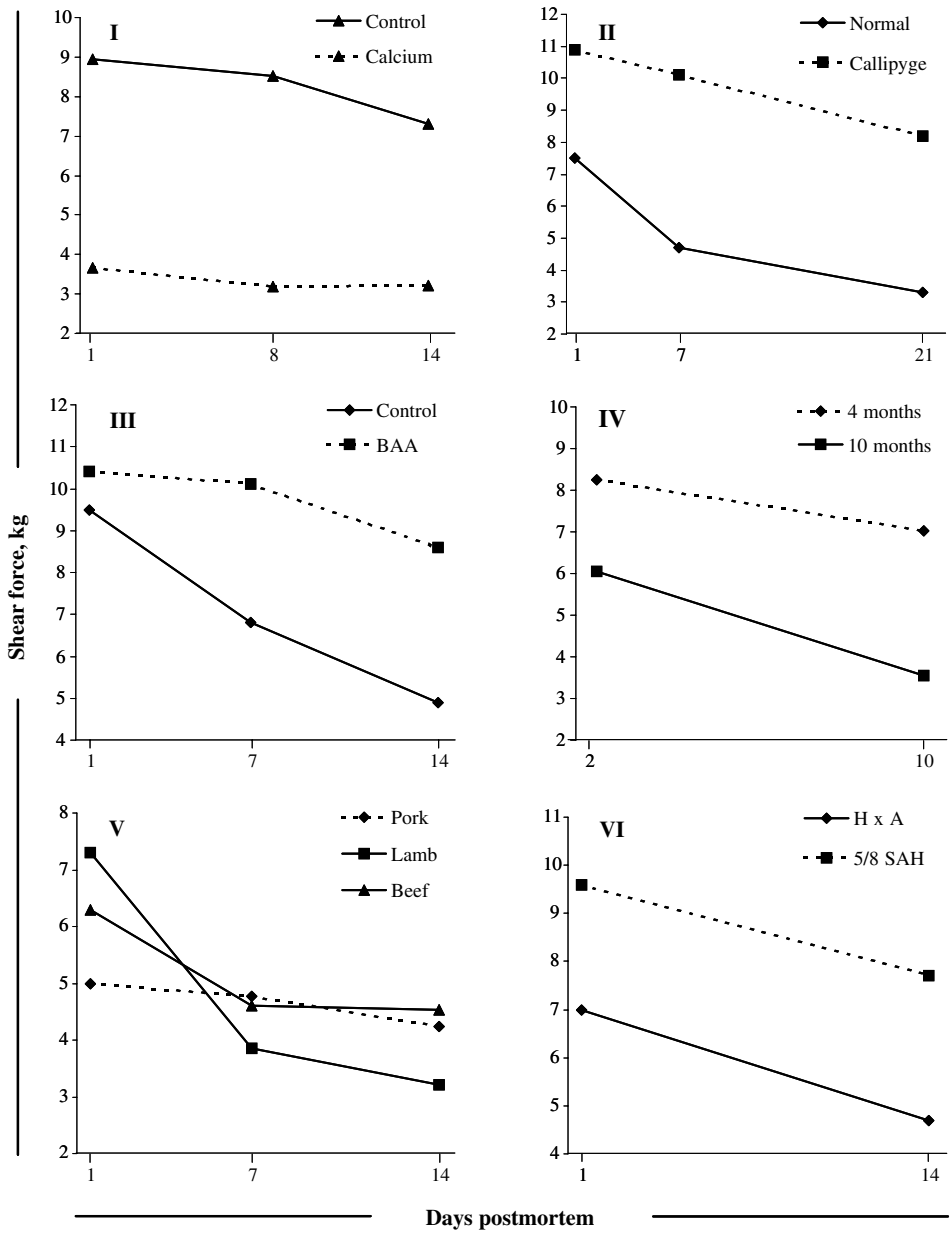

Fig. 3. Rate and extent of postmortem tenderization as affected by (I) $CaCl_2$ injection (data from Wheeler et al., 1991); (II) callipyge phenotype (data from Koohmaraie et al., 1995); (III) β-adrenergic agonist treatment (data from Koohmaraie et al., 1991b); (IV) animal age (data from Veiseth et al., 2004); (V) species (data from Koohmaraie et al., 1991a); and (VI) breeds (data from Whipple et al., 1990b).

study clearly showed that postmortem proteolysis was largely inhibited in μ-calpain knockout mice (Fig. 4). The limited proteolysis that did occur could be attributed to m-calpain, which is activated, as evidenced by autolysis, to some extent in postmortem murine skeletal muscle, contrary to what occurs with m-calpain in the muscles of meat-producing animals (Geesink et al., 2006; Kent et al., 2004).

8. Where do we go from here?

As stated previously, tenderness is the most important eating quality factor affecting consumer satisfaction. Thus, biological and other factors that determine meat tenderness have been the subject of intense research for almost a century. We have learned that essentially three factors can account for the great majority of the observed variation in meat tenderness, and they are proteolysis, connective tissue and sarcomere length (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004; Wheeler et al., 2000b). The relative importance of proteolysis, connective tissue and sarcomere length to tenderness is muscle dependent (e.g., while proteolysis is the major determinant of *longissimus* tenderness, sarcomere length is the major determinant of *psoas major* tenderness). There are a number of muscles for which all three factors contribute to the tenderness of these muscles.

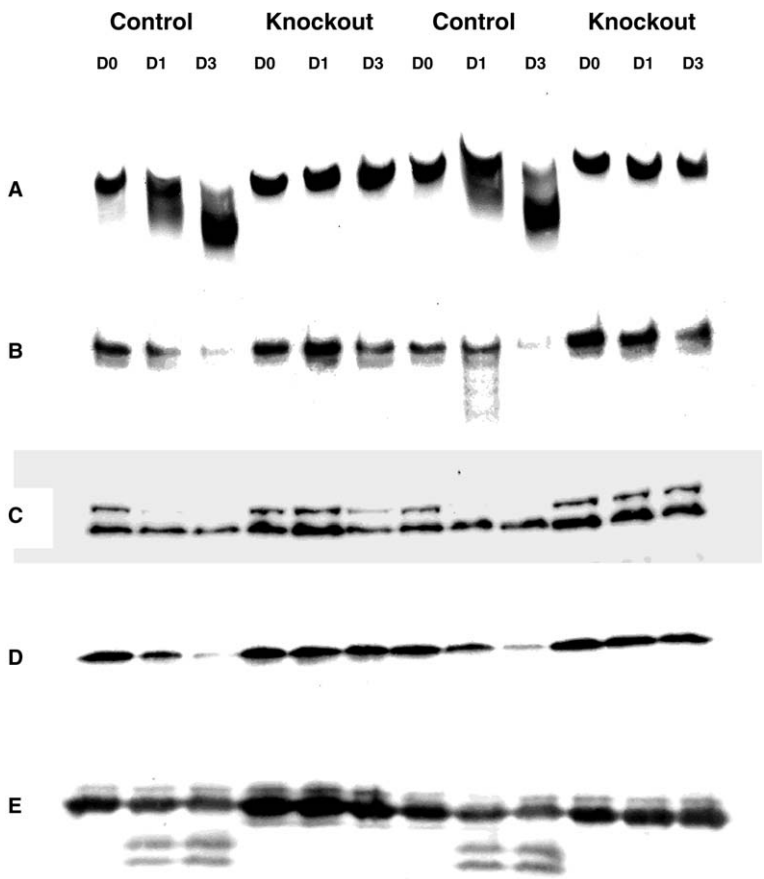

Fig. 4. Western blot analysis of nebulin (A), dystrophin (B), metavinculin and vinculin (C), desmin (D), and troponin-T (E) in whole muscle extracts of two control and two μ-calpain knockout mice at death (D0) and after 1 (D1) and 3 days' (D3) storage at 4 °C (from Geesink et al., 2006).

We, at the US Meat Animal Research Center, have tended to focus our efforts on determining the basis for tenderness of *longissimus* muscle, primarily due to its economic significance.

Because of the importance of tenderness, significant attempts have been made to ensure the tenderness of *longissimus* when presented to the consumer. Due to our inability to predict *longissimus* tenderness until very recently, one does not really know the degree of tenderness until the meat is eaten. The level of uncertainty is simply unacceptable and has led to efforts to develop methods to predict tenderness. Processing conditions and the time elapsed from exsanguination to meat on the plate varies throughout the world based on tradition, custom and culture. To the extent possible, it is the responsibility of researchers to enable industry to provide consumers with palatable meat regardless of variation in practices in various countries. As a result of decades of research, the meat industry in the United States (and undoubtedly in other countries) is well educated with respect to the importance of postmortem storage (5–7 days for pork, 7–10 days for lamb and 14 days for beef) to the ultimate tenderness. In the United States, 36–48 h after exsanguination carcasses are graded for quality (based on intramuscular fat or marbling) and yield. Any tenderness prediction will more than likely be done along with quality and yield grading. It is well established that the variation in the rate and extent of postmortem proteolysis and tenderization is the source of the variation in meat tenderness at the consumer level. Because of the variation in the rate of postmortem proteolysis, any prediction method will have to be able to predict the potential of a muscle to undergo postmortem proteolysis from the time of prediction until 14 days of postmortem storage. Because of the difficulty in developing an instrument that can predict tenderization that occurs beyond 36–48 h (the time at which tenderness will be predicted), there is no accurate method of predicting tenderness. It is highly likely that we can develop methods that can predict meat tenderness at the time of measurement. Therefore, if we could accelerate postmortem proteolysis so that much of it has happened by the time of tenderness prediction (36–48 h post exsanguination in the United States), the likelihood of developing a tenderness prediction method would dramatically increase.

We hope that the data presented in this review paper and in the original papers that are the basis for this review paper will once and for all convince the researchers in this field that postmortem tenderization is due to μ-calpain-induced degradation of key myofibrillar proteins. Acceptance of such a conclusion, which is rooted in sound

research, will enable the researchers to focus their efforts to elucidate the regulation of μ-calpain in postmortem muscle. If application of knowledge of μ-calpain regulation results in acceleration of μ-calpain activity, it should facilitate the development of accurate methods of predicting meat tenderness and ultimately in methods to ensure all meat is acceptably tender.

9. Conclusions

Data generated by numerous projects in our laboratory, as well as in laboratories around the world, have convinced us that μ-calpain-induced degradation of key myofibrillar proteins is the cause of postmortem proteolysis and, hence, μ-calpain activity is the primary source of variation in tenderness of muscles for which proteolysis is the major determinant of tenderness. Discovering the mechanisms of μ-calpain activity regulation in early postmortem muscle and, more importantly, the development of methods to accelerate μ-calpain activity within the constraints of highly regulated production practices should have a dramatic effect on the ability of the meat industry to produce a product that is consistently tender.

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