

Postmortem Proteolysis and Calpain/Calpastatin Activity in Callipyge and Normal Lamb Biceps Femoris During Extended Postmortem Storage^{1,2}

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ABSTRACT: The present experiment was conducted to determine whether calpastatin inhibits only the rate, or both the rate and extent, of calpain-induced postmortem proteolysis. Biceps femoris from normal (n = 6) and callipyge (n = 6) lamb was stored for 56 d at 4°C. Calpastatin activity was higher ($P < .05$) in the callipyge muscle at 0 and 14 d postmortem, but not at 56 d postmortem. The activity of μ -calpain did not differ between normal and callipyge biceps

femoris at 0 and 56 d postmortem ($P > .05$), but was higher at 14 d postmortem in the callipyge muscle ($P < 0.05$). The activity of m-calpain was higher in the callipyge muscle ($P < 0.05$). Western blot analyses of titin, nebulin, dystrophin, myosin heavy chain, vinculin, α -actinin, desmin, and troponin-T indicated that postmortem proteolysis was less extensive in callipyge than in normal biceps femoris at all postmortem times. The results of this experiment indicate that calpastatin inhibits both the rate and extent of postmortem proteolysis.

Key Words: Calpain, Calpastatin, Callipyge, Postmortem Changes, Proteolysis

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Introduction

The calcium-dependent protease μ -calpain seems to be a major cause of postmortem tenderization of skeletal muscle through degradation of key myofibrillar and associated proteins (for review see Koohmaraie, 1996). The activity of μ -calpain is mostly regulated by calpastatin, its endogenous inhibitor. Levels of calpastatin in muscle vary considerably between species (Ouali and Talmant, 1990; Koohmaraie et al., 1991), breeds (Whipple et al., 1990;

Shackelford et al., 1991, 1994), and muscles (Koohmaraie et al., 1988; Geesink et al., 1992). It is not surprising, therefore, that postrigor calpastatin activity accounts for a greater proportion of the variation in tenderness of aged beef longissimus (~ 40%) than any other single measure (Whipple et al., 1990; Shackelford et al., 1994).

It is not known whether increased levels of calpastatin only decrease the rate of postmortem proteolysis and tenderization or also limit the extent of tenderization. A theoretical model for calpain-induced tenderization (Dransfield, 1993) assumes the first possibility, but data on tenderness and proteolysis of lamb longissimus (Koohmaraie et al., 1995) aged for 21 d and tenderness of beef longissimus aged for 35 d (Wheeler et al., 1990) provided evidence that supports the latter. An animal model that can be used to test these possibilities is callipyge sheep. Callipyge muscles contain an increased amount of calpastatin and show reduced postmortem proteolysis and tenderization for up to 3 wk of postmortem storage (Koohmaraie et al., 1995). If an increased level of calpastatin only reduces the rate of postmortem proteolysis, and thereby tenderization, a considerable amount of postmortem proteolysis should occur in callipyge muscles beyond 3 wk postmortem.

Our objective in the current study, therefore, was to compare postmortem proteolysis and calpain and calpastatin activities of normal and callipyge muscle.

¹Mention of a trade name, proprietary product or specific equipment is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Materials and Methods

Animals

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Crossbred (1/2 Dorset × 1/2 Romanov) lambs ($n = 12$) were grain-fed and slaughtered at about 180 d of age. Carcasses were assigned to callipyge and normal as reported by Koohmaraie et al. (1995). After slaughter and dressing (within 30 min postmortem), the biceps femoris was removed from the left side of the carcass for determination of calpain and calpastatin activities. About 25 g of the remainder of the muscle was diced, frozen in liquid nitrogen, and stored (-70°C) for up to 2 mo before preparation of samples for SDS-PAGE and immunoblotting. At 24 h postmortem, the biceps femoris was removed from the right side of the carcass. The biceps femoris was cut into chops that were assigned to determination of calpain and calpastatin activities at 14 and 56 d postmortem, and SDS-PAGE and immunoblotting after 1, 3, 7, 21, 42, and 56 d of vacuum storage at 4°C . Samples for SDS-PAGE and immunoblotting were diced, frozen in liquid nitrogen, and stored (-70°C) for up to 2 mo before use.

Calpains and Calpastatin

Calpains and calpastatin were extracted from 25 g of muscle and quantified according to the procedure described by Koohmaraie (1990b), except that dialysis against elution buffer was used to reduce the ionic strength prior to ion-exchange chromatography. To be able to detect μ -calpain activity in the column fractions from 56 d postmortem samples, the standard assay mixture was incubated overnight at 15°C . Pooled μ -calpain activity from 56 d postmortem samples was determined using [^{14}C]casein according to Koohmaraie (1992b). Diluted calpain samples of known activity in the standard calpain assay were used in the radiolabeled casein assay to be able to express the activity in standard units.

SDS-PAGE and Immunoblotting

One gram of frozen muscle was homogenized in 10 volumes of extraction buffer (100 mM Tris-HCl, pH 7.5, 10 mM EDTA, .05% (vol/vol) 2-mercaptoethanol [MCE], 100 mg/L ovomucoid, 2 mM PMSF, and 6 mg/L leupeptin; 4°C) using a Polytron. The homogenate was centrifuged at $8,800 \times g$ for 10 min. The supernatant was filtered through a Whatman no. 1 filter, and the protein concentration was determined using the Coomassie Plus protein assay (Pierce, Rockford, IL). Samples were diluted to 3 mg/mL and mixed with half a volume of protein denaturing buffer (PDB; 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% MCE, .02% bromophenol blue, and 10% glycerol). The pellet

was extracted twice as described above and dissolved in PDB by heating at 95°C for 5 min. The protein concentration in these samples was determined according to Karlsson et al. (1994). The SDS-PAGE was performed according to Laemmli (1970) on .75 mm thick 12.5% (troponin-T; TnT), 10% (myosin heavy chain, vinculin, calpastatin, and desmin), 7.5% (α -actinin, μ -calpain), 6% (dystrophin), or 5% (nebulin) (37.5:1 ratio of acrylamide to N,N'-methylenebis [bisacrylamide]) separating gels with 4% (37.5:1) stacking gels. For electrophoresis of titin, continuous 5% gels (100:1) in 50% glycerol were used. For separation of α -actinin isoforms, discontinuous inverse gradient (DIG) gels with 4% (37.5:1) stacking gels were used. The separating gel consisted of, from top to bottom, layers of 10% (1.25 cm), 7.5% (1.25 cm), and 6% (3 cm) gels (37.5:1). All gels were run at 150 V. Gels for μ -calpain and α -actinin (DIG-gels) were run for an additional 30 min after the dye front reached the bottom of the gels. Proteins were electrophoretically transferred to nitrocellulose membranes (Amersham, Buckinghamshire, UK). Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (pH 7.4) containing .05% Tween-20 (TTBS) for 1 h. Primary antibodies used in these experiments included mouse anti-recombinant calpastatin (Doumit and Koohmaraie, 1999), anti- μ -calpain monoclonal antibody, produced according to standard protocols (Harlow and Lane, 1988) against gel-purified 80 kDa subunit of bovine skeletal muscle μ -calpain, mouse anti-titin, (9D10, Wang and Greaser, 1985), mouse anti-myosin heavy chain (MF20, Bader et al., 1982), mouse anti-desmin (D3, Danto and Fischman, 1984), mouse anti-troponin-T (JTL-12, Sigma Chemical Co., St Louis, MO), mouse anti- α -actinin (EA-53, Sigma Chemical), mouse anti-nebulin (NB-2, Sigma Chemical), mouse anti-dystrophin (NCL-DYS1, Novocastra, Newcastle upon Tyne, UK), and mouse anti-vinculin (V284, Accurate, Westbury, NY). The secondary antibody was alkaline phosphatase conjugated anti-mouse IgG (Sigma Chemical). Antibodies were diluted in blocking buffer and incubated for 1 h at room temperature with gentle rocking. Membranes were washed three times with TTBS after each incubation. Antibody binding was visualized by exposure to BCIP/NBT (Bio-Rad, Hercules, CA). The intensity of the bands was quantified using a ChemiImager 4000 (Alpha Innotech Corp., San Leandro, CA) digital imaging system.

Statistical Analysis

Analysis of variance was conducted for a split-plot design; phenotype served as the whole-plot treatment, carcass within phenotype served as the whole-plot error term, and postmortem storage period served as the split plot. For μ -calpain, variances were not homogenous across subclasses. Therefore, a Box-Cox transformation was conducted before ANOVA (Box

and Cox, 1964). Means were back-transformed for presentation (Table 1). Means were separated using the PDIFF procedure (a pairwise *t*-test) of SAS (1988).

Results and Discussion

Calpains and Calpastatin

The interaction of the main effects (phenotype and days postmortem) was significant for μ -calpain and calpastatin activities, but not for m-calpain activity (Table 1). In agreement with the results of Koohmaraie et al. (1995), the activity of m-calpain was higher in callipyge than in normal muscles. In accordance with the results of Koohmaraie et al. (1987) and Koohmaraie (1990a), the activity of m-calpain remained virtually constant throughout the aging period. When carcasses are infused with calcium chloride, m-calpain rapidly loses activity during aging (Koohmaraie et al., 1989). The stability of m-calpain during aging is, therefore, likely the result of insufficient free calcium to activate this enzyme. A theoretical model for postmortem tenderization proposed by Dransfield (1993) includes activation of m-calpain in postmortem muscle. As a result, m-calpain activity is predicted to decline about 24% during 14 d of postmortem storage. Continuation of this trend to 56 d

Table 1. Calpain and calpastatin activities of the biceps femoris of normal and callipyge lambs at death, 14, and 56 days postmortem

Item	μ -Calpain	m-Calpain	Calpastatin
Phenotype			
Normal	.30 ^b	1.11 ^b	1.34 ^b
Callipyge	.35 ^a	1.71 ^a	2.28 ^a
SEM	.03	.05	.12
Day			
0	.83 ^a	1.35 ^b	3.20 ^a
14	.12 ^b	1.63 ^a	1.49 ^b
56	.02 ^c	1.25 ^b	.76 ^c
SEM	.03	.06	.15
Interaction			
Probability level	.02	.74	.02
Normal d 0	.84 ^a	1.03	2.46 ^b
Normal d 14	.05 ^c	1.31	.93 ^c
Normal d 56	.02 ^d	.99	.64 ^c
Callipyge d 0	.82 ^a	1.67	3.93 ^a
Callipyge d 14	.19 ^b	1.95	2.05 ^b
Callipyge d 56	.03 ^d	1.51	.88 ^c
SEM	.05	.09	.21

a,b,c,d Means in the same column, within a main effect, with superscripts that do not have a common superscript letter differ ($P < .05$).

postmortem would lead to almost complete loss of activity. Clearly, this was not the case in the present study.

μ -Calpain activities were similar in callipyge and normal muscles at death. However, at 14 d postmortem, callipyge muscles had 23% of their at-death activity, whereas the activity in normal muscles had dropped to 6% of their at-death activity. In both muscles, the μ -calpain activities continued to decline between 14 and 56 d postmortem and were not significantly different at 56 d postmortem. The antibody against the large subunit of μ -calpain used in this study is specific for μ -calpain, and does not cross-react with other soluble muscle proteins (Figure 1). Western blots against the large subunit of μ -calpain are shown in Figure 2. Autolysis of μ -calpain was slower in callipyge muscles than in normal muscles. Both autolysis of the 80 kDa subunit to a 78 kDa fragment, and further autolysis from 78 to 76 kDa, proceeded at a slower rate in the callipyge muscle. In vitro, calpastatin inhibits autolysis of μ -calpain (Inomata et al., 1988; Koohmaraie, 1992a). The decreased rate of autolysis in the callipyge muscle was, therefore, likely the result of inhibition of this process by calpastatin. At 56 d postmortem, the extractable μ -calpain activity was 2 to 3% of the at-death activity. Clearly, the immunologically detectable amount of μ -calpain does not reflect this drop in activity. This indicates that only part of the immunologically detectable μ -calpain represents an active enzyme. μ -Calpain likely loses most of its activity during postmortem storage as a result of instability of the autolyzed enzyme, and not through extensive autoly-

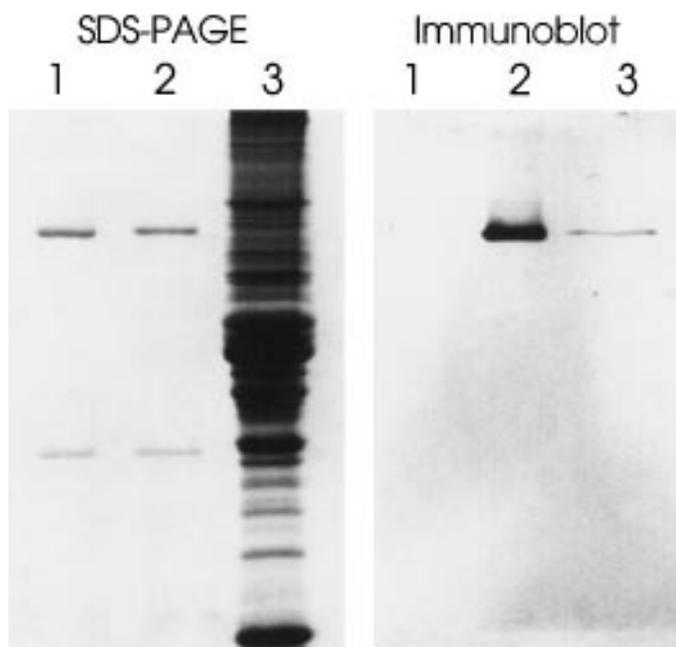


Figure 1. An SDS-PAGE and Western blot of purified m-calpain, μ -calpain, and soluble fraction of lamb biceps femoris probed with anti- μ -calpain monoclonal antibody. Lane 1: Purified m-calpain (.5 μ g), Lane 2: Purified μ -calpain (.5 μ g), Lane 3: Soluble fraction of at-death lamb biceps femoris (50 μ g).

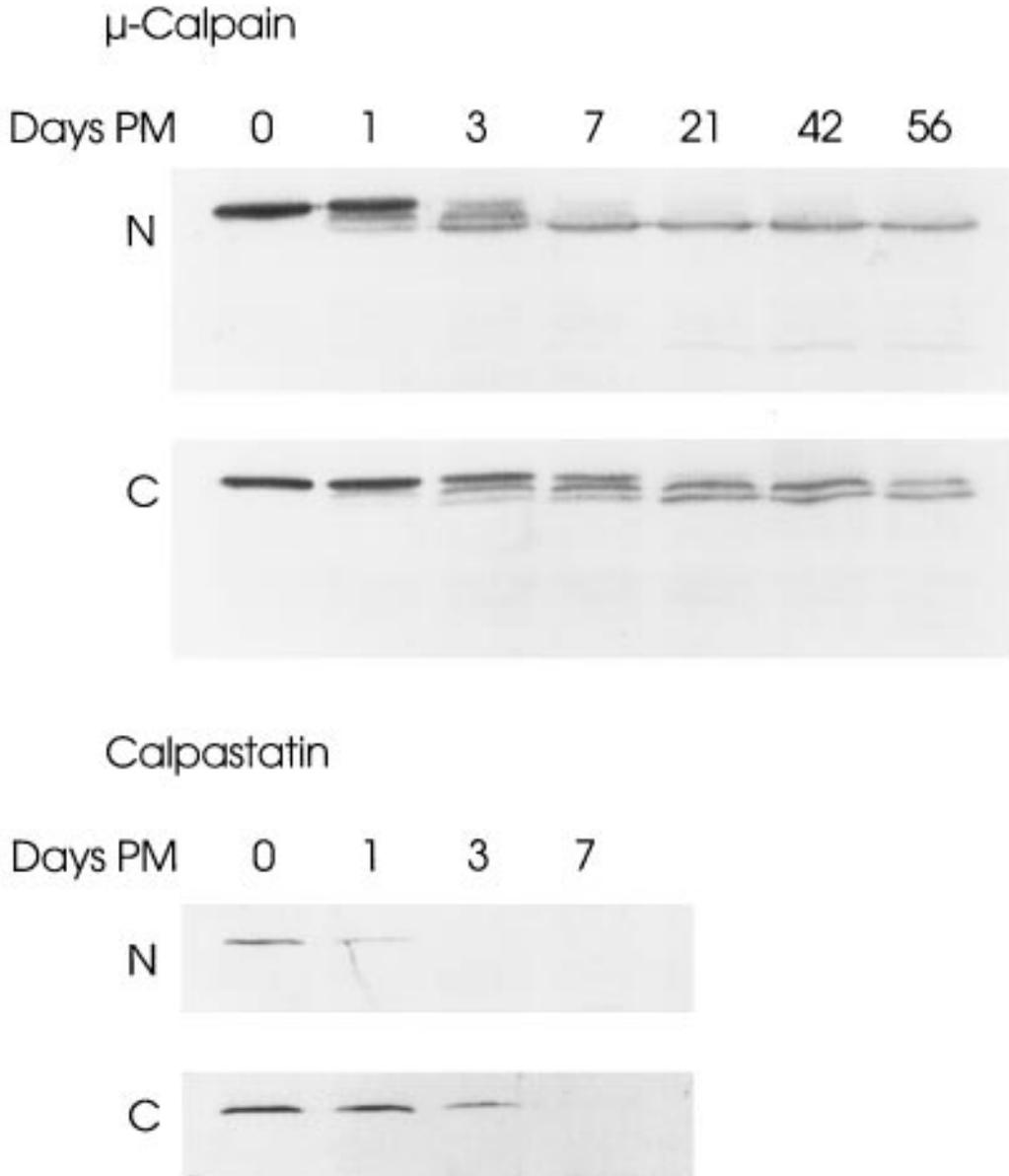


Figure 2. Western blot analysis of the large subunit of μ -calpain and calpastatin in the soluble fraction of normal (N) and callipyge (C) biceps femoris (PM = postmortem).

sis to inactive fragments (Geesink and Koohmaraie, unpublished). The present results, therefore, suggest that the rate of autolysis and subsequent loss of activity was reduced in the callipyge muscle.

At death, calpastatin activity was 60% higher in callipyge biceps femoris than in those from normal animals (Table 1). This increase is lower than previously reported (124%) by Koohmaraie et al. (1995), but confirms a significant elevation in calpastatin in this muscle in callipyge lamb. Calpastatin activity declined in both muscles during aging but, at 14 d postmortem, callipyge muscles contained more than twice the amount of calpastatin activity of normal muscles. The calpastatin activity in normal muscles did not decrease significantly between 14 and 56 d postmortem. The activity in callipyge muscles

continued to decline and was not significantly different from the activity in normal muscles at 56 d postmortem. In accordance with the results of Doumit and Koohmaraie (1999), calpastatin was degraded during postmortem storage (Figure 2). In fact, of the proteins tested, calpastatin was the most sensitive to postmortem proteolysis. However, some proteolytic fragments of calpastatin retain inhibitory activity (Mellgren and Carr, 1983; Imajoh et al., 1984; Doumit and Koohmaraie, 1999), and their activity could be detected up to 56 d postmortem (Table 1).

Postmortem Proteolysis

For a detailed comparison of postmortem proteolysis between normal and callipyge biceps femoris, one

muscle from each group was selected. The selection criterion was that these muscles had calpastatin levels at death that were closest to the mean for the respective groups. In addition, differences in the rate of proteolysis of all samples were quantified by measuring the amount of undegraded desmin at death and 21 d postmortem.

Titin

Titin is a high molecular weight ($M_r = \sim 3 \times 10^6$) protein that spans half the length of a sarcomere and connects Z-lines and M-lines in the sarcomeres of striated muscles (for reviews see Trinick, 1994; Labeit et al., 1997). As reviewed by Robson et al. (1997), degradation of titin, because of its structural role in the myofibril, may be instrumental for postmortem tenderization. In agreement with the results of Koohmaraie et al. (1995), degradation of titin occurred much slower in callipyge than in normal muscle (Figure 3). A minor amount of intact titin was detectable at 21 d postmortem in the normal muscle, whereas, intact titin was clearly present in the callipyge muscle after 56 d of postmortem storage.

Nebulin

Nebulin is a high molecular weight ($M_r = 6$ to 9×10^5) protein and is part of the thin filaments (Wang et al., 1996). Nebulin is very susceptible to postmortem proteolysis (Fritz and Greaser, 1991; Huff-Lonergan et al., 1995) and, even in callipyge longissimus, little intact nebulin remains after 7 d of postmortem storage (Koohmaraie et al., 1995). In the same time period, however, little tenderization occurred (Koohmaraie et al., 1995). It is clear, therefore, that degradation of intact nebulin contributes little to postmortem tenderization. It is possible that nebulin degradation products still contribute to the structural integrity of the myofibril and that further degradation of these fragments contributes to tenderization. In agreement with the results of Koohmaraie et al. (1995), degradation of nebulin occurred at a slower rate in callipyge than in normal muscles (Figure 3). No intact nebulin remained at 7 d postmortem in the normal muscle, and little intact nebulin remained in the callipyge muscle after the same aging period. The difference in the rate of proteolysis was more pronounced for some high molecular weight fragments of nebulin. In the callipyge muscle, these fragments were present from 7 to 56 d postmortem; whereas little of these fragments remained at 21 d postmortem in the normal muscle.

Dystrophin

Dystrophin is the protein product of the Duchenne muscular dystrophy gene (Hoffman et al., 1987). Dystrophin is composed of two subunits ($M_r = \sim 4 \times$

10^5) and is part of the costameres, which link myofibrils to the sarcolemma (Minetti et al., 1992). In accordance with the results of Taylor et al. (1995), dystrophin was highly susceptible to postmortem proteolysis (Figure 4). Dystrophin was largely degraded at 7 d postmortem in the normal muscle, and little intact dystrophin remained at 21 d postmortem in the callipyge muscle. Similar to degradation of nebulin, the difference in the rate of proteolysis between the callipyge and normal muscles was more pronounced for some dystrophin fragments (Figure 3C, see arrows). Considering its location in muscle, degradation of dystrophin likely contributes to sarcolemma detachment as observed in postmortem muscle (Taylor and Koohmaraie, 1998). In the latter study, 7 and 20% of the sarcomeres of normal and callipyge longissimus, respectively, remained attached to the sarcolemma at 14 d postmortem. However, the shear force of these muscles was 2.8 kg for normal and 9.0 kg for callipyge longissimus at 14 d postmortem. Proteolysis of dystrophin, therefore, seems to contribute little to tenderization.

Myosin Heavy Chain

Myosin heavy chain ($M_r = \sim 2 \times 10^5$) is the large subunit of the myosin molecule ($M_r = \sim 4.8 \times 10^5$), which is composed of two large and four small subunits. Myosin is the main constituent of the thick filaments (for a recent review see Cooke, 1997). In agreement with the results of Bandman and Zdanis (1988) on bovine muscle, very little postmortem degradation of myosin heavy chain is detectable during postmortem storage of ovine biceps femoris (Figure 4). Some degradation products (Figure 4, see arrows) are detectable after long-term storage, but the intensity of the bands corresponding to these fragments is minimal, compared with the intact protein. To our knowledge, no studies have been published in which degradation of the myosin light chains has been studied using Western blotting. On Coomassie-stained gels, however, the intensity of the bands corresponding to the myosin light chains remained unchanged during postmortem storage for 56 d (data not shown). These results indicate that proteolysis of myosin does not play a role in postmortem tenderization.

Vinculin

Vinculin ($M_r = \sim 1.16 \times 10^5$) is part of the costameres, which link myofibrils bordering the sarcolemma to this structure (Minetti et al., 1992). In agreement with the results of previous studies (Koohmaraie et al., 1995; Taylor et al., 1995), vinculin was degraded during postmortem storage (Figure 5). Proteolysis of vinculin occurred at a slower rate in the callipyge muscle. A degradation product with a molecular weight of about 90 kDa (Figure 5, see

arrow) was detectable at 3 d postmortem in the normal muscle, but not in the callipyge muscle at 7 d postmortem. In addition to vinculin, the antibody recognized an additional band above vinculin (see arrowhead). This band likely corresponds to metavinculin, a higher molecular weight variant of vinculin, which is more abundant in smooth and cardiac muscle (Strasser et al., 1993). It is not known whether degradation of vinculin contributes to postmortem tenderization. However, if vinculin is only involved in sarcomere-sarcolemma attachment, its degradation likely does not contribute to tenderization.

α-Actinin

α-Actinin is a cross-linking protein found in both muscle and nonmuscle cells. In striated muscle, *α-actinin* is localized at the Z-disk and is composed of two identical subunits ($M_r = \sim 1 \times 10^5$). *α-Actinins* from fish muscle, human T cells, and human platelets are susceptible to the action of calpain (Gache et al., 1984; Selliah et al., 1996; Taylor et al., 1997). However, *α-actinins* from rabbit and porcine muscle have been reported to be resistant to proteolysis by calpain (Goll et al., 1991). During postmortem storage, *α-actinin* is gradually released from the Z-

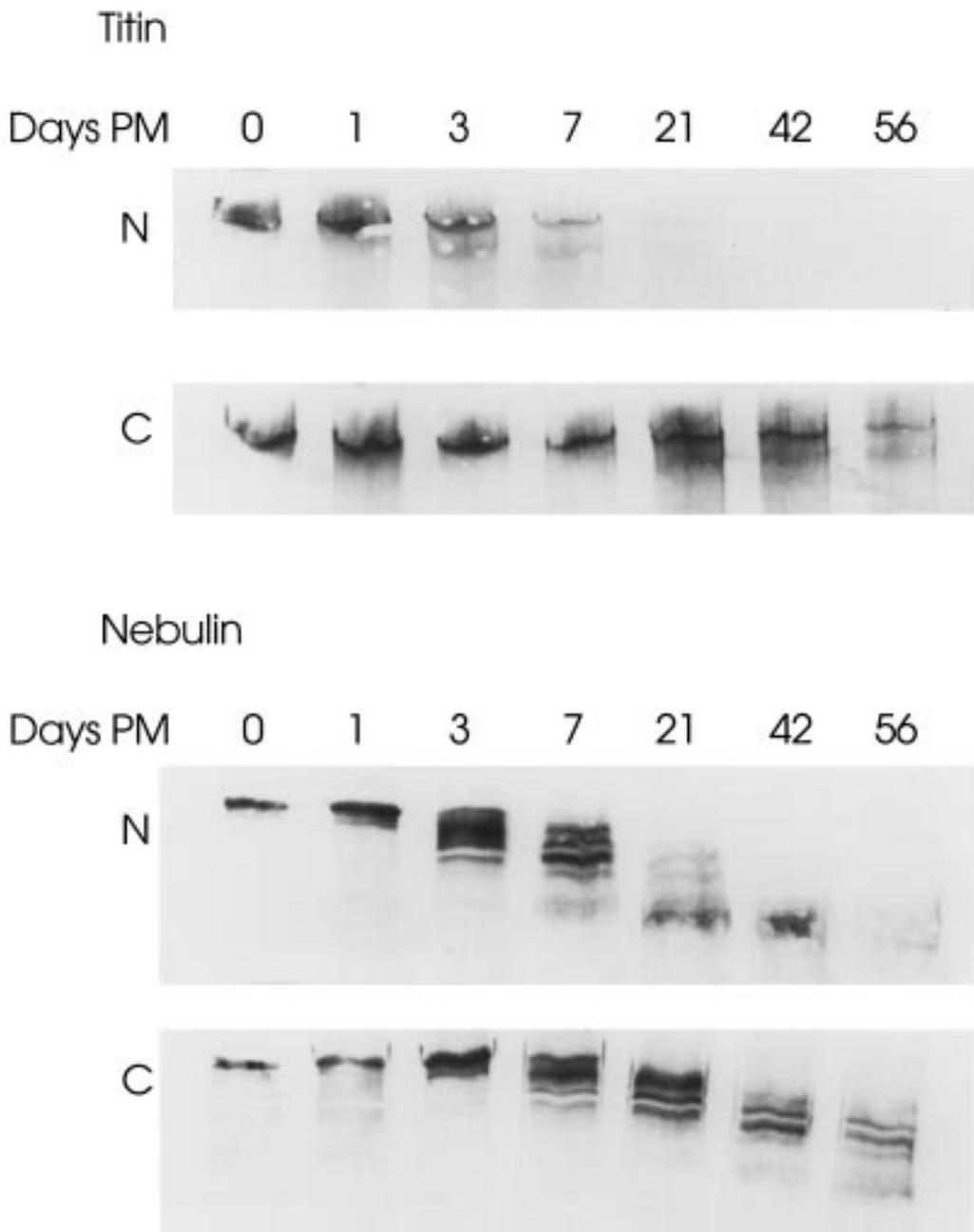


Figure 3. Western blot analysis of titin and nebulin degradation during postmortem (PM) storage of normal (N) and callipyge (C) biceps femoris.

disk, indicating that one or more of the proteins it attaches to is degraded during postmortem storage. It has recently been reported that titin binds to α -actinin (Ohtsuka et al., 1997a,b; Sorimachi et al., 1997). Degradation of titin, therefore, could cause the release of α -actinin from the Z-disk. The appearance of α -actinin in the soluble muscle fraction (Figure 6A) seems to parallel the degradation of titin (Figure 3) during postmortem storage. In accordance with the results of Hwan and Bandman (1989), some degradation of α -actinin was observed during long-term postmortem storage, as evidenced by the appearance of a minor degradation product (see arrow). In the normal muscle, the solubilized α -actinin appeared to be a doublet beyond 21 d postmortem. Degradation of α -actinin from fish muscle and human platelets by calpain results in a degradation product with a slightly lower molecular weight than the native protein (Gache et al., 1984; Taylor et al., 1997). We managed to separate the doublet by using a discontinuous inverted gradient gel (Figure 6B). The lower band of the doublet was present in at-death muscle and its intensity did not increase during aging (Figure 6B). The two bands, therefore, seem to be isoforms of α -actinin. The presence of two isoforms of α -actinin in skeletal muscle has been reported for pork, rabbit, and chicken (Suzuki et al., 1973; Kobayashi et al., 1984), and in humans these isoforms are the product of different genes (Beggs et al., 1992). Interestingly, the upper band was detectable in the soluble fraction as early as 3 d postmortem; whereas the lower band was not detectable during the first 7 d postmortem (Figure 6B). Although Suzuki et al. (1973) isolated the two isoforms from fast- and slow-twitch porcine muscle, the expression of the two isoforms does not reflect a clear-cut distinction between fast- and slow-twitch muscles in rabbit (Schachat et al., 1985). In the latter study, the expression of the α -actinin isoforms correlated with Z-disk width and the expression of troponin-T and tropomyosin isoforms. Recently, it was reported that the Z-disk region of titin is assembled from a 45 amino acid repeat that is expressed in variable copy numbers (Gautel et al., 1996). This "Z-repeat" contains the α -actinin binding site and seems to be important for the fiber type diversity of the Z-disk lattice (Sorimachi et al., 1997). The earlier appearance of the upper α -actinin band in the soluble muscle fraction possibly reflects a fiber type depen-

dent rate of titin degradation postmortem. This could be a result of differences in the susceptibility of titin isoforms to proteolysis, as suggested by Tanabe et al. (1997), or fiber type dependent differences in the relative amounts of μ -calpain and calpastatin.

Desmin

Desmin is an intermediate filament protein composed of four subunits ($M_r = \sim 5.3 \times 10^4$). Desmin intermediate filaments encircle the Z-disks and connect adjacent myofibrils at the Z-disk level (for review see Price, 1991). Degradation of desmin during postmortem storage has been observed in numerous studies, and its degradation seems to parallel tenderization (for review see Robson et al., 1997). In accordance with the results of Koohmaraie et al. (1995), desmin degradation progressed at a slower rate in callipyge than in normal muscle (Figure 5). To test whether the selected muscles properly reflect a difference in postmortem proteolysis between callipyge and normal muscles, the amount of desmin remaining after 21 d of storage was quantified for all muscles (Table 2). At 21 d postmortem, more than 80% of desmin was degraded in normal muscles, whereas less than 50% was degraded in callipyge muscles. The selected muscles, therefore, properly reflect a difference in postmortem proteolysis between the two groups.

Troponin-T

Troponin T ($M_r = \sim 3.5 \times 10^4$) is a regulatory muscle protein that, in complex with troponins I and C and tropomyosin, constitutes the Ca^{2+} -sensitivity switch that regulates the contraction of striated muscle fibers. In adult skeletal muscle, six major TnT isoforms can be distinguished by SDS-PAGE. Four of them are products of the TnT-fast gene, whereas the others are products of the TnT-slow gene (for a recent review see Schiaffino and Reggiani, 1996). Degradation of TnT, with simultaneous appearance of protein fragments with a molecular weight of 27 to 30 kDa, can be easily observed on Coomassie stained gels and is probably the most reported change in myofibrillar proteins during aging of muscle (for review see Robson et al., 1997). In accordance with the results of Koohmaraie et al. (1995), TnT degradation progressed at a slower rate in callipyge than in normal muscle (Figure 5). In the normal muscle, proteolysis of TnT results in two apparently stable fragments with a molecular weight of 27 to 30 kDa, whereas in the callipyge muscle degradation hardly proceeds beyond a doublet of intermediary proteolysis products (Figure 5).

Summary

The present results show that μ -calpain activity can be detected for up to 56 d postmortem and that

Table 2. Amount of desmin remaining after 21 d of postmortem storage

Phenotype	Percentage of d 0
Normal	19.5 ^b
Callipyge	53.6 ^a
SEM	6.4

^{a,b}Means with superscripts that do not have a common superscript differ ($P < .05$).

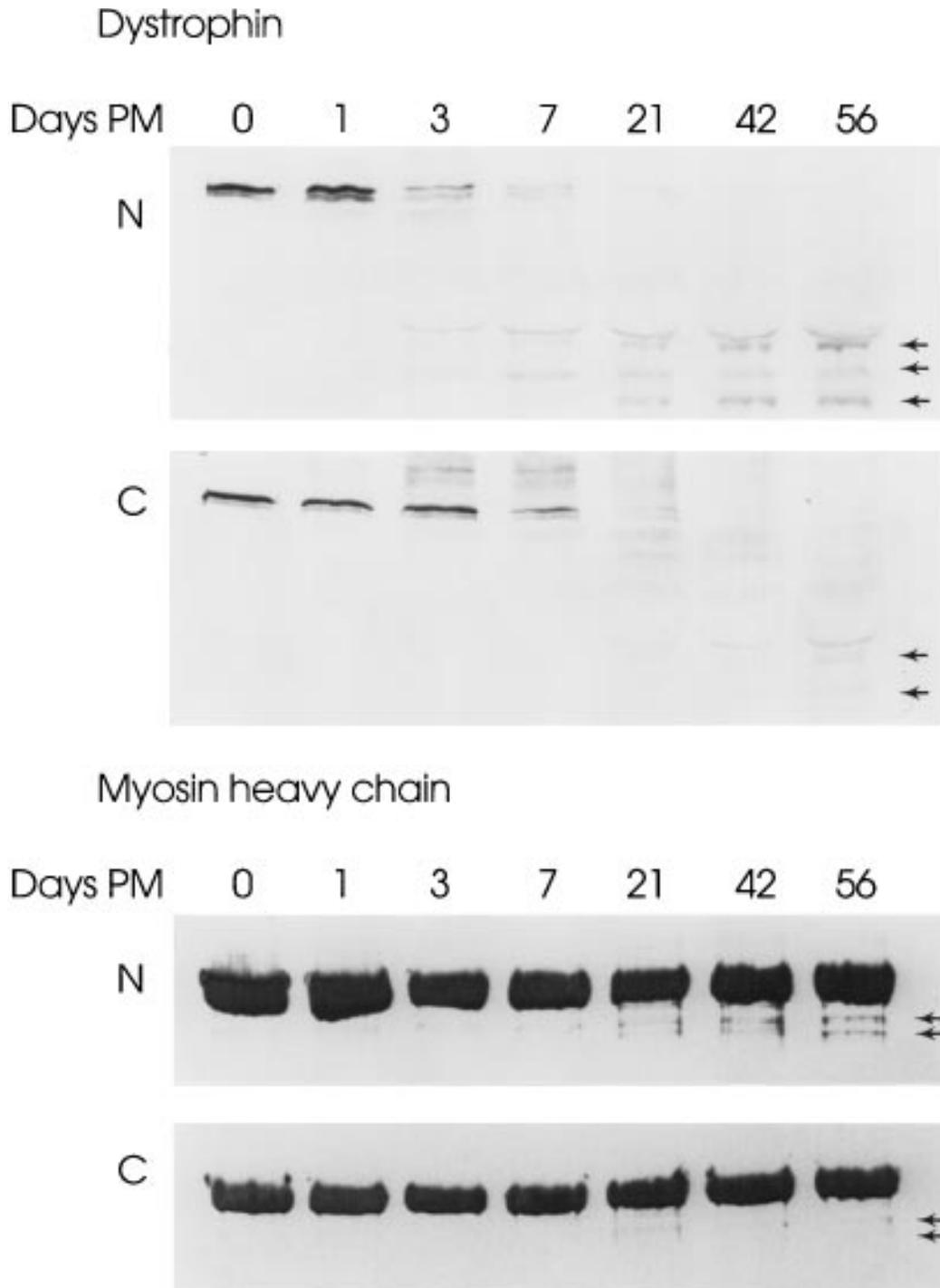


Figure 4. Western blot analysis of dystrophin and myosin heavy chain degradation during postmortem (PM) storage of normal (N) and callipyge (C) biceps femoris. Some degradation products of the respective proteins are indicated with arrows.

postmortem proteolysis progresses during the same period. However, at no time postmortem did the overall level of postmortem proteolysis in callipyge muscle equal that of normal muscle. Even though μ -calpain activity can be detected at 56 d postmortem, it is unlikely that the difference in postmortem proteolysis between normal and callipyge muscle would

diminish to a large extent beyond 56 d postmortem. First, postmortem proteolysis proceeds very slowly at this time postmortem, as evidenced by the extent of proteolysis between 21 and 56 d postmortem (Figures 3, 4, and 5). Second, μ -calpain and calpastatin activities are not different between normal and callipyge muscle at 56 d postmortem. The rate of

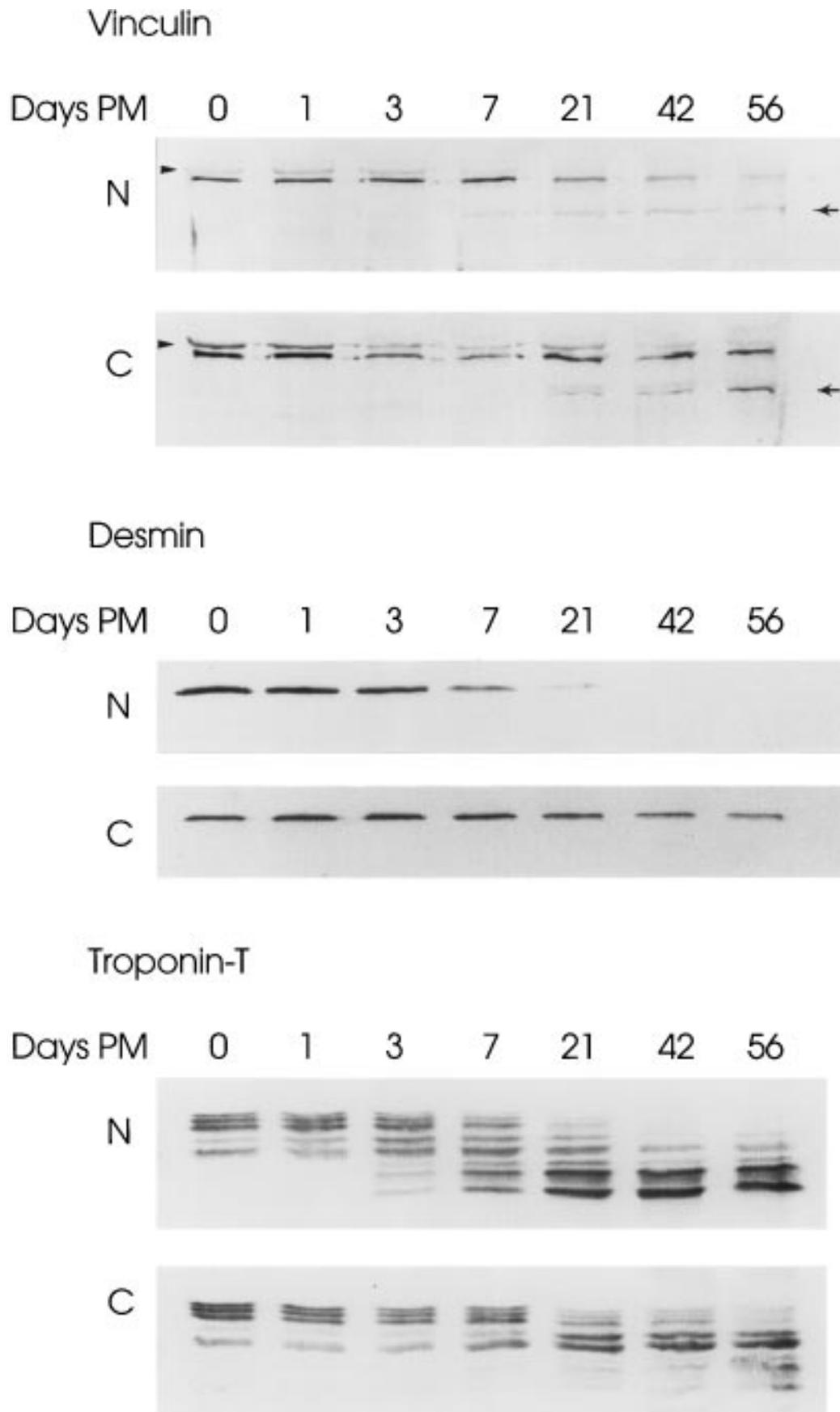


Figure 5. Western blot analysis of vinculin, desmin, and troponin-T degradation during postmortem (PM) storage of normal (N) and callipyge (C) biceps femoris.

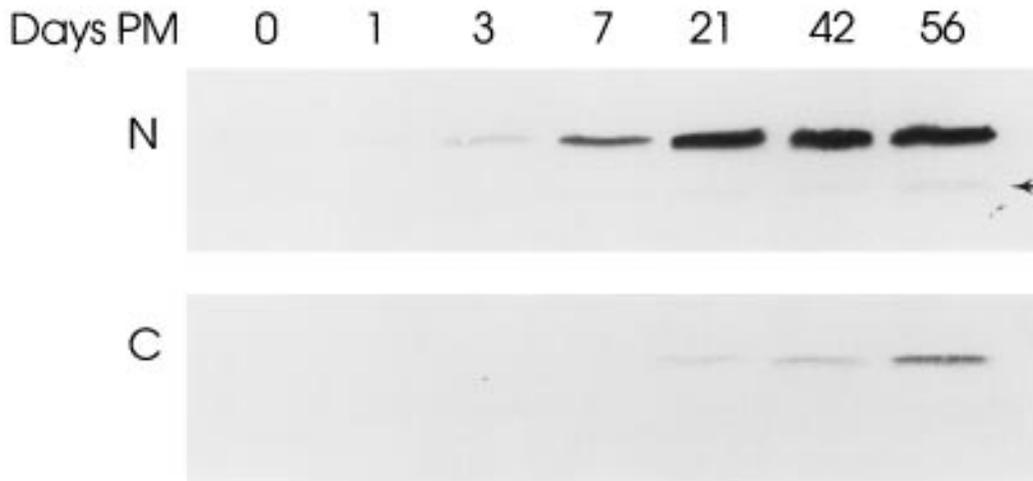
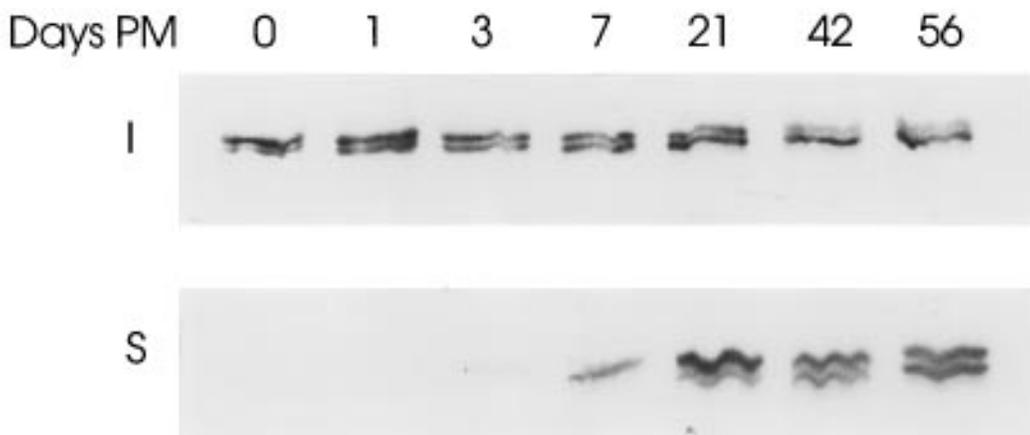
A α -Actinin (soluble)**B** α -Actinin

Figure 6. Western blot analysis of α -actinin solubilization during postmortem (PM) storage of normal (N) and callipyge (C) biceps femoris (A), and α -actinin isoforms in the insoluble (I) and soluble (S) muscle fraction of normal biceps femoris at different times postmortem (B).

postmortem proteolysis beyond 56 d postmortem would, therefore, likely be similar in both muscles. The present results do not support the conclusion of Dransfield (1993) that calpastatin only limits the rate of postmortem proteolysis. Our results indicate that calpastatin inhibits both the rate and extent of postmortem proteolysis.

Implications

Elevated calpastatin levels seem to be associated with a decreased rate and extent of postmortem proteolysis. Results support the hypothesis that postmortem proteolysis is probably calpain-mediated. Thus, strategies to improve postmortem tenderization

should focus on processes that maximize calpain-induced proteolysis. μ -Calpain activity can be detected for up to 56 d postmortem, and proteolysis proceeds during this time. Thus, extended aging may be a viable option to tenderize meat that is tough after 14 d of postmortem storage.

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