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Effects of Postmortem Aging Time, Animal Age, and Sex on Degradation of Titin and Nebulin in Bovine Longissimus Muscle

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ABSTRACT: This study was conducted to determine degradation of the giant myofibrillar proteins titin and nebulin in postmortem aged beef, with known tenderness values, from animals differing in sex (steers vs bulls) and age (cows vs steers and bulls). Ten bulls and 10 steers (both groups were approximately 14 mo old) and 10 cows (44 to 108 mo old) were slaughtered. Longissimus muscle samples were removed for determination of Warner-Bratzler shear force, sensory panel tenderness evaluation, and SDS-PAGE analysis at 3, 7, 14, and 28 d postmortem. The SDS-PAGE analysis of titin and nebulin revealed that titin often migrated as three closely-spaced bands (T1, T1-2, T2, in increasing order of migration) in 3-d postmortem samples. With increasing time postmortem, intact titin (T1) decreased and degraded titin (T2) increased in all samples. Within a class (i.e., steers, bulls, or cows) the rate of conversion of T1 to T2 was slower in the less-tender samples. The T1 to T2 conversion postmortem was slower in the intact males (bulls) than in the castrated males (steers). The T1 to T2 conversion postmortem also was slower in the older animals (cows) in comparison to the younger steers, but not in comparison to the younger bulls. Nebulin was degraded by 3 d postmortem in tender samples from steers, but some nebulin remained in the less-tender 3-d samples from steers and in all of the 3-d samples from bulls and older animals (cows). Intact nebulin was absent in all 7-d samples, regardless of the class of animal. Our results suggest that titin and nebulin are degraded at faster rates in more tender beef samples within each of the three classes of animals examined. The rate of degradation seems to differ when sex and age classifications are compared.

Key Words: Beef, Myofibrils, Postmortem Changes, Meat Quality

Introduction

It is well established that beef tenderness increases during postmortem storage of carcasses at refrigerated temperatures, and a significant part of this increase in tenderness is thought to involve postmortem loss of structural integrity of the myofibril (Parrish et al., 1973; Koohmaraie et al., 1987) and other cytoskeletal elements (Robson et al., 1984, 1991) of the muscle cell. Two of the structural components of the myofibril that have been shown to change during postmortem aging include the giant proteins titin and nebulin (Lusby et al., 1983; Bandman and Zdanis, 1988; Fritz and Greaser, 1991), but the role these two proteins may have in influencing tenderness is not yet clear (Anderson and Parrish, 1989; Fritz et al., 1993). Titin, the third most abundant protein in the myofibril, is the largest protein yet discovered (approximately 3,000 kDa) and likely serves at least two functions in striated muscle cells in vivo by providing a template for thick myosin filaments and an elastic connecting element between thick filaments and the sarcomeric Z-lines (Koretz et al., 1993; see Robson et al., 1991 and Robson, 1995 for reviews). Nebulin, another significant component of the skeletal muscle myofibril, is a quite large (approximately 800 kDa), insoluble protein that constitutes a set of inextensible filaments in conjunction with the thin actin filaments (see Robson, 1995 for review). Both titin and nebulin are anchored at one of their ends to the Z-line and are considered to be important contributors to myofibrillar integrity (Robson, 1995).

The primary objectives of this study were to examine the effects of postmortem aging time, sex condition of males (bulls vs steers), and age of beef animals at slaughter on the degradation patterns of...
the myofibrillar proteins titin and nebulin in longissimus muscle samples having known tenderness values obtained from Warner-Bratzler shear force measurements and sensory panel evaluations.

Materials and Methods

Source and Storage of Longissimus Muscle Samples

All animals in this study were slaughtered according to standard humane procedures. Longissimus muscles were removed from the carcasses of 10 intact males (bulls) and 10 castrated males (steers) that were all approximately 14 mo old (A maturity) at slaughter. The longissimus muscles were also removed from the carcasses of 10 cows that ranged in age from 44 to 108 mo old (C50 to E maturity) at slaughter. The carcasses in this study were not electrically stimulated. Marbling scores for the carcasses ranged from Slight\(^6\) to Small\(^7\). The longissimus muscles were removed from all of the carcasses at approximately 24 h postmortem to follow industry practices. Each of the 30 longissimus muscles was individually vacuum-packaged and stored at 2°C. Four steaks were removed from each longissimus muscle at 3, 7, 14, and 28 d postmortem, vacuum-packaged, frozen, and stored at -20°C until they were analyzed. Two 2.86-cm steaks were used for Warner-Bratzler (WB) shear force analysis and sensory panel evaluation. Those data were reported in Huff and Parrish (1993). Two 1.27-cm steaks were used for biochemical analysis herein.

Tenderness Analysis

The 2.86-cm steaks were analyzed by a trained sensory panel as described in Huff and Parrish (1993). Panelists rated the samples for several characteristics, including fiber fragmentation, an evaluation of how easily the sample broke down into smaller pieces upon chewing. Samples were rated on a 150-mm line scale with possible scores ranging from 0 (very difficult to fragment) to 150 (very easy to fragment). The WB shear force determinations of tenderness were done by using the WB shear force attachment of the Instron Universal Testing Machine (Instron, Canton, MA). The first yield point of the curve obtained from the Instron was identified as the compression force and corresponded to the "myofibrillar component of tenderness" (Huff and Parrish, 1993) and is the WB shear force value reported herein. The tenderness values (i.e., the WB shear force values [reported as kilograms/square centimeter] and the sensory fiber fragmentation scores) will be given with every sample shown in the Results section.

Preparation of Myofibrils

Purified myofibrils used for analysis by SDS-PAGE were isolated from raw muscle samples taken from the 1.27-cm-thick steaks, which were adjacent to the 2.86-cm-thick steaks used for WB and sensory evaluations in Huff and Parrish (1993), from samples in each sex (steers and bulls), age (cows vs steers and bulls), and postmortem aging category. Myofibrils were purified at 2°C by using differential centrifugation according to the method of Goll et al. (1974) with the following modifications. After the second wash and centrifugation with 1% Triton X-100 (vol/vol), 100 mM KCl, 2 mM MgCl\(_2\), 1 mM EGTA, 1 mM Na\(_2\)HPO\(_4\), and 20 mM potassium phosphate, pH 6.9, resuspension of the myofibrils in all succeeding steps was accomplished by vigorous stirring with a small plastic spatula rather than by homogenization in a Waring blender. This was done to minimize structural damage that may occur with extremely large proteins. Following the final wash with 100 mM KCl, the myofibrils were washed twice by resuspension in 10 volumes (wt/vol; weight in grams of the original ground muscle sample) of 5 mM Tris-HCl, pH 8.0, and were centrifuged at 3,020 \(\times\) g for 10 min. Following the second 5 mM Tris-HCl, pH 8.0, wash, the myofibrils were resuspended in four volumes (wt/vol; weight in grams of the original ground muscle sample) of 5 mM Tris-HCl, pH 8.0. The samples were immediately subjected to protein determination. The modified biuret procedure of Robson et al. (1968) was used to determine the protein content of the final myofibril suspension. Directly after protein determination and adjustment of protein concentration, 1 mL of protein solution was mixed with 5 mL of SDS sample buffer (30 mM Tris-HCl, 3 mM EDTA, 3% [wt/vol] SDS, 30% [vol/vol] glycerol, and 30 \(\mu\)g of pyronin Y/mL, pH 8.0) (Wang, 1982) and .1 mL of 2-mercaptoethanol. The samples were heated at 50°C for 20 min and were loaded onto the gels.

Electrophoretic Separation of Myofibrillar Proteins

The SDS-PAGE analysis was done according to the procedure of Laemmli (1970), with modifications to accommodate separation of proteins having widely different molecular weights. A 5% polyacrylamide slab separating gel, without a stacking gel, was used to monitor changes in proteins having extremely high molecular weights (i.e., titin and nebulin), and was made from a 30% stock solution of acrylamide (acrylamide:N,N’-bis-methylene acrylamide = 100:1) and .375 M Tris-HCl, pH 8.0, 2 mM EDTA, .1% (wt/vol) SDS, .67% (vol/vol) glycerol, and 30 \(\mu\)g of pyronin Y/mL, pH 8.0) (Wang, 1982) and .1 mL of 2-mercaptoethanol (APS). A 12% polyacrylamide separating gel was used to monitor changes in myofibrillar proteins having lower molecular weights, and was made from a 30% stock solution of acrylamide (acrylamide:N,N’-bis-methylene acrylamide = 37:1) and .375 M Tris-HCl, pH 8.0, 2 mM EDTA, .1% (wt/vol) SDS, .67% (vol/vol) TEMED, and .1% (wt/vol) APS. A 5% stacking gel prepared as previously
described herein was used over the 12% separating gel. The running buffer used in both the upper and lower buffer chambers of the SE 400 Sturdier Vertical Slab Gel Unit (gel dimensions = 180 mm high × 160 mm wide × 1.5 mm thick) (Hoefer Scientific Instruments, San Francisco, CA) consisted of 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 1% SDS. Eighty micrograms of protein was loaded per lane onto the slab gel. Gels were run at room temperature at a constant current setting of 20 mA. Gels were stained for 6 h in an excess of 0.1% (wt/vol) of Coomassie brilliant blue R-250, 50% (vol/vol) methanol, and 30% (vol/vol) glacial acetic acid. Gels were destained in an excess of the same solution excluding the Coomassie brilliant blue R-250. Titin and nebulin, purified from bovine longissimus muscle according to the method of Wang (1982), were used for identification of proteins in the 5% gels. Sigma High Molecular Weight Standards were used to aid in identification of protein bands in the 12% gels.

**Results**

All of the 120 samples (30 animals with four postmortem aging periods each) used in this study were characterized for tenderness by Warner-Bratzler (WB) shear force measurements and sensory panel evaluations and for postmortem degradation of myofibrillar proteins by SDS-PAGE. The overall tenderness results were included with other sensory measurements in a recent report by Huff and Parrish (1993). From the 30 animals, we selected samples representative of the tenderness differences that were observed.

The samples analyzed and shown in Figures 1 and 2 were selected as representative of two animals (steers) from the study (Huff and Parrish, 1993) that differed considerably from each other in both their WB shear force values and sensory panel scores for ease of fiber fragmentation at 3 d postmortem. The "tough" (higher shear force) sample was representative of 2 steers with similar tenderness scores out of the 10 steers. The "tender" (lower shear force) sample was representative of 3 steers with similar tenderness scores out of the 10 steers in the study. The other 5 of the 10 steers had tenderness scores that were intermediate. Analysis of the myofibrils from longissimus samples from these two animals on 12% gels (Figure 1), which were used to examine myofibrillar proteins with subunit molecular weights ranging from myosin heavy chains and smaller, verified that no detectable degradation occurred in the major myofibrillar proteins myosin and actin in muscle stored at 2 to 4°C, in agreement with other studies (e.g., Bechtel and Parrish, 1983; Koohmaraie et al., 1986; Troy et al., 1986). Upon examination of samples from all 30 animals, the only consistent differences identified by SDS-PAGE with 12% gels were the well-known postmortem 1) loss of bands in the troponin-T region migrating between actin and tropomyosin and 2) coincident increase in polypeptide(s) migrating at approximately 30 kDa (MacBride and Parrish, 1977; Olson et al., 1977; Wheeler and Koohmaraie, 1994). Olson et al. (1977) originally reported that a 30-kDa component in bovine muscle was a degradation product of troponin-T. Also, by using antibodies to troponin-T and Western blots, Ho et al. (1994) recently have shown that a 30-kDa polypeptide in postmortem beef is derived from troponin-T. Overall, we observed that a 30-kDa component intensified with increasing time postmortem in all samples from all animals, regardless of the animal's sex or age. However, the rate of the increase in intensity of this 30-kDa band differed among animals; the more tender samples (low WB shear force values and high sensory scores for fiber fragmentation) exhibited a more rapid postmortem appearance than in less tender samples, in agreement with earlier studies (Olson and Parrish, 1977).

Examination of the identical samples shown in Figure 1, but on a 5% polyacrylamide separating gel (Figure 2), demonstrated the changes that occurred in titin and nebulin over the four postmortem storage periods. At 3 d postmortem, after limited degradation had occurred, titin migrated primarily as a doublet with the uppermost (slowest migrating) band commonly referred to as T1 (compare to intact titin standard prepared from at-death bovine skeletal muscle) and the lowermost (fastest migrating) band, referred to as T2, which is considered to be a degradation product of T1 (Wang et al., 1979; Boles et al., 1992). The samples examined in this study, using our SDS-PAGE conditions, revealed a third band that migrated between T1 and T2, which probably also is a degradation product of T1. A titin band migrating between T1 and T2 recently was observed and referred to as T1-2 (Fritz et al., 1993). This nomenclature has been adopted herein for describing the three-band titin pattern in this study. Although our T1-2 band and their T1-2 band (Fritz et al., 1993) may represent similar or identical titin polypeptides, this identification must be made with considerable caution because different samples and procedures were used in the two studies. A notable difference in the titin pattern between the two animals shown in Figure 2 was already evident in the 3-d samples; intact T1 composed the major band in the less-tender animal, and degraded T2 composed the major band in the more-tender animal (Figure 2). As postmortem aging time was increased further, the T1 band of titin decreased in intensity and was absent by 7 d in the tender animal, and was nearly absent by 14 d in the less-tender animal. The T1 band of titin was gone by 28 d postmortem in all 30 animals examined (results not shown). Coincident with the postmortem decrease in T1, a corresponding increase in T2 occurred (Figure 2), indicating that the T2 band arises from the
**Figure 1.** The SDS-PAGE analysis on a 12% polyacrylamide gel of purified myofibrils from two beef animals (steers) differing considerably in tenderness as evaluated by Warner-Bratzler shear and sensory panel evaluations. Samples were taken from longissimus steaks aged 3, 7, 14, and 28 d postmortem. Note that decreased Warner-Bratzler shear values (kg = kilograms/square centimeter), but increased sensory scores for fiber fragmentation, correspond to increased tenderness. The standard lane (STD) contains the following proteins with corresponding molecular weights (as seen by migration under SDS-PAGE conditions) in parentheses: myosin heavy chains (205,000), β-galactosidase (116,000), phosphorylase b (97,400), bovine albumin (66,000), egg albumin (45,000), and carbonic anhydrase (29,000).

Degradation of T1 into lower-molecular-weight (faster migrating) components. Additional faint bands are present in the region of the gel just below T2, and also immediately above the position in the gel where the intact nebulin band migrates (Figure 2, “tough” 7- to 28-d and “tender” 3- to 28-d samples). These bands recently have been shown to be titin degradation products by probing Western blots with monospecific titin antibodies (our unpublished observations, E. Lonergan, T. Mitsuhashi, R. M. Robson, and F. C. Parrish, Jr.). The same general trend in postmortem degradation of titin observed in Figure 2 was seen in all samples in our study, although the rate of the conversion of T1 to T2 differed among animals, as will be described subsequently.

Nebulin also was degraded postmortem, with a notable difference observed at 3 d postmortem. An example of this is illustrated by the samples from two animals differing significantly in tenderness shown in Figure 2. In the less-tender samples at 3 d postmortem, the intact nebulin band was still easily detectable, but the more-tender samples contained at most only a trace of intact nebulin (Figure 2). Intact nebulin was not present in either of the samples shown at 7 d postmortem. Intact nebulin was not apparent at or beyond 7 d postmortem in any of the 30 animals examined in this study (results not shown).

Taking both the titin and the nebulin results together (Figure 2), at 3 d postmortem the relatively more tender samples had less intense bands of both intact T1 and nebulin, whereas the relatively tougher samples had more prominent T1 and nebulin bands. This indicated that titin and nebulin generally were degraded faster in those samples that had been classified as more tender. However, detailed comparisons of tenderness values with the state of titin and nebulin revealed that the degree of their degradation did not always match precisely. For instance, the tenderness scores for the 3- and 7-d “tough” samples were nearly identical even though the 7-d sample exhibited more degraded T2 and no intact nebulin.

A comparison of the degree of degradation of titin and nebulin in 3-d postmortem samples from examples of animals differing in sex (steers vs bulls) and age (cows vs steers and bulls) are shown in Figure 3. Samples from three separate animals, which were selected as representative of differing overall tenderness scores (more tender, intermediate, less tender) within each class, are shown for each of the three classes of animals (Figure 3). First, it was noted within each class (e.g., steers, bulls) that degree of titin and nebulin degradation was related to the...
Figure 2. The SDS-PAGE analysis on a 5% polyacrylamide gel of purified myofibrils from two beef animals (steers) differing considerably in tenderness as evaluated by Warner-Bratzler shear and sensory panel evaluations. Samples were taken from longissimus steaks aged 3, 7, 14, and 28 d postmortem. The samples loaded on each lane are identical to the samples loaded on each corresponding lane in the gel in Figure 1. Note that decreased Warner-Bratzler shear values [kg = kilograms per square centimeter], but increased sensory scores for fiber fragmentation, correspond to increased tenderness. The standard (STD) lane contains the following proteins with corresponding estimated molecular weights in parentheses: purified bovine skeletal muscle titin (T1 is approximately 3,000,000) and purified bovine skeletal muscle nebulin (is approximately 800,000) (reprinted from Huff-Lonergan et al., 1994, p 106, by courtesy of Marcel Dekker, Inc.).

Tenderness scores. For example, the tougher steer sample shown in lane 3 exhibited a higher proportion of intact T1 and a more noticeable nebulin band in comparison to the more tender steer samples shown in lanes 2 and 1, which contain less T1, but more degraded T2, and consistently less nebulin. Likewise, the tougher bull sample shown in lane 5 contained a larger proportion of intact T1 and a very evident nebulin band in comparison to these corresponding bands in the progressively more tender bull samples shown in lanes 6 and 4. This overall trend also was evident, but less consistently so, within the cow samples. The tougher cow sample shown in lane 9 had more prominent T1 and nebulin bands than in those more tender samples shown in lanes 7 and 8. However, as an example of inconsistency observed in the cow samples, the sample shown in lane 7 had more intact T1 than did the sample shown in lane 8, but the sample in lane 7 was slightly more tender. When comparisons were made between animals differing in sex (steers vs bulls), some notable differences were found at 3 d postmortem (Figure 3). In general, more intact T1 and at least some intact nebulin was evident in the samples from the bulls, but little intact T1 and a virtual absence of intact nebulin was more commonly found in the samples from steers. As described above, these differences were related to the overall tenderness scores of the respective samples. When comparisons were made between the older animals (cows) and one of the two younger classes of animals (steers), some general differences were noted (examples are shown in Figure 3). At least some intact nebulin was consistently present in 3-d postmortem samples from older animals (cows, lanes 7 to 9), but this band was not always present at 3 d postmortem in samples from the younger animals (steers, lanes 1 to 3; for instance, the nebulin band is essentially absent in the sample shown in lane 1). At least some
of the T₁ band of titin was present in all samples at 3 d postmortem (Figure 3), but again it tended to be less prominent in the steer samples than in the cow samples, and it was more intense in those samples (steers, lane 3; cows, lane 9) that also showed a more significant nebulin band. The differences observed in animal age when comparisons were made between the older cows and younger steers were not evident when comparisons were made between the older cows and younger bulls (i.e., the sex status of the bulls negated differences due to age between these two latter classes of animals). For instance, in comparing the samples from bulls (lanes 4 to 6) and cows (lanes 7 to 9), at least some nebulin was seen in all of the 3-d postmortem samples, and its intensity was greatest in those samples (bulls, lane 5; cows, lane 9) that represented the upper extremes for toughness in this study as measured by both shear force values and sensory scores. Likewise, an easily detectable intact T₁ band of titin was consistently present in samples from both bulls and cows at three days.

Examination of samples from the identical animals shown in Figure 3, but at 14 d postmortem, are shown in Figure 4. Nebulin already was absent from all samples examined at 7 d postmortem in our study, so differences observed are limited to titin. For all samples, the major band evident in the titin region of the gel (Figure 4) is the degraded product of T₁, T₂. Comparison of animals differing in sex (steers vs bulls) indicated that T₁ was absent in all three of the steer samples at 14 d postmortem, but that at least a trace of T₁ remained in all three bull samples. This was most noticeable in the bull sample shown in lane 5, which had been the least tender sample at 3 d postmortem (see Figure 3). By 14 d postmortem, however, there was little difference among the three bull samples (lanes 4 to 6) in tenderness scores. Comparison of animals differing in age (older cows vs younger steers) indicated that although T₁ was absent in the steer samples, at least trace amounts of T₁ remained in the cow samples. Few, if any, differences, however, were observed when comparisons were made between samples from cows vs samples from bulls. The two samples exhibiting the least tenderness, as measured by sensory fiber fragmentation, also were the two samples exhibiting the most prominent T₁ band (bulls, lane 5; cows, lane 9) remaining at 14 d postmortem. As was evident in results shown in

Figure 3. The SDS-PAGE analysis on a 5% polyacrylamide gel of purified bovine myofibrils prepared from 3-d postmortem longissimus steaks. Samples (three examples are shown for each class) represent animals differing in sex [steers vs bulls] and age [older cows vs younger bulls and steers]. Note that decreased Warner-Bratzler shear values (kg = kilograms per square centimeter), but increased sensory scores for fiber fragmentation, correspond to increased tenderness [reprinted from Huff-Lonergan et al., 1994, p 107, by courtesy of Marcel Dekker, Inc.].
Figure 4. The SDS-PAGE analysis on a 5% polyacrylamide gel of purified bovine myofibrils prepared from 14-d postmortem longissimus steaks. Samples [three examples are shown for each class] represent animals differing in sex (steers vs bulls) and age (older cows vs younger bulls and steers). The 14-d samples loaded on each lane are from the identical animals used for the 3-d samples loaded on each corresponding lane in Figure 3. Note that decreased Warner-Bratzler shear values (kg = kilograms per square centimeter), but increased sensory scores for fiber fragmentation, correspond to increased tenderness [reprinted from Huff-Lonergan et al., 1994, p 108, by courtesy of Marcel Dekker, Inc.].

Figure 2, additional bands migrating near, but below T2, and also to a position near, but above, the position where intact nebulin migrates in our gel system (see Figure 2) are present to varying degrees in all of the 14-d samples shown in Figure 4. These bands are labeled in Western blots with monospecific antibodies to titin, indicating that they originate from titin degradation (our unpublished observations).

Discussion

Beef tenderization is a complex phenomenon, but it is well known that postmortem storage of beef at refrigerated temperatures (0 to 4°C) results in an increase in tenderness. Although all the factors responsible for the postmortem increase in tenderness have not been identified, proteolytic changes in the key proteins responsible for the integrity of the overall muscle cell cytoskeleton are likely involved (Robson et al., 1991; Koohmaraie, 1992; Uytterhaegen et al., 1994; Robson, 1995). These changes include degradation of proteins 1) within the myofibril, such as the large structural proteins titin and nebulin (Lusby et al., 1983; Fritz and Greaser, 1991) and the thin filament regulatory protein troponin-T (Olson and Parrish, 1977; Ho et al., 1994; Uytterhaegen et al., 1994), 2) in the desmin/synemin intermediate filaments that connect adjacent myofibrils at their Z-line levels and the Z-lines of the peripheral layer of myofibrils to the cell membrane skeleton (Robson et al., 1984, 1991; Koohmaraie et al., 1991), and 3) in at least some of the proteins composing the cell membrane skeleton such as the costameric protein vinculin (Taylor et al., 1995a).

This study was focused on the postmortem changes of titin and nebulin in beef samples of known tenderness from different sex and age classifications and upon the relationship of the degradation of these large proteins to the tenderness of the samples. With these objectives, it was important that the gel system used give good separation and resolution of major titin.
polypeptides and considerably more separation of those bands from the intact nebulin band than has sometimes been observed (c.f. results herein with those in Lusby et al., 1983; Zeece et al., 1986; Fritz and Greaser, 1991; Fritz et al., 1993). This was necessary to help reduce the confusion that can occur when inadequate separation causes high-molecular-weight titin degradation products (e.g., the 1,200 kDa band of Matsuura et al., 1991) to migrate very closely when inadequate separation causes high-molecular-weight titin degradation products (e.g., the 1,200 kDa band of Matsuura et al., 1991) to migrate very closely to T2 or nebulin. The system used in our study (5% separating gel, acrylamide:N,N'-bis-methylene acrylamide = 100:1) also permitted easier identification of the T1 and T2 bands due to their greater separation (Huff-Lonergan et al., 1994). Although Fritz and Greaser (1991) suggested that somewhat more degradation of titin and nebulin occurred in myofibrils than in whole muscle samples, we found that the myofibril preparation technique we used did not cause any detectable further degradation of the titin and nebulin bands in comparison to procedures using whole muscle preparations from samples taken at the same time postmortem, nor were any nondegraded or degraded products of titin or nebulin noticeably lost during myofibril preparation (our unpublished results, E. Lonergan, T. Mitsuhashi, R. M. Robson, and F. C. Parrish, Jr.). The rate of titin and nebulin degradation that we have described with myofibrils is also similar to the rate observed by Taylor et al. (1995a), who used whole muscle samples.

Granzier and Wang (1993) have recently compared their gel system for separation of large proteins, which we in part have employed herein, with that of others such as that described by Fritz et al. (1989) and used in Fritz et al. (1993). For instance, the different gel sample buffer and lower temperature (50°C vs the more commonly used 100°C) used in our study for solubilizing the myofibrils before loading onto the gels have a less detrimental effect on the extremely heat labile protein titin (Granzier and Wang, 1993 and our unpublished results). Granzier and Wang (1993) demonstrated that 50% or more of the intact form of titin (T1) can be degraded by using a procedure such as that used by Fritz et al. (1993). Granzier and Wang (1993) also showed little degradation of titin and nebulin using procedures similar to those utilized herein. Fritz et al. (1993) reported slower degradation of T1 to T2 and little, if any, correlation between titin degradation and beef tenderness, in contrast to the results reported herein. It is possible that the sample buffer (8 M urea, 2 M thiourea, 3% [wt/vol] SDS, 75 mM dithiothreitol, 25 mM Tris-HCl, pH 6.8) and heating conditions (100°C for 3 min) used by Fritz et al. (1993) could cause premature conversion of T1 to T2. As also suggested by Taylor et al. (1995a), T2 could have been the primary band examined and identified as T1 by Fritz et al. (1993). This is difficult to ascertain, however, because an intact titin standard and a zero-time myofibril sample were not in in Fritz et al. (1993) to clearly locate where T1 migrated in their gels. These factors (sample buffer composition, heating conditions, and the use of controls), combined with much less separation of protein bands seen in the 8% separating gels (acrylamide:N,N'-bis-methylene acrylamide = 200:1) of Fritz et al. (1993), make it difficult to make more detailed comparisons between the two studies.

We have found that both titin and nebulin are progressively degraded with increased time postmortem. For all samples, the majority of the intact form of titin, T1, was degraded before or by 14 d postmortem, whereas all of the detectable intact nebulin was degraded by 7 d. Examination of the more tender samples in the study showed that the majority of the T1 was degraded by 7 d, whereas nebulin was degraded by 3 d. In all cases, nebulin was degraded more rapidly than T1. A similar overall rate of titin and nebulin degradation has been found in a study by Taylor et al. (1995a) in which whole muscle samples were used for analysis. Comparisons between the sex classifications revealed that samples from bulls generally exhibited slower postmortem degradation of both titin and nebulin than those from steer samples. Samples from older animals (cows) also showed a slower rate of postmortem degradation than seen in the younger steer samples. However, the postmortem degradation patterns of the older animals were similar to those obtained in the younger bull samples. We observed an increase in variability in tenderness scores, due to inherent background toughness in the cows (Huff and Parrish, 1993), which made direct comparisons between the degradation patterns and the given tenderness scores in the cow samples less definitive. It is likely that background toughness, due to increased cross-linking of connective tissue in older animals, may play a proportionately larger role in influencing tenderness of steaks from older animals than it does in younger animals.

Fragmentation or breakage of the myofibril is believed to play a significant role in determining the tenderness of beef (Olson and Parrish, 1977). Although some of the degradation occurs within the Z-line region (for review see Koohmaraie, 1992), recent electron microscopy studies of postmortem muscle samples have shown that considerable breakage of the myofibril occurs within the nearby I-band regions (Taylor et al., 1995a,b). Each titin molecule spans the distance between the Z-line and M-line (Furst et al., 1988). It is now likely that nebulin composes part of the skeletal muscle thin filaments (the most prominent longitudinal structure in the I-band), which were heretofore considered only to be made up of actin, tropomyosin, and troponin (Wright et al., 1993). It is well known that troponin-T, which is positioned periodically along the thin filaments, is degraded in postmortem beef (e.g. Olson et al., 1977). Degradation of troponin-T is often considered an indicator of tenderness rather than a causative factor; however, this must be reevaluated considering that its degrada-
tion could contribute to loss of overall thin filament integrity and of thin filament/Z-line attachment (Ho et al., 1994; Uytterhaegen et al., 1994). Thus, all three of these proteins, titin, nebulin, and troponin-T, are present within the I-band regions of the intact myofibril. Because all three of the proteins are degraded postmortem, their combined disruption may contribute significantly to myofibril fragmentation. This increased fragmentation would lead to improved tenderness, described as “myofibril fragmentation tenderness” (MacBride and Parrish, 1977).

The evidence on degradation of the giant sarcomeric proteins presented herein suggests that decreased activity of a protease such as calpain, or possibly increased activity of its inhibitor, calpastatin, may be responsible for the slower postmortem proteolysis and decreased tenderness seen in some animals (e.g., bulls and cows). The calpains, endogenous muscle cell proteinases, catalyze the degradation of titin (Zeece et al., 1986; Kimura et al., 1992), nebulin (Thompson et al., 1993), desmin (O'Shea et al., 1979), synemin (our unpublished observations, S. Sernett, E. Lonergan, and R. Robson), troponin-T (Olson et al., 1977), and the costameric protein vinculin in vitro (Evans et al., 1984). The calpain proteolytic system is generally regarded as playing the major role in the proteolysis that occurs during postmortem storage at 0 to 4°C (Goll, 1981; Koohmaraie, 1992; Dransfield, 1993; Uytterhaegen et al., 1994). Any conditions or parameters that enhance postmortem activity of this proteolytic system would be expected to result in more degradation of the key cytoskeletal proteins. Morgan et al. (1993) recently have found that calpastatin activity is higher in longissimus samples from young bulls than in those from young steers, which may explain at least in part the differences in degradation of titin and nebulin observed in our study between castrated and intact males. A similar situation with the calpain system may exist in samples from older animals and would explain the slower rate of degradation of titin and nebulin observed in samples from these animals, but this remains to be shown.

Overall, by combining sensory and WB tenderness data with SDS-PAGE data, we have shown that progressive degradation of titin and nebulin follows the same trend in improvement of tenderness over postmortem time (i.e., both titin and nebulin were degraded faster in those samples within a class that were more tender); however, the precise relationship between the degree of degradation of titin and nebulin in a particular sample and its tenderness value at a precise point in time was not always exact.

Tenderization of beef is a very complex process involving many factors, such as protein degradation, increased ionic strength, and pH changes. Given the complexity of the muscle/meat system, it is not surprising that no single property or event by itself has yet been shown to be responsible for, or a perfect predictor of, meat tenderness at a specific time point. Rather, it is more likely the summation of several properties and events that must be considered in toto before meat tenderness can be understood and predicted accurately at a given point in time.

**Implications**

Two key structural myofibrillar proteins, titin and nebulin, were shown to be degraded faster in more tender beef than in less tender beef within each classification studied (young steers, young bulls, old cows). In addition, titin and nebulin were degraded at different rates postmortem in beef animals from different classifications (young steers vs young bulls; young steers vs old cows). This study supports the concept that degradation of key structural myofibrillar proteins is related to postmortem tenderization of beef.

**Literature Cited**


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