Meat Toughening Does Not Occur When Rigor Shortening Is Prevented[1]

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ABSTRACT: The objective of this experiment was to test the hypothesis that meat toughening during the first 24 h postmortem results from sarcomere shortening during rigor mortis development. Eleven market-weight lambs were used to measure changes in shear force of clamped longissimus during rigor development. Within 15 min of exsanguination, while attached at both ends, each longissimus was separated from the vertebrae body and clamped between three sets of metal plates to prevent muscle shortening (six clamped sections per lamb). Five of the clamped sections were placed at –1.1°C for 0, 3, 6, 12, or 24 h. After storage at their respective times at –1.1°C, the samples were placed at –30°C for 90 min and then at –5°C for 8 d. The sixth section (168-h section) was stored at –1.1°C for the first 24 h, at 4°C for 144 h, and then treated the same as other sampling times. Sections were sampled for pH, sarcomere length, shear force, and Western blot analyses before and after storage at –5°C. Shear force values were the same ($P > .05$) from 0 to 24 h (4.5 kg at 0 h to 4.9 kg at 24 h) then declined ($P < .05$) to 3.3 kg at 168 h postmortem. As evident by lack of statistical difference in the sarcomere lengths, we were successful in holding the muscle length constant. Western blot analyses of nebulin, vinculin, and troponin-T indicated that minimum degradation occurred through 12 h, was slightly increased by 24 h, and was relatively extensive by 168 h postmortem. Although limited proteolysis occurred during storage at –5°C for 8 d, this by itself had no effect on shear force. Results indicate that shear force values do not increase during rigor development when muscle is prevented from shortening; thus, the toughening that occurs during the first 24 h of slaughter is most likely due to sarcomere shortening.

Key Words: Tenderness, Sarcomeres, Shortening, Rigor, Proteolysis

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Introduction

In an experiment designed to determine the inherent level of meat tenderness at the time of slaughter, we demonstrated that lamb longissimus has intermediate shear force value immediately after slaughter (5.07 kg), toughens during the first 24 h (maximum toughness was achieved at 9 to 24 h; 8.66 kg), and then becomes tender during postmortem storage at 4°C (3.10 kg). These data were interpreted to mean that rigor shortening was the cause of toughening and postmortem proteolysis was the reason for tenderization (Wheeler and Koohmaraie, 1994; Figure 1). There is considerable evidence to support the idea that proteolysis of key myofibrillar proteins is responsible for the decline in shear force during postmortem storage (for review see Goll et al., 1983, 1995; Koohmaraie 1992a,b, 1994, 1995). Numerous studies have demonstrated that muscles that shorten less during rigor or are stretched (i.e., long sarcomeres) are more tender (for review see Locker, 1985; Marsh, 1985). However, no direct evidence exists to support the hypothesis that sarcomere shortening during rigor development is the cause of meat toughening from time of slaughter to 24 h postmortem. Previously, we demonstrated that shear force increased as sarcomere lengths decreased from at-death lengths (2.24 mm) to 24-h lengths (1.69 mm; Wheeler and Koohmaraie, 1994). Thus, the objective of this study was to determine the consequence of preventing sarcomere shortening on shear force of lamb longissimus during rigor development and extended postmortem storage at 4°C.

[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of other products that may also be suitable. The authors greatly acknowledge the technical assistance of P. Ekeren, S. Hauver, K. Mihm, P. Tammen, and T. Wycoff for execution of these experiments; the secretarial assistance of M. Bierman; and the photographic assistance of P. Bures. We are also grateful to Darrel Goll, Marion Greaser, Robert Merkel, and Richard Taylor for their comments on the draft of the manuscript.

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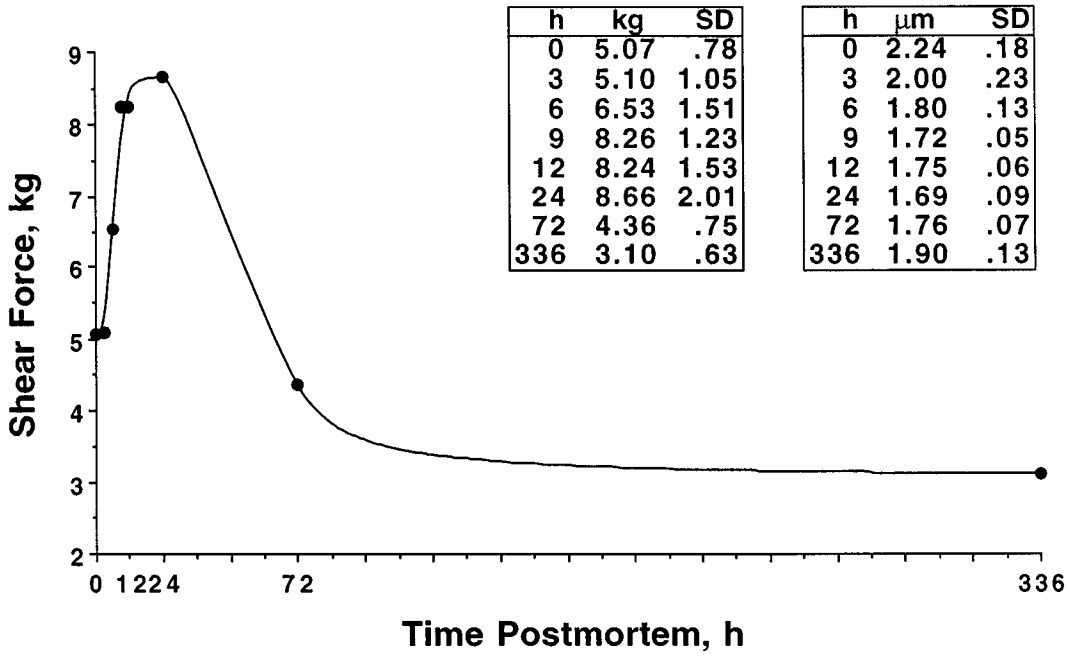

h	kg	SD
0	5.07	.78
3	5.10	1.05
6	6.53	1.51
9	8.26	1.23
12	8.24	1.53
24	8.66	2.01
72	4.36	.75
336	3.10	.63

h	μm	SD
0	2.24	.18
3	2.00	.23
6	1.80	.13
9	1.72	.05
12	1.75	.06
24	1.69	.09
72	1.76	.07
336	1.90	.13

Figure 1. Changes in Warner-Bratzler shear force and sarcomere length of lamb longissimus during postmortem storage (adapted from Wheeler and Koohmaraie, 1994).

Materials and Methods

First Experiment

Animals. The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Eleven market weight wether lambs were used (eight Romanov and three Dorset × Romanov). Samples were prepared as described by Wheeler and Koohmaraie (1994). Briefly, within 15 min of exsanguination, while attached at both ends, each longissimus was separated from the vertebrae body and placed between three pairs of metal plates. The plates were clamped with Vise Grip locking welding clamps (model 9R, Petersen Manufacturing, DeWitt, NE) with enough pressure to prevent muscle shortening. The sections were cut at each end of the plates to give three 15-cm-long samples per longissimus. To remove location effects, sections were labeled as 1 through 6 (Figure 2) and sample time assignment was rotated from animal to animal so that all sample times were represented in all locations. The clamped sections were placed at –1.1°C for 0, 3, 6, 12, or 24 h. The 168-h section was stored at –1.1°C for the first 24 h, at 4°C for 144 h, and then treated the same as other sampling times. At each of these times, a section was removed, sampled for temperature, pH and Western blot analyses, placed at –30°C with rapid air circulation for 90 min, unclamped, wrapped in aluminum foil, and stored at –5°C. At this temperature, glycolysis proceeds and ATP is depleted (Moran, 1930; Smith, 1930; Marsh and Thompson, 1958); thus shortening does not occur during thawing. After 8 d at –5°C, sections were removed and sampled for pH, sarcomere length, shear force, and Western blot analyses.

Second Experiment

A second experiment was conducted to determine the effect of limited proteolysis, which occurs during storage at –5°C, on shear force. Two market-weight lambs were slaughtered as described above and carcasses were stored at –1.1°C for 18 h. At 18 h postmortem, longissimus from both sides were removed and each was divided into three sections. All sections were frozen at –30°C for 90 min as described above. Treatment (storage for 8 d at –5°C, or storage at –30°C for 7 d then at –5°C for 1 d) assignments were alternated among sides. To prevent the effect of meat temperature variation at the initiation of cooking on shear force (Wheeler et. al., 1996), samples from –30°C treatment were equilibrated at –5°C for 1 d before cooking. Proteolysis, as measured, is virtually undetectable during this equilibration period. Samples were analyzed for pH, sarcomere length, shear force, and Western blot.

Longissimus pH and Temperature. pH was determined by homogenizing longissimus in 10 volumes of neutralized 5 m*M* sodium iodoacetate containing 150 m*M* potassium chloride (Bendall, 1973). Temperatures were determined with a hand-held thermocouple thermometer (model 85280-30, Cole-Palmer, Chicago, IL).

Sarcomere Length. Sarcomere length was determined by neon laser diffraction according to Cross et

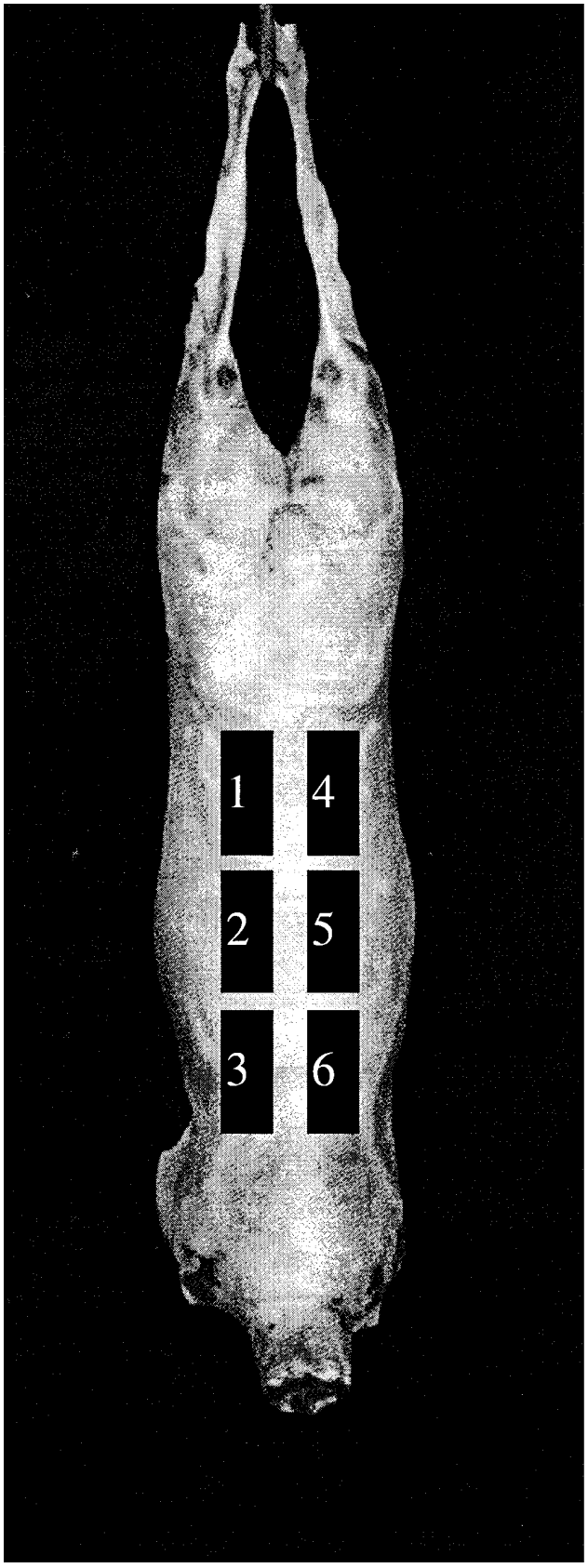

Figure 2. A lamb carcass depicting the six sample locations. In the first experiment, sample locations were rotated between animals and sampling times. In the second experiment, sides (locations 1, 2, and 3 or 4, 5, and 6) were rotated between animals and treatments.

al. (1980). Samples from lateral, central, and medial locations within transverse sections of the longissimus were measured and averaged.

Immunoblotting. One gram of muscle was homogenized in 10 mL of 50 m*M* Tris, 10 m*M* EDTA, pH 8.3 for 20 s using a polytron on setting 4. The muscle homogenate (.5 mL) was diluted 1:1 with 2× protein denaturing buffer (**PDB**) excluding mercaptoethanol and bromophenol blue (1× PDB consists of 2% SDS, 10% glycerol, 62.5 m*M* Tris, pH 6.8). Samples were heated at 50°C for 20 min and protein concentrations were determined using the micro-BCA assay (Pierce, Rockford, IL). Samples were diluted to contain 3 mg/mL protein in PDB containing 10% mercaptoethanol and .008% bromophenol blue. For electrophoresis, 30 mg of protein/lane was loaded, electrophoresed, transferred to Immobilon-P, and immunoblotted as described by Koohmaraie et al. (1995). Vinculin was separated on 10% gels (37.5:1), and troponin-T was separated on 12.5% gels (37.5:1) with 4% (37.5:1) stacking gels. Discontinuous gels were run at 200 V for approximately 45 min. Gels (10 and 12.5%) were transferred to Immobilon-P (Millipore, Bedford, MA) membranes for 2 or 1 h, respectively, at 4°C and 200 mA in buffer containing 25 m*M* Tris, 193 m*M* glycine, and 15% methanol. Nebulin was separated on continuous 5% gels (100:1) and transferred overnight at 170 mA in Tris-glycine buffer containing .005% SDS and no methanol. Lanes containing molecular weight markers were stained with amido black. To prevent nonspecific antibody binding, membranes were blocked with 2.5% sheep serum (Sigma Chemical, St. Louis, MO) in Tris-buffered saline, pH 7.4, containing .05% Tween-20 (**TTBS**) for 90 min. Antibody incubations were performed in blocking buffer at room temperature and membranes were washed three times with blocking buffer after each incubation. Membranes were incubated for 90 min with primary antibody as follows: anti-nebulin 1:1,000 (clone NB2; Sigma Chemical), anti-vinculin 1:500 (clone V284; Boehringer Mannheim, Indianapolis, IN), and anti-troponin-T 1:20,000 (clone JLT-12, Sigma Chemical). Secondary antibodies were alkaline phosphatase conjugates of anti-mouse IgG 1:1,000 (A-5153, Sigma Chemical). Antibody binding was visualized by exposure to BCIP/NBT (Bio-Rad, Richmond, CA).

The Western blot analysis was conducted for all animals. The blot using sample from the animal whose shear force values were nearest to the mean was photographed and reported.

Warner-Bratzler Shear Force. Chops (three 2.54-cm-thick chops per sample) were broiled from the frozen (−5°C) state on Farberware Open Hearth electrical broilers (Farberware, Bronx, NY) to an internal temperature of 40°C, turned, and broiled to an internal temperature of 75°C. Chops were cooled (4°C) for 24 h before removal of six (two per chop)

Table 1. Longissimus temperature and pH before and after 8 days at –5°C

Trait (n = 11)	pH, Before –5°C	pH, After –5°C	Temperature, °C
0 h postmortem	6.6[a]	5.4	34.2[a]
3 h postmortem	6.3[b]	5.5	3.2[b]
6 h postmortem	6.2[b]	5.5	1.8[bc]
12 h postmortem	6.0[c]	5.6	2.0[bc]
24 h postmortem	5.8[d]	5.6	1.2[c]
168 h postmortem	5.7[d]	5.7	2.2[bc]

[a,b,c,d]Means in a column without a common superscript differ ($P < .05$). SEM were .06, .13, and .67 for pH before, pH after, and temperature, respectively.

cores (1.27 cm diameter) parallel to the longitudinal orientation of the muscle fibers. Each core was sheared once with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine (Instron, Canton, MA) with a 50-kg load cell and crosshead speed of 20 cm/min.

Statistical Analysis. The data were analyzed by analysis of variance using the GLM procedure of SAS (SAS, 1988) for a repeated measures design. Mean separation for significant ($P < .05$) treatment effects was accomplished with PDIFF option (pair-wise *t*-test) of the least squares procedures (SAS, 1988).

Results and Discussion

During –5°C storage glycolysis continues, albeit at a slow rate, and results in a slow decline in muscle pH. However, due to the frozen state of muscle, shortening does not occur. To confirm that pH declined at –5°C, we measured pH before and after storage at –5°C (Table 1). After storage at –5°C for 8 d, the pH of all samples was similar (Table 1), indicating that glycolysis had reached its final stages after this storage and thus should not shorten during thawing. During storage at –1.1°C, temperature of muscle sections decreased much faster and pH declined much slower than longissimus in intact carcasses (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1995). The reasons for rapid decline in temperature, which most likely are the reasons for the slow pH decline, are the size of these sections and the fact that these muscle sections were clamped between metal plates allowing more efficient heat transfer than muscle attached to the carcass.

Results shown in Table 2 indicate that the protocol employed (clamping, freezing, unclamping, and storage at –5°C before shear force measurement) did not allow sarcomere shortening to occur. However, sarcomere length was shortened during cooking by about .4 mm at all postmortem times (Table 2). We routinely observe a .4-mm reduction in sarcomere length during cooking as described in the Materials and Methods. Similar results were obtained by Lewis et al. (1977) and Bouton et al. (1973).

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Table 2. Longissimus shear force and sarcomere length (SL; before and after cooking)

Trait (n = 11)	Shear force, kg	SL before, mm	SL after, mm
0 h postmortem	4.49[a]	2.36	1.89
3 h postmortem	4.23[a]	2.40	1.94
6 h postmortem	4.40[a]	2.34	1.96
12 h postmortem	4.89[a]	2.28	2.05
24 h postmortem	4.81[a]	2.42	1.94
168 h postmortem	3.34[b]	2.36	1.90

[a,b]Means in a column without a common superscript differ ($P < .05$). SEM were .21, .13, and .09 for shear force, SL before and after, respectively.

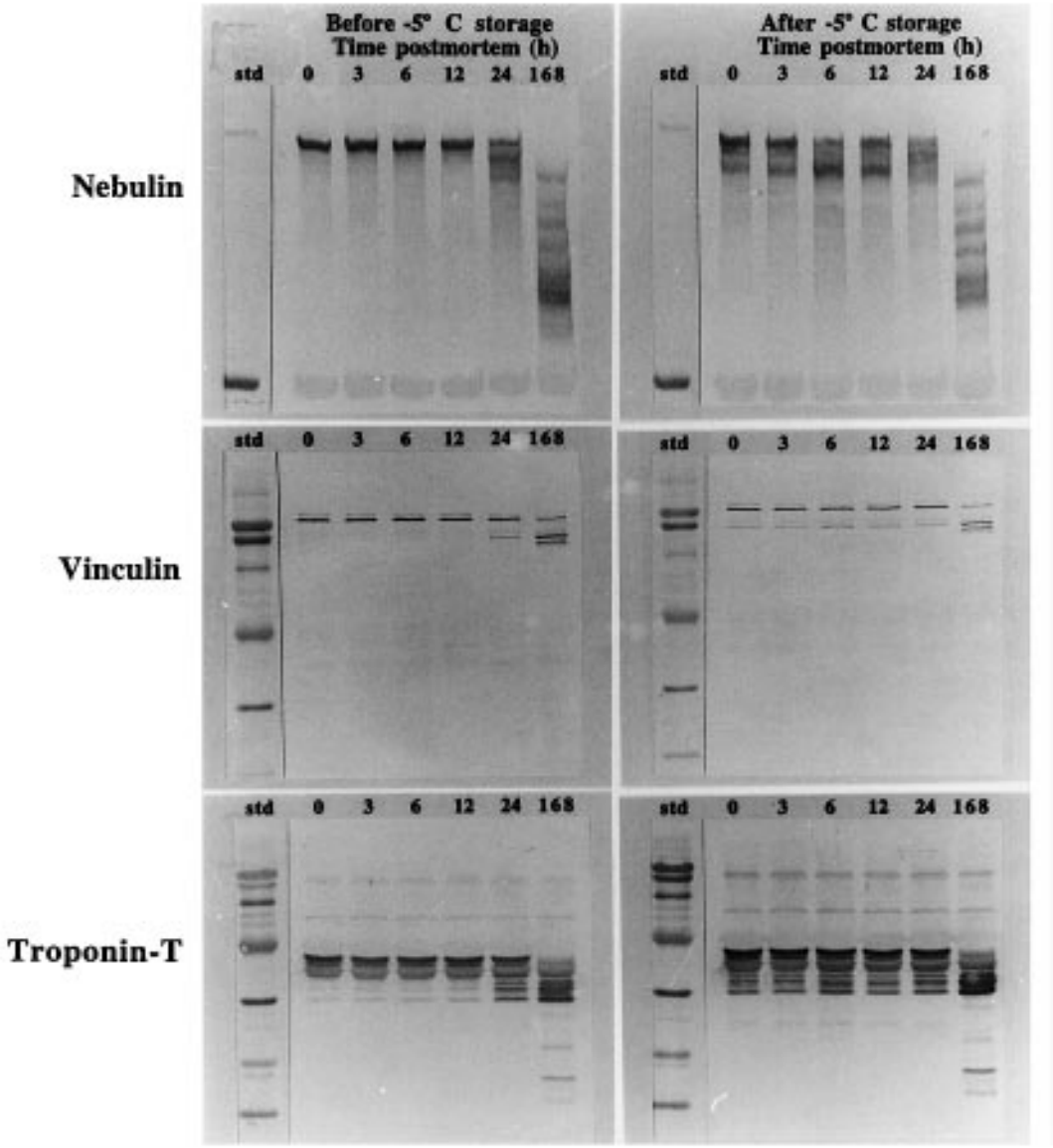

Figure 3. Western blot analysis of nebulin (top), vinculin (middle), and troponin-T (bottom) degradation at different postmortem times and storage at –5°C. Longissimus homogenates were prepared from samples taken at 0, 3, 6, 12, 24, and 168 h of postmortem storage at –1.1°C (the 168-h sections were transferred to 4°C at 24 h) or the same samples after 8 d at –5°C. Each lane was loaded with 30 mg of protein, electrophoresed, and blotted as described in Materials and Methods. Standard (std) lanes consist of molecular weight markers, including Myosin (200 kDa), b-galactosidase (116.25 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg white ovalbumin (45.0 kDa), bovine carbonic anhydrase (31 kDa), and soybean trypsin inhibitor (21.5 kDa). Only the 200-kDa marker is seen in the nebulin blots. The 200-kDa marker did not transfer efficiently and is therefore not clearly visible in the vinculin and troponin-T blots.

shear force value (i.e., tender meat), these and other key myofibrillar and associated proteins (e.g., titin and desmin) are gradually degraded during postmortem storage at refrigerated temperatures. As expected, before storage at –5°C, these proteins were degraded

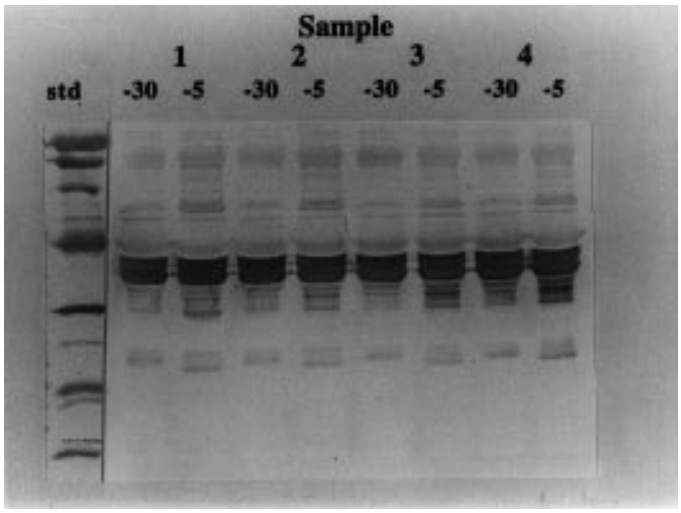

Figure 4. Western blot analysis of troponin-T from longissimus removed from the carcasses at 18 h postmortem, then stored for 8 d at –30°C or –5°C. Longissimus homogenates were prepared and each lane was loaded with 30 mg of protein, electrophoresed, and blotted as described in Materials and Methods. Standard (std) lanes are the same as in Figure 3. Mean shear force value did not differ between samples stored at –5°C or –30°C (8.29 kg vs 8.49 kg, respectively).

proteolysis, unpublished data). Therefore, it seemed unlikely that the limited proteolysis that was detected in samples stored at –5°C in the current experiment could affect shear force. Nonetheless, to determine the effect of this level of proteolysis on the observed shear force value, a second experiment was conducted in which longissimus were kept on the carcass for 18 h at –1.1°C and then removed and stored at –5°C or –30°C. In agreement with the results of the first experiment, the level of proteolysis was slightly higher in samples stored at –5°C than samples stored at –30°C (Figure 4). Mean shear force value for samples stored at –5°C was similar ($P > .05$) to those stored at –30°C (8.29 kg vs 8.49 kg, respectively, at 18 h postmortem in unclamped muscle sections). Therefore, it can be concluded that, although a limited amount of proteolysis occurs during 8 d of storage at –5°C, this level of proteolysis has little or no effect on shear force. The question is, why? The reason could be that 1) degradation of these proteins has no effect on meat tenderness or 2) more extensive degradation of these proteins is required before any change in tenderness is observed. It is highly unlikely that the first answer is correct. However, there is evidence to support the second scenario. Taylor et al. (1995) reported that during the first 24 h postmortem, a period when little or no tenderization occurred, about 25% of nebulin was degraded. They also reported that the ratio of degraded to undegraded vinculin was 4- to 50-fold higher in tender muscle than in tough muscle. Therefore, it seems that the relationship between protein degradation and improvement in meat tenderness is not linear and that some minimum threshold of degradation is necessary for proteolysis to have any measurable effect on meat tenderness.

General Discussion

Solving the problem of inconsistent meat tenderness has been identified as a top priority by the meat industry. Results of various studies conducted in this laboratory have indicated that differences in the rate and extent of postmortem tenderization are the principal sources of variation in meat tenderness and are probably the source of inconsistency in meat tenderness at the consumer level (for review see Koohmaraie, 1995). To solve the tenderness problem, even greater understanding of the mechanisms regulating meat tenderness and tenderization must be gained.

Clearly, changes that take place in longissimus during the first 24 h postmortem are very important in determining the ultimate meat tenderness (for review see Bendall, 1973; Locker, 1985; Marsh, 1985). It is well established that longissimus muscles from different carcasses tenderize differently during postmortem storage and this causes variation in longissimus tenderness (for review see Koohmaraie, 1992a). This assumes that postrigor longissimus is initially tough and that differences in the extent of postmortem tenderization are the sole source of variation in tenderness of longissimus after storage.

The principal source of error in attempting to measure tenderness in prerigor meat is excision- and heat-induced shortening. Locker (1985) stated that "tenderness relates to cooked meat, but cooking in a prerigor state involves such a dramatic modification of the myofibrils that the resultant material is sheer artifact." Thus, to prevent excision- and heat-induced shortening, we developed and used a novel approach to measure tenderness of meat having prerigor sarcomere lengths (Wheeler and Koohmaraie, 1994). The protocol involved clamping of muscle sections while on the carcass, excision, brief storage at –30°C, unclamping, and storing muscle sections at –5°C for 8 d. At this temperature, glycolysis will proceed and ATP will be depleted (Moran, 1930; Smith, 1930; Marsh and Thompson, 1958), thus shortening does not occur during thawing. At present, we do not fully understand the biochemistry of muscle stored at –5°C, but it seems to be different from storage at or below –10°C. Davey and Gilbert (1976) demonstrated that, during storage at –10°C, glycolysis does not occur, whereas the ATP concentration declines through enzymatic hydrolysis to levels insufficient to cause contraction during thawing. Smith (1930) detected accumulation of lactic acid in frog muscle at –5°C, but not at –10°C.

We previously demonstrated that lamb longissimus has intermediate shear force value immediately after

slaughter. If allowed to shorten in situ in the carcasses, lamb longissimus toughens during the first 24 h postmortem and then becomes increasingly more tender with increasing time of postmortem storage at 4°C (Wheeler and Koohmaraie, 1994). During the toughening phase, sarcomere length decreased and, thus, we concluded that sarcomere shortening is the cause of longissimus toughening from 0 to 24 h postmortem. Mean shear force value of at-death longissimus was 5.07 kg, and after 14 d of storage at 4°C shear force value was 3.10, which was 61.1% and 35.8% of the at-death and 24-h values, respectively. There is ample evidence indicating proteolysis of key myofibrillar and associated proteins whose function is to maintain the structural integrity of the myofibrils is the cause of tenderization that occurs during storage of meat at 4°C (e.g., Goll et al., 1983, 1995; Koohmaraie, 1992a,b, 1994, 1995; Koohmaraie et al., 1995; Taylor et al., 1995). However, there was no direct evidence to support the conclusion that sarcomere shortening was the cause of the increase in shear force between 0 and 24 h after slaughter. Thus, the present experiment was conducted to determine shear force value at various postmortem times in lamb longissimus that is not allowed to shorten. Our results indicate that in the absence of sarcomere shortening, shear force does not increase during the first 24 h and, therefore, provides support for the conclusion that the increase in shear force during the first 24 h postmortem was likely caused by the decrease in sarcomere length.

Goll et al. (1995) hypothesized that a change in the actin/myosin interaction from a weak binding state to a strong binding state may contribute to meat toughening during the first 24 h postmortem, and that this increase in toughness does not require, but may be accompanied or exacerbated by, sarcomere shortening. Although the nature of the actin/myosin interaction was not examined in the current study, our findings indicate that meat toughness, normally associated with rigor, does require sarcomere shortening. The contribution, if any, of actin/myosin binding state to tenderness of meat with short sarcomeres is unclear and may be technically difficult to address.

On the basis of the results of this study and those of Wheeler and Koohmaraie (1994), we conclude that very little variation in tenderness exists between longissimus of different animals at slaughter, then a toughening occurs due to sarcomere shortening during rigor development, and finally a variable tenderization process takes place during extended postmortem storage. It is this variability of the tenderization that results in variation in meat tenderness.

Implications

Results indicate that meat toughening that occurs in lamb longissimus during the first 24 h postmortem is caused by sarcomere shortening, because it did not occur when sarcomere shortening was prevented. This rigor-induced toughening occurs uniformly in all carcasses. The toughening phase coincides with, or is followed by, a tenderization process that involves postmortem proteolysis during storage at refrigerated temperatures. The tenderization process, however, is not uniform and results in carcasses having different levels of meat tenderness after storage and, thus, variation in meat tenderness.

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