Prerigor and Postrigor Changes in Tenderness of Ovine Longissimus Muscle[1,2]

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ABSTRACT: A novel approach was used to measure the tenderness of prerigor ovine longissimus thoracis et lumborum by avoiding the confounding effects of heat-induced shortening resulting from cooking prerigor meat. The objective was to determine the tenderness of the muscle at the time of slaughter and to monitor changes in tenderness during rigor development and postmortem aging. Nine Romanov and 12 Finnsheep rams were slaughtered at 49.3 kg live weight. Samples of longissimus thoracis et lumborum were removed at 0, 3, 6, 9, 12, 24, 72, or 336 h after exsanguination. Five of the eight sample times were represented in each carcass and all carcasses were sampled at 0, 12, and 24 h. Prerigor muscle samples (0, 3, 6, 9, and 12 h) were clamped between two metal plates before excision to prevent shortening.

The samples were frozen at −30°C then stored at −5°C for 10 d to allow glycolysis to proceed to completion, and thus ultimate pH and complete rigor mortis were attained. The longissimus thoracis et lumborum was then cut into chops and cooked and shear force was determined. Sarcomere length decreased through 24 h postmortem, then increased slightly through 336 h postmortem. Warner-Bratzler shear force values were 5.1 kg at 0 and 3 h, increased to 8.3 kg from 3 to 9 h, and then declined to 3.1 kg from 24 to 336 h postmortem. These data imply that longissimus thoracis et lumborum at slaughter is intermediate in tenderness, rigor shortening toughens the meat, and proteolysis tenderizes the meat, resulting in more tender meat after 14 d of aging than at slaughter.

Key Words: Muscles, Sheep, Postrigor, Prerigor, Tenderness

Introduction

Tenderness is an important palatability trait in meat. A large amount of variation in meat tenderness occurs under current production and postmortem handling systems (Morgan et al., 1991). A more complete understanding of factors that contribute to variation in meat tenderness is needed to better control meat tenderness. Little is known about the inherent level of meat tenderness at the time of slaughter or about changes that occur in tenderness during the first 24 h postmortem. Attempts have been made to measure tenderness of meat in the prerigor state, but the rate of heating prerigor meat determines whether it will be tough or tender (Paul et al., 1952; Weideman et al., 1967; Dransfield and Rhodes, 1975; Cia and Marsh, 1976). A few studies have attempted to avoid the confounding effect of muscle shortening during heat treatment of prerigor muscle by restraining the muscle at the ends (Weideman et al., 1967; Klose et al., 1970); however, it has been shown that shortening can still occur in the middle of the muscle (Marsh and Leet, 1966). To our knowledge, no measurement of prerigor tenderness has been reported that was not at least partially confounded by excision- or heat-induced shortening. Therefore, the objectives of this study were to determine 1) the tenderness of ovine longissimus thoracis et lumborum (**LTL**) at the time of slaughter and 2) changes in tenderness during rigor mortis development and postmortem aging.

Materials and Methods

Sampling Protocol

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved use of animals for this experiment. At 49.3 ± 2.1 kg live

weight, 9 Romanov and 12 Finnsheep rams were humanely slaughtered and dressed according to standard procedures. Carcass weight was 24.6 ± .8 kg. All sample times could not be represented in every carcass. Therefore, the sampling scheme shown in Figure 1 was followed to maximize the information obtained. In addition, sample time was confounded with sample location due to the sampling technique used. Jeremiah and Murray (1984) indicated there was no location effect within the LTL, so our sampling technique should not affect the results.

To avoid shortening of prerigor muscle after excision and during cooking, the LTL was clamped between two plates, excised, frozen, and then allowed to undergo rigor mortis before cooking. The details of the protocol used are described below. The 0-h LTL sample (15 min after exsanguination) was obtained from the left side between the first and last lumbar vertebrae (Figure 1). The s.c. fat was removed from this section of LTL. This section of LTL was separated from the vertebral body and the lateral spinous processes but was not cut at either end. Two metal plates (15 cm × 9 cm) 3 mm thick were used; one was placed between the muscle and the lateral spinous processes and the other on the outside of the muscle to sandwich the LTL (plates were ambient temperature, approximately 25°C). These two plates were then clamped on both ends with 225-mm Vise Grip locking welding clamps (model 9R, Petersen Manufacturing, DeWitt, NE). Enough pressure was applied with the clamps to prevent the LTL from shortening after the muscle was excised. Muscle thickness was reduced to 2.0 to 2.5 cm after clamping, depending on unclamped muscle thickness. Slightly more pressure was required on the 12-h sample than on earlier samples, although the exact clamping pressure was not measured. The clamped muscle section was removed by cutting transversely across the LTL at the end of the plates to obtain a section of muscle 15 cm long. The clamped section of LTL was immediately placed into a –30°C freezer with rapid air circulation on all sides for 90 min. After removal of the 0-h sample, the carcass was placed in a 1°C chill cooler for 24 h with air velocity of 1 m/s. At either 3, 6, or 9 h postmortem, the second LTL sample was clamped then cut from the left side between the 6th and 11th thoracic vertebrae, leaving approximately 5 cm between the first and second sample locations to avoid any shortening that may have occurred after the first section was removed (Figure 1). The skeletal and connective tissue restraints on the LTL for the second sample were maintained after removal of the first sample. In addition, the sarcomere length measurements of the second sample (at 3, 6, or 9 h) were appropriate, based on literature values, for their respective times postmortem. Thus, it was concluded that no shortening occurred in the second sample due to removal of the first sample. At 12 h postmortem, the third LTL sample was clamped then cut from the right side between the first and last lumbar vertebrae as described above (Figure 1). At 24 h postmortem, the
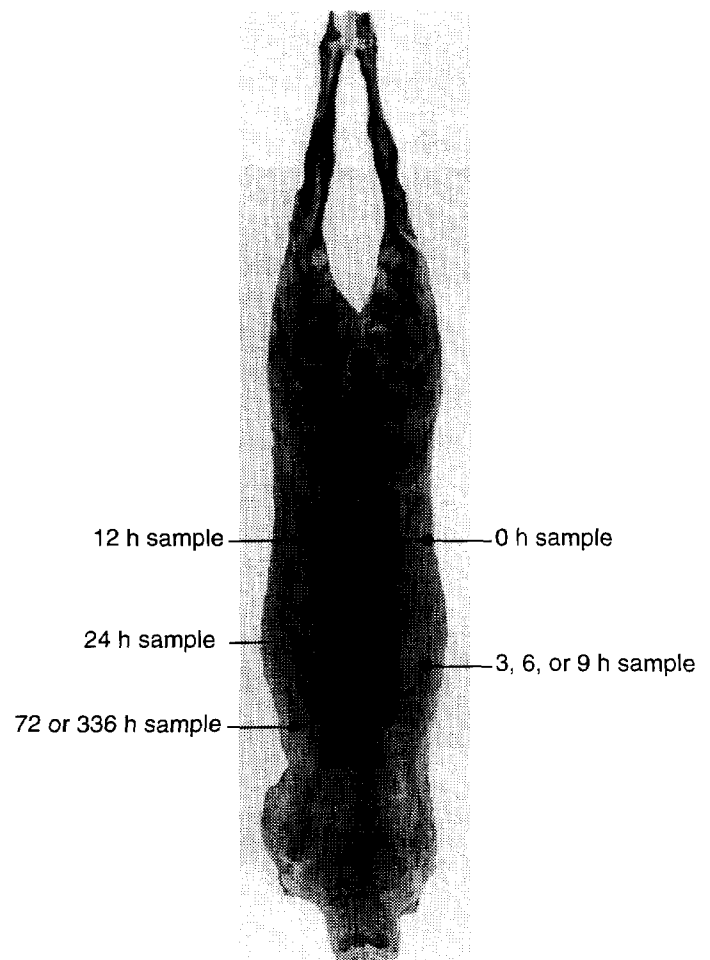

Figure 1. A lamb carcass depicting the five sample locations. All nine sample times could not be represented in every carcass. Therefore, each carcass was sampled at 0, 12, and 24 h; at 3, 6, or 9 h; and at either 72 or 336 h postmortem. Twenty-one lambs were used. The alternating sample times resulted in the following numbers of samples per sample time: 0 h, n = 21; 3 h, n = 7; 6 h, n = 7; 9 h, n = 7; 12 h, n = 21; 24 h, n = 21; 72 h, n = 10; and 336 h, n = 11.

remainder of the LTL from the right side was removed (without clamping, because rigor mortis was complete) and cut into 15-cm-long sections for 24 h and either 72- or 336-h sample times. The 24-h sample was frozen immediately at –30°C as described above. The 72- and 336-h samples were vacuum-packaged and stored at 2°C until the appropriate time postmortem then frozen at -30°C as described above.

Completion of Rigor Mortis

The LTL sections were removed from the –30°C freezer after 90 min. Fat and epimysium were removed from the exterior of the muscle. A 5-mm-thick slice was removed from the cranial end of the section and discarded, then another 5-mm-thick slice was removed for pH determination. The remainder of the section was wrapped in foil and stored

Table 1. Longissimus thoracis et lumborum pH decline
at −5°C frozen storage at various postmortem times

Postmortem sample time, h	Initial[a]		Time at −5°C			
			5 d		10 d	
	Mean	SD	Mean	SD	Mean	SD
0	6.66	.16	5.81[b]	.23	5.54	.10
3	6.31	.16	5.58[c]	.14	5.53	.06
6	6.15	.23	5.56[c]	.15	5.53	.10
9	6.01	.13	5.47[c]	.07	5.49	.04
12	5.91	.14	5.49[c]	.08	5.52	.08
24	5.81	.07	5.57[c]	.08	5.61	.07

[a]pH at the time sample removed from carcass and frozen.
[b,c]Means in a column without a common superscript differ ($P < .05$).

at −5°C for 10 d. At this temperature, glycolysis can proceed and rigor mortis will develop, but shortening cannot occur (Marsh and Thompson, 1958; Davey and Gilbert, 1976). At 5 and 10 d of storage at −5°C, another 5-mm-thick slice was removed from the cranial end for pH determination. At the end of the 10-d storage at −5°C, three 2.54-cm-thick chops were cut from the cranial end of the section for shear force measurement. Two additional 1-cm-thick transverse sections were removed, wrapped in foil, and stored at −20°C for sarcomere length and SDS-PAGE analysis.

Temperature and pH Determination

Temperature at the geometric center of the LTL was measured with a metal thermometer at each sample location immediately before sample removal. Muscle pH was measured after homogenizing 2.5 g of LTL in 25 mL of 5 mM iodoacetate and 150 mM KCl, pH 7.0 (Bendall, 1973). Three pH measurements were made: immediately after freezing at −30°C, after 5 d at −5°C, and after 10 d at −5°C.

Sarcomere Length Determination

Sarcomere length was measured by neon laser diffraction as described by Cross et al. (1981). Samples from lateral, central, and medial locations within a transverse section of the LTL were measured and averaged.

Warner-Bratzler Shear Force Determination

Chops were cooked from the frozen state (−5°C) on Farberware Open Hearth (Farberware, Bronx, NY) electric broilers to a 75°C final internal temperature. Chops were turned after reaching 40°C. Three chops were cooked for each sample time within each ram. Chops were cooled 24 h at 3°C and two cores 1.27 cm in diameter, parallel to longitudinal muscle fiber orientation, were obtained from each chop. The six cores were sheared once each with a Warner-Bratzler shear attachment for an Instron Universal Testing Machine (Instron, Canton, MA). A 50-kg load cell

with crosshead and chart paper speed of 5 and 20 cm/min, respectively, was used.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Isolated LTL myofibrils (Goll et al., 1974) were electrophoresed on 7.5 to 15% discontinuous gradient gels (Hames and Rickwood, 1982). Eighty micrograms of protein was loaded per lane. The resolving gel consisted of 30% (wt/vol) acrylamide with a 75:1 ratio of acrylamide to bis-acrylamide, 50% glycerol, pH 8.8. The sample buffer consisted of 8 M urea, 2 M thiourea, .05 M Tris (pH 6.8), 75 mM dithiothreitol, 3% SDS, and .05% bromophenol blue (Fritz et al., 1989). After the electrophoretic run was complete, the gels were fixed for 15 min in 25% trichloroacetic acid. The gels were then stained for 15 h with .1% Coomassie brilliant blue R-250, 50% methanol, and 7% acetic acid. The gels were destained with 40% methanol and 10% acetic acid.

Statistical Analysis

Data were analyzed with ANOVA by GLM procedures of SAS (1985) for a repeated measures design. Least squares means were computed and mean separation for a significant ($P < .05$) main effect of postmortem time was accomplished with the PDIFF option of the least squares procedures (a pairwise t-test).

Results and Discussion

To confirm that glycolysis was proceeding during storage at −5°C, pH was monitored at the time of sampling and after 5 and 10 d at −5°C (Table 1). Muscle from all sample times except 0 h had declined to ultimate pH after 5 d, and all samples were at ultimate pH after 10 d at −5°C. These data indicate that glycolysis had proceeded to completion and that the muscle was in complete rigor mortis. Thus, the muscle could be cooked and sheared without the possibility of fiber shortening interfering with the

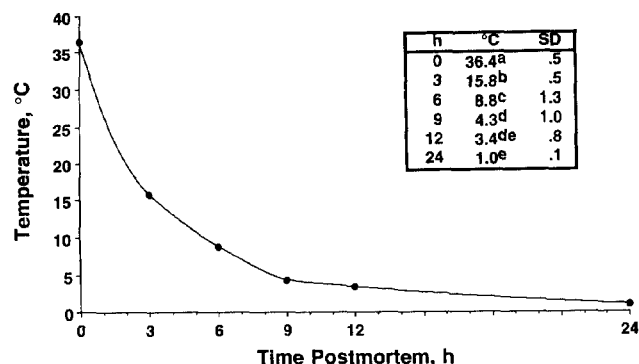| h	°C	SD
0	36.4[a]	.5
3	15.8[b]	.5
6	8.8[c]	1.3
9	4.3[d]	1.0
12	3.4[de]	.8
24	1.0[e]	.1

Figure 2. Postmortem changes in temperature of lamb longissimus thoracis et lumborum. Mean separation and SD are shown in the inset. Means without a common superscript differ $(P < .05)$.

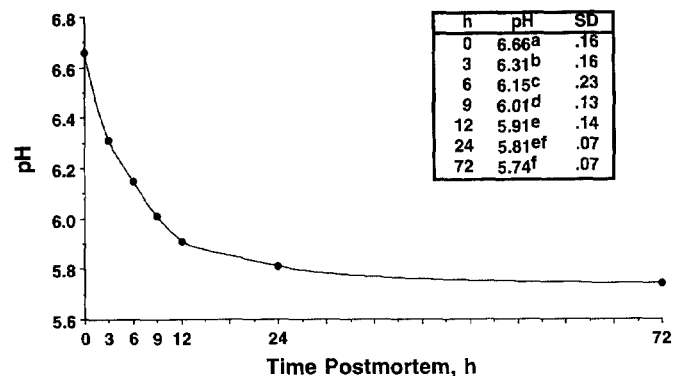| h	pH	SD
0	6.66[a]	.16
3	6.31[b]	.16
6	6.15[c]	.23
9	6.01[d]	.13
12	5.91[e]	.14
24	5.81[ef]	.07
72	5.74[f]	.07

Figure 3. Postmortem changes in pH of lamb longissimus thoracis et lumborum. Mean separation and SD are shown in the inset. Means without a common superscript differ $(P < .05)$.

tenderness measurement. It previously has been shown that muscle frozen prerigor and then stored at temperatures just below freezing for several days was capable of undergoing glycolysis and rigor development, yet ice formation was still sufficient to prevent muscle fiber shortening (Marsh and Thompson, 1958; Davey and Gilbert, 1976). Marsh and Thompson (1958) reported that muscle frozen prerigor at -70°C and then stored for 4 d at -3.5°C did not shorten or produce excessive drip loss upon thawing. Davey and Gilbert (1976) found that storage of prerigor frozen muscle at -12.5°C for 2 wk gave the same result.

Temperature and pH decline of the LTL were typical for the chilling conditions used and are shown in Figures 2 and 3. Muscle from all carcasses reached normal ultimate pH.

Sarcomere length at 0 h was 2.24 μm; it reduced through 24 h (1.69 μm) and then increased through 14 d (1.9 μm) postmortem (Figure 4). Resting sarcomere length for bovine semitendinosus of carcasses suspended from the hind limb has been reported to be 2.25 μm (Herring et al., 1967). Gothard et al. (1966) reported that bovine LTL sarcomere length was 2.0 μm immediately following death, shortened to 1.49 μm, and then lengthened to 1.72 μm by 7 d postmortem. Stromer et al. (1967) reported that bovine semitendinosus sarcomere length was 2.7 μm immediately following death, shortened to 1.5 μm at 1 d, and lengthened to 1.8 μm by 13 d postmortem. These values agree well with the sarcomere length obtained immediately postmortem in this experiment. The present experiment also agrees with earlier work indicating that LTL sarcomere length shortens during rigor mortis development, then lengthens with additional postmortem storage. The initiation of rigor mortis and its concomitant muscle shortening has been reported to begin when ATP concentration has been reduced by 20% (Bendall, 1951). In addition, the time course of rigor development and extent of rigor shortening has also been reported to vary due to variation in temperature, pH at death, and glycogen

reserves (Bate-Smith and Bendall, 1949; Busch et al., 1967; Honikel et al., 1983). Faster rigor onset results in greater muscle shortening (Bate-Smith and Bendall, 1949; Marsh, 1954; Sink et al., 1965). Marsh and Thompson (1958) reported that rigor mortis progresses faster in lamb than in beef. Locker (1959, 1960) first reported the importance of muscle shortening to tenderness. Hertzman et al. (1993) reported that rigor shortening is temperature-, not pH-, dependent due to the temperature dependence of calcium release, and that rigor shortening was very important to ultimate meat tenderness in muscle stored at 37°C. The highly tender bovine psoas major had long sarcomeres (3.7 μm) when the carcass was suspended by the hind limb (Herring et al., 1965a,b). But when excised and allowed to shorten (Herring et al., 1965b) or when the carcass was maintained in a horizontal position during rigor (Herring et al., 1965a), psoas major sarcomere lengths were 2.4 and 2.7 μm, respectively, and in both cases much tougher than

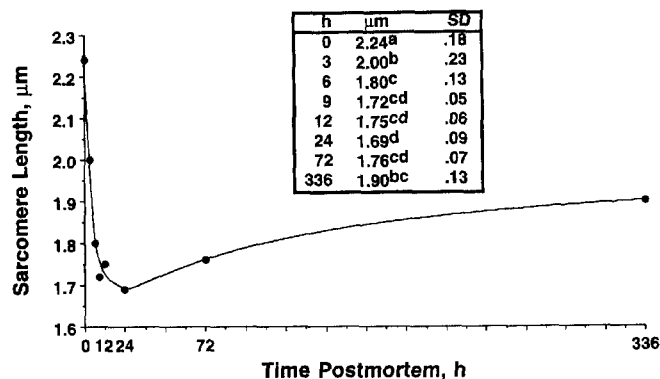| h	μm	SD
0	2.24[a]	.18
3	2.00[b]	.23
6	1.80[c]	.13
9	1.72[cd]	.05
12	1.75[cd]	.06
24	1.69[d]	.09
72	1.76[cd]	.07
336	1.90[bc]	.13

Figure 4. Sarcomere length of lamb longissimus thoracis et lumborum at specific times postmortem. Mean separation and SD are shown in the inset. Means without a common superscript differ $(P < .05)$.

under normal conditions. Thus, there is little doubt that the muscle shortening that accompanies rigor mortis results in reduced tenderness. Marsh and Leet (1966) demonstrated that up to 20% shortening had little effect on tenderness of beef sternomandibularis, 20 to 40% shortening resulted in dramatic decreases in tenderness, and 55 to 60% shortening resulted in tenderness similar to that with < 20% shortening.

Warner-Bratzler shear force was not different between 0 and 3 h postmortem (Figure 5). The muscle shortened only 11% from 0 to 3 h postmortem. Shear force increased through 9 h (23% shortening) and was not different between 9, 12 (22% shortening), or 24 h (25% shortening) postmortem. These results demonstrate the increase in shear force value with decreased sarcomere length (i.e., rigor shortening). Shear force declined from 24 h to 14 d postmortem. Simultaneously, sarcomere length increased from 24 h to 14 d, resulting in 21% shortening at 3 d and 15% shortening at 14 d postmortem. However, aging from 24 h to 14 d resulted in tenderness improvement beyond the initial level, implying that factors other than the lengthening of the muscle sarcomeres were contributing to the tenderization. The additional tenderization most likely resulted from proteolysis by the calpain proteolytic system (Koohmaraie, 1992a,b), which eventually overcame the rigor shortening-induced toughening and produced meat with lower shear force at 14 d postmortem than at slaughter.

Tenderness immediately postmortem was intermediate (5 kg). Previous work has reported that prerigor meat was both more tender and less tender than postrigor meat. However, these discrepancies can be explained by data indicating that prerigor meat cooked rapidly was tender and that cooked slowly was very tough (Paul et al., 1952; Weideman et al., 1967; Dransfield and Rhodes, 1975; Cia and Marsh, 1976; Montgomery et al., 1977; Ray et al., 1980). Cia and Marsh (1976) indicated that the superior tenderness of rapidly cooked prerigor meat was the consequence of disrupture of fiber structure in some areas, brought about by extreme shortening in other areas. More recently these findings have been confirmed by Abugroun et al. (1985a) and Silva et al. (1993), who also reported that the superior tenderness of prerigor meat cooked rapidly results from heat-induced contraction that causes physical disruption of the tissue. However, Abugroun et al. (1985b) found that in electrically stimulated muscle, even rapidly cooked prerigor meat was tough due to the inability to contract sufficiently to cause tissue disruption. These findings are consistent with those of Weideman et al. (1967) and Klose et al. (1970), who reported that if prerigor muscle was restrained at the ends during rapid cooking it was less tender than if it was left to shorten unrestrained. Thus, heating prerigor muscle without regard for heat-induced contraction results in an inaccurate measure of its tenderness at that point in time. The protocol used in the present experiment to stop shortening at various times postmortem and

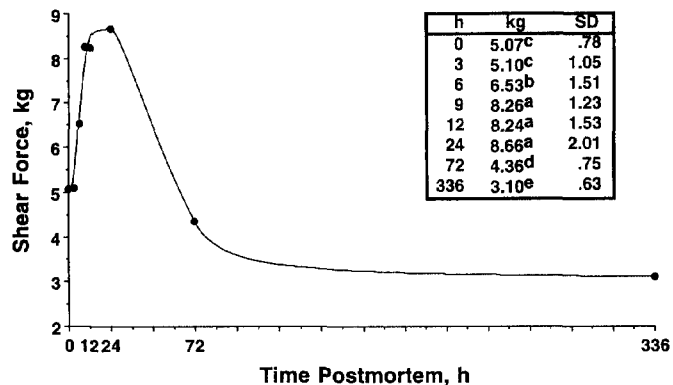| h	kg	SD
0	5.07c	.78
3	5.10c	1.05
6	6.53b	1.51
9	8.26a	1.23
12	8.24a	1.53
24	8.66a	2.01
72	4.36d	.75
336	3.10e	.63

Figure 5. Warner-Bratzler shear force of lamb longissimus thoracis et lumborum at specific times postmortem. Mean separation and SD are shown in the inset. Means without a common superscript differ (P < .05).

allow the muscle to go into rigor before cooking provides a more accurate measure of tenderness at that time. Clamping the entire muscle section between two plates prevented any shortening along the entire length of the muscle section. Clamping the ends (Weideman et al., 1967; Klose et al., 1970) is not sufficient; Marsh and Leet (1966) showed that shortening can occur in the middle of a muscle section when it is clamped on both ends.

Our interpretation of the research on meat tenderness is that ultimate tenderness in any one muscle (attached to the carcass during chilling) from young animals depends on a combination of rigor shortening-induced toughness and the extent of proteolytic tenderization by the calpain proteolytic system. Similarly, Hertzman et al. (1993) stated that myofibrillar toughness is influenced by the development of rigor mortis and tenderization by enzymatic breakdown. This experiment was designed to measure the tenderness of prerigor muscle independent of excision and heating effects and to identify the contributions of initial tenderness at slaughter, rigor toughening, and aging to ultimate meat tenderness. It seems that initial tenderness (0 h) does not vary greatly in sheep (Figure 5), and, thus, variation in initial tenderness contributes little to the variation in ultimate tenderness (r = .38). However, muscle shortening during rigor mortis development reduced tenderness. In addition, this increase in toughness due to rigor development was eliminated with continued storage at 2°C (aging), such that ultimate tenderness was greater than at slaughter.

Electrophoresis of myofibrillar proteins did not detect evidence of proteolysis before 12 h postmortem (Figure 6). Degradation products from 28 to 32 kDa appeared at 12 h postmortem. Desmin and Troponin-T degradation were detectable at 24 h and were extensive by 72 h. Assuming that tenderness variation at 24 h postmortem results from differences in muscle
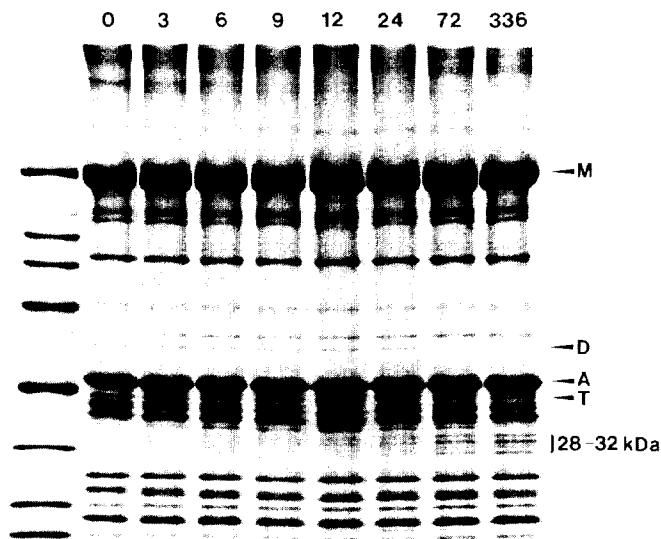
Figure 6. SDS-PAGE of myofibrils from ovine longissimus thoracis et lumborum obtained at 0 to 336 h postmortem. Lane 1 contains the following molecular weight standards: myosin, 200 kDa; *E. coli* β-galactosidase, 116.3 kDa; rabbit muscle phosphoralase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; hen egg-white ovalbumin, 45 kDa; bovine carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and hen egg-white lysozyme, 14.4 kDa. M = Myosin, D = Desmin, A = Actin, T = Troponin-T.

shortening and the extent of proteolysis, the decreased variation in sarcomere length and simultaneous increased variation in shear force from 0 to 24 h (Figure 5), combined with degradation of key myofibrillar proteins (Figure 6), indicate that a sufficient amount of proteolysis may have occurred by 24 h postmortem to affect tenderness. In support, Troy et al. (1986, 1987) reported that myofibrillar protein breakdown commences within 4 to 6 h postmortem. Koohmaraie et al. (1987) stated that tenderization begins soon after exsanguination. However, Dransfield et al. (1992) concluded that tenderization starts when a muscle pH of approximately 6.1 is attained (approximately 7 h postmortem in the present study) and predicted that meat tenderness at this point was approximately 12.5 kg of shear force. This predicted shear force value (12.5 kg) was much greater than that observed in the present experiment (5 to 6 kg). Although the present experiment was performed with lamb muscle rather than beef, it is doubtful that species differences would be so great. The more likely explanation is that the predicted value of 12.5 kg was overestimated because it did not account for rigor shortening effects on tenderness during the first 24 h postmortem.

The initiation of tenderization is dependent on intracellular free calcium concentration reaching sufficient levels (10 μM) to activate μ-calpain, resulting in

degradation of specific myofibrillar proteins. Jeacocke (1993) reported that after the onset of rigor, intracellular free calcium concentration increased to more than $10^{-4} M$. Honikel et al. (1983) reported that rigor shortening starts at pH 6.3 to 6.0 with approximately 2 μM ATP/g of muscle, and Hertzman et al. (1993) obtained similar results. Koohmaraie et al. (unpublished observation) found that calcium concentration increased and calpastatin activity decreased in a crude longissimus muscle homogenate such that μ-calpain activity was detected at approximately 18 h postmortem in lamb and 9 h postmortem in beef. This would indicate that tenderness measured at 24 h postmortem is highly dependent on the extent of rigor shortening and the amount of proteolytic tenderization that has occurred 24 h postmortem. That proteolysis has an important role in ultimate tenderness is also indicated by the finding that, although shear force decreased to one-half (from 8.66 to 4.36 kg) from 24 to 72 h postmortem, sarcomere length did not change significantly (from 1.69 to 1.76 μm) during this same period. Conversely, during the first 24 h postmortem, shear force increased 71% (from 5.07 to 8.66 kg), whereas sarcomere length decreased 25% (from 2.24 to 1.69 μm). These data indicate the importance of sarcomere length to tenderness before extensive proteolysis and the decreased importance after proteolysis.

In conclusion, to our knowledge this paper presents the first data on the tenderness of prerigor meat that are not confounded by excision- or heat-induced contraction. The data imply that ovine LTL at the time of slaughter is intermediate in tenderness, rigor shortening toughens the meat, and proteolysis tenderizes the meat resulting in more tender meat after 14 d of aging than at slaughter. For ovine muscle, it seems that tenderness differences at the time of slaughter are not closely related to ultimate meat tenderness and that ultimate meat tenderness depends on the extent of proteolysis and its ability to overcome rigor toughening. In addition, the initiation of proteolysis occurred by 12 h postmortem. Further experiments are planned to better differentiate the contributions of rigor toughening and proteolytic tenderization to ultimate tenderness.

Implications

Changes in tenderness of prerigor meat have been poorly documented. These data represent the first tenderness measurement of muscle at the time of slaughter and during the first 24 h postmortem that are independent of heat- and excision-shortening. The contribution of initial tenderness was minimal, but that of rigor shortening and proteolysis was significant to ultimate meat tenderness.

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