The Extent of Proteolysis Is Independent of Sarcomere Length in Lamb Longissimus and Psoas Major1,2,3

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ABSTRACT: The objective of this experiment was to determine the effect of sarcomere length on postmortem proteolysis and meat tenderness. Eighteen Dorset market-weight sheep were slaughtered conventionally. The longissimus thoracis et lumborum and psoas major from each carcass were either left intact on the carcass (control), which was chilled at 0°C, or excised from the carcass and chilled in an ice slurry (0°C). At 24 h, control muscles were excised, and all muscles were cut into sections and assigned to 1 or 10 d of postmortem storage at 2°C. Sarcomere length was shorter (P < .01), as intended, in the shortened relative to the control treatment and in longissimus relative to psoas major (1.36 vs 1.69 μm, raw longissimus; 1.45 vs 3.03 μm, raw psoas major). Sarcomere length was not affected (P > .05) by aging time. Western blot analysis of troponin-T and desmin indicated no effect (P > .05) of the shortened treatment compared to the control on the extent of proteolysis. Regardless of aging time or treatment, troponin-T was more degraded (P < .01) in longissimus than in psoas major (38.1 vs 23.5%) and desmin tended to be more degraded (P = .08) in longissimus than in psoas major (50.4 vs 35.1%). Regardless of muscle or treatment, aging 10 d compared to 1 d increased degradation of troponin-T (46.3 vs 15.3%) and desmin (69.3 vs 16.1%). Warner-Bratzler shear force was greater (P < .01) in the shortened treatment than in control (6.9 vs 3.8 kg), greater (P < .01) in longissimus than in the psoas major (6.5 vs 4.2 kg), and greater (P < .01) with 1 d than with 10 d of aging time (6.1 vs 4.6 kg). A muscle × aging time interaction (P < .05) indicated shear force declined more in longissimus than in psoas major during aging. We conclude that sarcomere length did not affect the extent of proteolysis. However, sarcomere length may have an indirect effect on tenderization during aging due to its effect on initial tenderness.

Key Words: Lamb Meat, Muscles, Proteolysis, Sarcomeres, Tenderness

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Introduction

The importance of muscle shortening to meat tenderness is well established (for review see Locker, 1985; Marsh, 1985). More recently, the impact of in situ rigor shortening on meat tenderness was demonstrated (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). In addition, current information suggests that the increase in tenderness during refrigerated, postmortem storage of meat is due to calpain proteolysis of key myofibrillar and cytoskeletal proteins (for review see Goll et al., 1983; Koohmaraie, 1994, 1996). Thus, the tenderness after aging of longissimus from achilles-hung carcasses that were chilled intact depends on sarcomere length and the extent of proteolysis (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996).

However, there are conflicting data regarding the tenderization of excised, cold-shortened meat during aging (for review see Locker, 1985). Perhaps the increased overlap of thick and thin filaments reduces the availability of proteolytically susceptible sites. Furthermore, the tenderization response during aging of psoas major may be reduced relative to that of longissimus (Koohmaraie et al., 1988). The relative contributions of sarcomere length and proteolysis to tenderness variation among muscles has not been
Materials and Methods

Animals

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use and treatment of animals in these studies according to guidelines established by the USDA. Eighteen Dorset wether lambs, 6 to 8 mo of age, were humanely slaughtered and dressed (three or four per day on 5 d) according to standard procedures. Mean carcass weight was 31.2 kg.

Treatments

The psoas major from one side of each carcass was assigned to be excised and shortened, and the psoas major from the other side was assigned to remain intact on the carcass as a control. The longissimus thoracis et lumborum from the two sides were assigned to two of three treatments: intact control, excised and shortened, or excised and stretched. Within 15 min after exsanguination, the psoas major from one side of each carcass (n = 18) and the longissimus thoracis et lumborum from one-third of the carcass sides (12 of 36 sides) were removed, placed in a plastic bag, and immersed in an ice-water bath (0°C) to induce shortening. The psoas major from the other side (n = 18) and the longissimus thoracis et lumborum from one-third of the carcass sides (12 of 36 sides) were left intact on the carcass as a control. The longissimus thoracis et lumborum from one-third of the carcass sides (12 of 36 sides) was removed, clamped at both ends, and stretched. However, the stretch treatment failed to produce sarcomere lengths longer than resting length; thus, the data from that treatment were discarded. Carcasses were chilled at 0°C for 24 h. Thus, both treatments were chilled at the same temperature.

Samples

At 24 h postmortem, control longissimus thoracis et lumborum and psoas major were removed from the carcasses, and shortened muscles were removed from the ice-water bath. Each muscle was cut into seven 2.54-cm-thick chops, and three chops each were assigned to 1 or 10 d of postmortem aging for Warner-Bratzler shear force measurement. Chops assigned to the 1-d aging time were vacuum-packaged and frozen at −30°C. Chops assigned to the 10-d aging time were vacuum-packaged, stored at 3°C until d 10 postmortem, then frozen at −30°C. The seventh chop was used for raw sarcomere length measurement and Western blot analysis of desmin and troponin-T.

Warner-Bratzler Shear Force

The chops for shear force were thawed to 6°C and then cooked on a Farberware model 450N open hearth electric broiler (Farberware, Bronx, NY) to a 75°C internal temperature. The chops were turned after reaching 40°C. Temperature was monitored with iron constantan thermouple wires inserted into the geometric center of each chop and attached to a Beckman Industrial model 205 data logger (Beckman Industrial, San Diego, CA). The cooked chops were chilled 24 h at 3°C, then six 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation (two from each chop). Cores were sheared once each at the center on an Instron Universal Testing Machine model 1132 (Instron, Canton, MA) with a Warner-Bratzler attachment and 200 mm/min crosshead speed. Sheared cores also were used to obtain sarcomere length of cooked samples.

Sarcomere Length

Three muscle cubes (raw measurements), one each from lateral, central, and medial locations within transverse sections (the 7th chop) of both muscles, and three cores randomly selected from the six cores used for Warner-Bratzler shear force measurement (cooked measurements) of both muscles were fixed according to Koolmees et al. (1986). From each cube or core, sarcomere length of eight fiber samples was determined (24 total measurements per observation) by neon laser diffraction as described by Cross et al. (1981).

Immunoblotting

A subsample of six animals per treatment was used for Western blot analysis. Whole muscle extracts were prepared by homogenizing 1 g of muscle in 10 mL of 50 mM Tris, 10 mM EDTA, pH 8.3, for 20 s using a polytron on speed setting 4 (Brinkmann Instruments, Westbury, NY). The muscle homogenate (.5 mL) was diluted 1:1 with 2x protein denaturing buffer (PDB) excluding mercaptoethanol and bromophenol blue (1× PDB consists of 2% SDS, 10% glycerol, 62.5 mM Tris, pH 6.8). Samples were heated at 50°C for 20 min, remixed and reheated 5 min, then centrifuged 20 min at 16,000 x g. 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, 20 μg of protein per lane was loaded. Desmin was separated on 10% gels (37.5:1 ratio of acrylamide to bisacrylamide), 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
Chemiluminescence substrate (Pierce) diluted 50% in TTBS for 60 min. Membranes were incubated with SuperSignal ULTRA antibodies diluted 1:25,000 (Pierce, Rockford, IL). IgG horseradish peroxidase conjugated secondary antibody as follows: monoclonal anti-desmin 1:25 (clone D3; developed by D. A. Fischman and obtained from the Developmental Studies Hybridom Bank); anti-troponin-T 1:10,000 (clone JLT-12, Sigma Chemical Co., St. Louis, MO). Membranes were washed three times (5 min) with TTBS after each incubation. Bound primary antibodies were labeled (60 min at room temperature) with Immunopure goat anti-mouse IgG horseradish peroxidase conjugated secondary antibody.

The extent of desmin and troponin-T degradation was determined by expressing the density of protein bands of the treatments relative to that of the reference standard within each blot. The anti-troponin-T monoclonal reacted with a number of polypeptides (3 to 5) in at death samples. The three predominant bands were used for quantification. The numbers reported in Table 2 are the percentages of the at-death protein that were degraded in each treatment. Figure 3 contains a representative example of Western blots used to quantify desmin and troponin-T degradation.

Statistical Analyses

Data were analyzed with mixed models analyses of variance using PROC MIXED of SAS (1997) for a split, split, split-plot design (Steel and Torrie, 1980). Random effects were animal and animal × muscle. Animal was the whole-plot treatment. The first split-plot treatment was muscle (longissimus thoracis et lumborum and psoas major), the second split-plot treatment was treatment (control or shortened), and the third split-plot treatment was aging time (1 or 10 d). The effect of muscle was tested with animal × muscle as the error term. The effect of treatment was tested with animal × muscle × treatment as the error term. The effect of aging time was tested with the overall error term. The effect of cooking on sarcomere length was tested with a split, split, split-plot design using the overall error term. Mean separation for significant (P < .05) interactions was accomplished with the PDIF option (a pairwise t-test) of the least squares procedures (SAS, 1997).

Results

As designed, sarcomere lengths of raw and cooked muscle from the shortened treatment were shorter (P < .01) than muscle from the control treatment (Table 1). Raw and cooked sarcomere length was shorter (P < .01) in longissimus than in psoas major. Sarcomere lengths of raw and cooked muscle were not affected (P = .60 and .28, respectively) by aging time. The three-way interaction was not significant for sarcomere length of raw (P = .92) or cooked (P = .34) muscle (Table 1). However, a substantial two-way interaction of treatment and muscle was detected (P < .01) for sarcomere length of both raw and cooked muscle. This interaction occurred due to the longer sarcomere length of control psoas major relative to control longissimus and, thus, a greater response to the shortening treatment occurred in the psoas major relative to that of the longissimus (Figure 1). In raw muscle from the shortened treatment were shorter (P < .01) than muscle from the control treatment (Table 1). Raw and cooked sarcomere length was shorter (P < .01) in longissimus than in psoas major. Sarcomere lengths of raw and cooked muscle were not affected (P = .60 and .28, respectively) by aging time. The three-way interaction was not significant for sarcomere length of raw (P = .92) or cooked (P = .34) muscle (Table 1). However, a substantial two-way interaction of treatment and muscle was detected (P < .01) for sarcomere length of both raw and cooked muscle. This interaction occurred due to the longer sarcomere length of control psoas major relative to control longissimus and, thus, a greater response to the shortening treatment occurred in the psoas major relative to that of the longissimus (Figure 1). In raw muscle from the shortened treatment were shorter (P < .01) than muscle from the control treatment (Table 1). Raw and cooked sarcomere length was shorter (P < .01) in longissimus than in psoas major. Sarcomere lengths of raw and cooked muscle were not affected (P = .60 and .28, respectively) by aging time. The three-way interaction was not significant for sarcomere length of raw (P = .92) or cooked (P = .34) muscle (Table 1). However, a substantial two-way interaction of treatment and muscle was detected (P < .01) for sarcomere length of both raw and cooked muscle. This interaction occurred due to the longer sarcomere length of control psoas major relative to control longissimus and, thus, a greater response to the shortening treatment occurred in the psoas major relative to that of the longissimus (Figure 1). In raw
Figure 1. Two-way interaction between treatment and muscle on sarcomere length in (a) raw and (b) cooked muscle. Means lacking a common superscript differ (P < .05). The SEM for raw control longissimus = .035, raw shortened longissimus = .033, raw control psoas major = .028, raw shortened psoas major = .030. The SEM for cooked control longissimus = .028, cooked shortened longissimus = .026, cooked control psoas major = .029, cooked shortened psoas major = .033.

Raw control sarcomere lengths were much longer (P < .01) in psoas major than in longissimus, and the shortening treatment reduced (P < .01) sarcomere lengths in both muscles (Figure 1). Although the difference in sarcomere length between muscles was greatly reduced by the shortening treatment, the difference still was significant. The effect of cooking on sarcomere length in control longissimus was approximately a .2-µm reduction (P < .01). However, cooking further reduced (P < .01) sarcomere length of shorted longissimus by only .15 µm. In addition, cooking reduced (P < .01) control psoas major sarcomere length by approximately .6 µm, with < .1 µm (P = .51) further reduction in shortened psoas major due to cooking. The correlation between raw and cooked sarcomere length was .97.

The shortening treatment resulted in greater (P < .01) Warner-Bratzler shear force compared to the control treatment (Table 2). Warner-Bratzler shear force of the longissimus was higher (P < .01) than that of the psoas major. Aging muscle for 10 d resulted in lower (P < .01) shear force than that in muscle aged for 1 d postmortem. The only interaction detected for Warner-Bratzler shear force was a magnitudinal two-way interaction of muscle × aging (P < .03). This interaction involved a greater reduction in Warner-Bratzler shear force of longissimus relative to psoas major when aged 10 d compared to 1 d. Because the interaction was magnitudinal in nature, the main effect of treatment was meaningful.

Western blots of whole muscle homogenates were probed with monoclonal antibodies against desmin and troponin-T (Figure 3). The anti-desmin antibody recognized a major polypeptide in at death samples and reacted primarily with three degradation products after 10 d of postmortem storage. The anti-troponin-T monoclonal reacted with a number of polypeptides (3 to 5) in at death samples. These polypeptides are not proteolytic products, rather they are troponin-T isoforms (for review see Schiaffino and Reggiani, 1996). After postmortem storage, the anti-troponin-T antibody reacted with a series of polypeptides with molecular mass of 28 to 32 kDa (Koohmaraie, 1990). These results are consistent with previous results from various laboratories (Koohmaraie et al., 1995, 1996; Taylor et al., 1995; Huff-Lonergan et al., 1996).

Western blot analyses indicated that the shortening treatment did not affect (P > .05) the extent of degradation of troponin-T or desmin (Table 2). A greater (P < .05) proportion of troponin-T was degraded from longissimus than from psoas major, and a tendency (P = .09) was detected for more desmin degradation in longissimus than in psoas major. Furthermore, 10 d of aging time resulted in greater (P < .01) degradation of both troponin-T and desmin compared to 1 d of aging time. No interactions (P > .05) were detected for troponin-T or desmin degradation. The high correlation between desmin and
troponin-T degradation ($r = .87$) indicates that both measures provide similar information.

**Discussion**

It is well established that a majority of the variation in tenderness after aging of longissimus from young, grain-fed cattle and sheep is due to the extent of proteolytic degradation of key myofibrillar and cytoskeletal proteins (for review see Goll et al., 1983; Koohmaraie, 1994, 1996). The acute impact of prerigor muscle shortening on tenderness also is well documented (for review see Locker, 1985; Marsh, 1985). More recently, the effect of in situ rigor shortening on longissimus tenderness was established (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). Those data indicate that longissimus tenderness depends largely on the extent of rigor shortening and postmortem proteolysis during refrigerated aging. Much less clear is the effect of postmortem refrigerated aging time on tenderness improvement in muscle varying in sarcomere length. Different muscles vary in sarcomere length due to differences in muscle fiber orientation and in the amount of tension placed on them in a hanging carcass during chilling and rigor mortis development (Herring et al., 1965a). Our objective was to determine the extent of proteolysis and resulting tenderization when sarcomere length varies either due to shortening induced by prerigor excision or due to differences in tension between longissimus and psoas major induced by carcass suspension during chilling. These data provide greater understanding of the relative contribution of the sources of variation in tenderness among muscles.

Conflicting data in the literature indicate that aging may (Goll et al., 1964; Herring et al., 1967; Newbold and Harris, 1972) or may not (Davey et al., 1967, 1976; Locker et al., 1975) improve tenderness of shortened meat. These observations could indicate that shortened sarcomeres reduce access to proteolytically susceptible proteins, thereby reducing their degradation and subsequent improvement in tenderness. However, data in Table 2 indicate that shortening did not affect the extent of troponin-T or desmin degradation in either longissimus or psoas major. This
The result agrees with the results of Koohmaraie et al. (1984) in longissimus and Young et al. (1980-81) in sternomandibularis. Thus, we would expect a similar amount of tenderization from 1 to 10 d of aging between control and shortened treatments in both longissimus and psoas major. In fact, this is the result that occurred in the longissimus (Table 2). The slightly lower reduction in shear force from 1 to 10 d in control vs shortened psoas major was likely due to the fact that the control psoas major was already so tender because of the very long sarcomeres that there was little room for improvement. These results agree with those of Jaime et al. (1992), who concluded that the tenderizing effect of aging was independent of muscle shortening.

The slightly lower extent of degradation of tropinin-T and desmin in psoas major relative to the longissimus in all treatment comparisons (except for shortened muscle aged 1 d) is consistent with the slightly higher ratio of calpastatin to \( \mu \)-calpain in psoas major relative to longissimus (Koohmaraie et al., 1988). Thus, the reduced extent of tenderization in psoas major relative to longissimus was partially due to reduced proteolysis and partially the result of the fact that the initial level of tenderness influences the extent to which tenderness can be improved. Koohmaraie et al. (1988) speculated that sarcomere length might be related to proteolysis during aging because the aging response in three muscles (longissimus, biceps femoris, and psoas major) was inversely related to sarcomere length. It now seems apparent that the small effect sarcomere length has on tenderization during aging occurs due to the effect of

Figure 2. Two-way interaction between muscle and aging time on Warner-Bratzler shear force. Means lacking a common superscript differ (\( P < .05 \)). The SEM for longissimus 1 and 10 d = .38. The SEM for psoas major 1 and 10 d = .34.

Figure 3. Representative example of Western blots that were used to quantify degradation of desmin and troponin-T. STD = at-death reference standard. Within each blot, desmin or troponin-T was quantified (Table 2) relative to the STD and expressed as the percentage of the STD that was degraded.
shortening on initial tenderness, not by affecting the extent of proteolysis.

Taylor et al. (1995) also have quantified differences in degradation between muscles and between proteins using Western blotting. They reported that the proportion of degraded protein (100 minus their values, which were expressed as percentage undegraded) after 6 d postmortem in bovine biceps femoris and semimembranosus was 9.5 and 74.8%, 46.3 and 85.4%, and 77.6 and 96.5%, respectively, for desmin, vinculin, and nebulin. In the present experiment, after 10 d of postmortem aging, desmin was 80% (longissimus) and 60% (psoas major) degraded. The correlation between Warner-Bratzler shear force with desmin \( (r = .27) \) and troponin-T \( (r = .20) \) degradation was relatively low, most likely because much of the variation in Warner-Bratzler shear force was related to sarcomere length \( (r = -.66, \text{raw}; r = -.70, \text{cooked}) \) in this study. Furthermore, it seems that there is considerable variation among muscles in sarcomere length (Herring et al., 1965a), calpastatin activity (Koohmaraie et al., 1995), proteolytic degradation of specific proteins (Taylor et al., 1995; Table 2), and, thus, postmortem calpain proteolytic capacity. Thus, the relatively poor relationship among muscles for tenderness (Shackelford et al., 1995) potentially results from variation in connective tissue, sarcomere length, and proteolysis.

There is little doubt that the tenderness difference between control longissimus and control psoas major, and between control longissimus and shortened longissimus, was mostly due to sarcomere length. However, after 1 d of aging, the shortened longissimus had much higher shear force than shortened psoas major, despite both having quite short sarcomere lengths \( (1.36 \text{ vs } 1.45 \mu\text{m, raw}) \) and a similar level of proteolysis. Herring et al. (1965b) reported that psoas major was more tender than the semitendinosus after both were excised prerigor and shortened to \( -1.8 \mu\text{m} \). This difference in the toughening effect of shortening among muscles may be partially explained by the results of Dutson et al. (1976), who reported that shortening causes a greater reduction in tenderness in muscles with higher connective tissue content. Thus, the higher connective tissue content of longissimus relative to psoas major (Boccard, 1981; Seideman et al., 1989) may partially explain the difference in tenderness at the same sarcomere length.

Sarcomere lengths in control and shortened treatments from both muscles are similar to those reported previously (Herring et al., 1965b; Bouton et al., 1973a; Dutson et al., 1976; Lewis et al., 1977). Our data were similar to data in previous reports of the effect of cooking on sarcomere length of control longissimus (Bouton et al., 1973b; Koohmaraie et al., 1996) and the increased effect of cooking on sarcomere length of control psoas major relative to longissimus (Lewis et al., 1977).

We have shown that shortened and unshortened muscle go through similar proteolysis and tenderization. The lack of tenderization during aging in the psoas major that has been reported by others (Koohmaraie et al., 1988; Seideman et al., 1989) did not occur in this experiment, although less tenderization occurred in the psoas major than in the longissimus. However, this result may simply be due to the fact that the long sarcomeres in psoas major make it so tender that it has little room for improvement. These data highlight the roles of rigor shortening, proteolysis, and initial tenderness level on meat tenderness and tenderization during aging. They also indicate that information for each muscle on the relative contribution of sarcomere length, proteolysis, and connective tissue will be needed to understand variation in tenderness among muscles.

**Implications**

Sarcomere length has a direct effect on meat tenderness; however, it does not affect the extent of proteolysis. Thus, any effects sarcomere length may have on tenderization during aging occur because of its effect on initial tenderness.

**Literature Cited**


