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Tenderness and Associated Characteristics of Stretched and Contracted Bovine Muscles

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SUMMARY

The effects of muscle contraction state, carcass maturity and post-mortem aging on tenderness were studied on excised semitendinosus muscles of six A and six E maturity bovine carcasses. Fiber diameter was shown to be curvilinearly related with sarcomere length (R = .95 and .87 for A and E maturity groups, respectively). As muscles were shortened they had a larger percent area of fibers and a smaller percent area of both endomysial and perimysial material. Muscles of the A maturity group were significantly more tender ($P < .01$) than those of the E maturity group. Post-mortem aging resulted in tenderization in both A and E maturity groups at all states of contraction (-48 to +48% of the pre-excised length), however tenderness of contracted muscles did not reach acceptable levels, even after 240 hr. aging. Tenderness was shown to be linearly related to fiber diameter (r = .82 and .87 for A and E maturity group, respectively), however the relationship with sarcomere length was curvilinear (R = .90 and .75 for A and E maturities, respectively). Post-mortem contraction of muscles was very effective in causing decreased tenderness whereas the magnitude of tenderness increase was smaller when muscles were stretched.

INTRODUCTION

Locker in 1959 described a simple technique for measuring the length of post-rigor sarcomeres in muscle. Subsequently this same worker (Locker, 1960) suggested a relationship between the post-rigor sarcomere length of the muscle and its ultimate tenderness. In our extension of this work (Herring et al., 1965a) it was observed that if a muscle were excised and permitted to shorten during the development of rigor mortis it would not be tender upon thermal processing. Conversely, if a strip of the same excised muscle were restrained so that it could not shorten during the development of rigor mortis it would ultimately be more tender than the excised muscle. In related work, we (Herring et al., 1965b) have more recently shown that the vertical suspension of the carcass releases tension on some muscles and increases tension on others -- thereby influencing the ultimate tenderness of the muscles. These studies, by themselves, have not shown whether it is necessary to actually stretch a muscle for maximum tenderness or whether it is more important to merely prevent shortening during rigor mortis onset.

The following study was therefore conducted to quantitatively determine how the tenderness of a single muscle varies from a 48% shortening upwards to a 48% stretch (based on its pre-excised length). Because it was obvious that "background tenderness" (Marsh, 1966), might influence the effect of contraction state on ultimate tenderness, the present study was conducted on muscle from young (A maturity) and old (E maturity) bovine animals.

MATERIAL AND METHODS

Animal and sample selection. Six bovine carcasses of A maturity and six of E maturity (U.S.D.A., 1965) were used. The semitendinosus muscle was selected for these studies as the uniform, longitudinal fiber arrangement is suitable for *in vitro* experiments involving stretch and contraction. Both semitendinosus muscles of the carcass were excised approximately 45 min post-mortem and each muscle was divided into four portions about 25 x 5 x 5 cm.

Sample treatment. Four of the resulting portions were caused to contract with the phenomenon of cold shortening (Locker and Hagyard, 1963) by 12, 24, 36 and 48% of the pre-excised length and the remaining four portions were stretched by 12, 24, 36 and 48% of the pre-excised length. The muscle portions were held firmly at the specified length in a specially designed apparatus (Fig. 1 and 2) for 48 hr. Samples were removed from the muscle portions at 48, 120 and 240 hr post-mortem for tenderness evaluation. Histological examinations were made on samples of the same portions (uncooked) at 48 hr post-mortem only.

Histological evaluation. Sarcomere length was determined as previously described (Herring *et al.*, 1965a). Fiber diameter determinations were made on samples which had been fixed for 48 hr in 10% calcium-formol. Sections were cut 16 u thick in a cryostat, stained with neutral red and mounted in glycerol jelly. The diameter of 100 fibers was measured with an ocular micrometer. The relative fraction of muscle occupied by muscle fibers, endomysium and perimysium was determined on the same sections used

for fiber diameter measurement. The "hit" method of Chalkley (1943) as modified by Enesco and Puddy (1964) was used. The 81 points formed by the intersecting lines (except the four lines which formed the perimeter of the grid) of a 100-square ocular grid were used as hit points. Five hundred hits were required for reasonable accuracy, and the frequency of hits gave a figure for the relative fraction of the three components.

Tenderness evaluation. Samples (approximately 5 x 5 x 5 cm) for tenderness evaluation were roasted to an internal temperature of 66°C in a 177°C oven. Organoleptic evaluations were conducted by a six-member laboratory panel using a hedonic scale from 1 (very tough) to 9 (very tender). Corresponding samples were chilled at 5°C for 4 hr, prior to objective tenderness evaluation by Warner-Bratzler shear on 1.27 cm cores.

Statistical treatment. The data were subjected to analysis of variance and linear and curvilinear regression analysis (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Mean values for sarcomere length and fiber diameter as influenced by muscle contraction or stretch, within each maturity group are presented in Table 1. Gross manipulation of muscle length (treatment) greatly influenced the microscopic determination of the resultant contraction state (sarcomere length). Additionally, a decrease in muscle length gave rise to a large increase in fiber diameter. Figure 3 shows the effect of sarcomere length on fiber diameter. Regression analysis showed curvilinear regression lines with R's of .95 and .87 for A and E maturity

groups, respectively. For the A maturity group X^4 contributed significantly ($P < .01$) to reducing variation from the regression. At lengths less than 2.0 u, each increment decrease in sarcomere length resulted in a marked increase in fiber diameter. The regression line for the E maturity group was also curvilinear and the additional reduction attributable to inclusion of X^2 in the equation was significant ($P < .05$). Herring et al. (1965b) showed a linear relationship between changes in sarcomere length versus changes in fiber diameter, but this was established using twelve different muscles and a variable of carcass position was also involved.

Table 2 shows the analysis of variance for sarcomere length and fiber diameter. The treatment mean square accounted for a higher portion of the total variance than the other factors for both parameters. The effect of age on sarcomere length was highly significant ($P < .01$) but this result should be interpreted with caution. The experiments were done on excised pre-rigor portions and we prefer to speculate that the age difference is dependent in part upon a differential response of the muscle to stretch or contraction. As shown in Table 1, at each degree of stretch, the A maturity group had a longer sarcomere length than the E maturity group. This apparently was due to some inherent property of the muscle that was different between the two age groups.

There was also animal variation within each of the two age groups (Table 2) and the interactions were highly significant ($P < .01$) indicating a differential in response to treatment between and within age groups. The effects due to age and to

treatment on fiber diameter were highly significant ($P < .01$). Muscle fiber diameter has been shown by many workers (Hiner et al., 1953; Joubert, 1956; Tuma, et al., 1962; Romans et al., 1965) to generally increase with an increase in animal age.

The mean values for relative percent fibers, endomysium and perimysial material are given in Table 3. Analysis of variance (Table 4) showed a highly significant effect of treatment on all three components. Muscle which had been caused to contract had a larger percent area of fibers and a smaller percent area of both endomysial and perimysial material than rest length or stretched muscle. This observation is interesting in view of the greater toughness of the contracted muscle (Table 5), even though less percent endomysial and perimysial connective tissue was observed in the contracted muscles as compared to the stretched muscles. These data further support the contention of Marsh (1966) that an "actomyosin" type toughness is important in contracted muscle as compared to the contribution of so-called "background" type toughness contributed by connective tissue. It has been apparent, and our data further support the observation, that a shearing force through a unit area of stretched muscle must cut more fibers, more endomysium and more perimysium than a similar shear through contracted muscle. The possibility of changes in muscle connective tissue in regard to drastic changes in muscle length requires investigation.

Significantly ($P < .01$) more perimysium was observed in the muscle from E maturity carcasses as compared to those of A maturity. This observation is of interest since chemical studies

have not shown a significantly greater total connective tissue content in the muscles of old than in young animals. (Goll et al., 1963, Hill, 1966). The high elastin content is an important feature of bovine semitendinosus, Standine et al., 1949, Venable, 1962).

Table 5 contains mean values for shear force and panel tenderness. A simple correlation of -.81 ($P < .01$) was found between the two tenderness measurement methods. Age had a highly significant ($P < .01$) effect (Table 6) on shear force and panel tenderness. Possibly the higher percent perimysium in the E maturity group had some influence. Jacobsen and Fenton (1956) and Goll et al., (1963) reported that shear force values increase and organoleptic scores for tenderness and juiciness decrease with increased animal age. Brady (1937) and Cline et al. (1932) found cows to be significantly less tender than steers. Tuma et al. (1963) also reported that tenderness decreased as the age of the animals increased. Biochemical studies (Wilson et al. 1954) revealed that the collagen content of veal longissimus dorsi muscle was greater than that from older animals. Goll et al. (1963) suggested that a structural change may take place in the collagen as an animal matures. More recently, Hill (1966) provided presumptive evidence of an increase with age in number or strength of cross-links of intramuscular collagen. Cross-linking influenced the solubility of collagen, that is more collagen was solubilized in muscle from younger animals than from older animals. Data are not available on the effect of maturity on properties of the myofibrillar proteins.

Post-mortem aging (Table 6) resulted in tenderization as

measured by shear and panel score. The animal age x aging period interaction was significant ($P < .05$) for panel tenderness. This significant interaction was due to increased tenderization as indicated by the panel score differences being higher between the 48 and 120 hr aging period than between the 120 and 240 hr aging period for the A maturity group. Similar results were noted for treatments 3 and 2-8 in the E maturity group. However, treatments 1, 2, and 4 showed little tenderization as revealed by panel score during the 48-120 hr period, but increased tenderization during the 120-240 hr aging period. Similar trends were revealed in the shear force values. Hanson et al. (1942) and Ramsbottom and Strandine (1949) suggested that aging affected muscle fibers as well as connective tissue fibers. Hoagland (1917) in an extensive study drew attention to enzymatic degradation. Recently, Davey and Gilbert (1966) found that differences in the rate of tenderization are not paralleled by similar differences in the rates of proteolysis. Partmann (1963) feels the role of actomyosin complex dissociation is important in tenderness changes. Increased fibrillar protein solubility with post-mortem aging has been shown by Aberle and Merkel (1966) to be positively related to decreased shear force values.

The effect of treatment on tenderness and shear force (Table 6) was highly significant ($P < .01$). There was a doubling of shear force as the sarcomere length decreased by 50%. At intermediate states, the panel found large differences in tenderness between the two age groups. On the other hand, greatest differences in shear force were observed between age groups in the con-

tracted states (1-4) at all aging periods.

Marsh and Leet (1966) have recently shown a complex relationship between shortening and tenderness with shortening from 20% up to 40% resulting in decreased tenderness and with further shortening up to 60%, the muscle became progressively more tender. However, in our experiments, after measuring actual sarcomere lengths. we have been unable to achieve shortening greater than what would actually correspond to Marsh and Leet's 40% shortening of excised strips of bovine sternomandibularis muscles.

Figure 4 shows the relationship between fiber diameter and shear force for both age groups. Linear regression equations gave simple correlations of .82 and .87 for A and E maturity groups, respectively. This is in contradiction with Hiner et al. (1953) who showed, on using a number of muscles, a curvilinear relationship between shear force and fiber diameter. In the present study, however, all of our measurements were made on the same muscle. Panel tenderness was also linearly related with fiber diameter. The relationship between shear force and sarcomere length (48 hr post-mortem) is shown in Fig. 5 for A and E maturities. With respect to the regression line of best fit for the A maturity group, the additional reduction due to fitting the fourth degree polynomial was significant ($P < .025$). At sarcomere lengths up to 2.0 u, the slope was depicted as being very steep, however, at lengths between 2.0 and 3.25 u, the slope was relatively flat indicating little change in shear force with increased sarcomere length in the latter range. Similar patterns were seen with the E maturity group. The difference in the shape of the regression

lines between the two maturity groups was probably due to an age effect. Possibly the muscle fibers and connective tissue of the E maturity group are less flexible as reflected by the relationship between sarcomere length and fiber diameter in Fig. 1. Also, greater variability in shear force existed in the E maturity group.

CONCLUSION

On the basis of these results, it is readily apparent that it is more important, from the standpoint of ultimate tenderness, to prevent post-mortem shortening than to insure a maximum stretch. The difference in tenderness of muscles stretched from 12 to 48%, although apparent, was not of the magnitude that was evident with the various stages of shortening. Shortened muscles, even after 10 days of aging were not acceptable in tenderness. This was most apparent in the E maturity group.

Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by a research grant from the Campbell Soup Company, Camden, New Jersey. Acknowledgement is also made of the use of equipment purchased through Public Health Service Research Grant EF-81 (C7) from the Division of Environmental Engineering and Food Protection. Department of Meat and Animal Science manuscript No. 454.

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Table 1. Fiber Diameter and Sarcomere Length in Relation to Contraction State (Semitendinosus)

Treatment[b]	Sarcomere length[a] Maturity A	Sarcomere length[a] Maturity E	Fiber diameter[a] Maturity A	Fiber diameter[a] Maturity E
1 (-48%)	1.61	1.44	68.01	79.64
2 (-36%)	1.72	1.56	61.34	75.45
3 (-24%)	1.83	1.75	57.81	68.12
4 (-12%)	2.04	1.90	57.44	61.44
5 (+12%)	2.52	2.26	50.26	56.68
6 (+24%)	2.79	2.59	46.10	49.97
7 (+36%)	2.98	2.85	43.64	46.86
8 (+48%)	3.21	2.99	40.90	44.18

[a]Values presented in microns.

[b]Semitendinosus portions (excised 45 min. post-mortem) were permitted to contract to specific percentages of pre-excised lengths as shown in treatments 1-4; similar portions were stretched-restrained to specific percentages of pre-excised lengths as shown in treatments 5-8.

[c]According to USDA Beef Grading Standards, 1965. (Six animals per maturity group).

Table 2. Analysis of Variance for Sarcomere Length and Fiber Diameter

Source	df	Sarcomere length[a]	Fiber diameter[a]
Age	1	57.57**	20,146.32**
Animal/age	10	3.66**	3,117.17**
Treatment	7	365.24**	24,257.5**
Age X treatment	7	.762**	833.24**
Animal X treatment/age	70	1.137**	154.60**
Error	2304 (9504 for fiber diameter)	.08	22.38

** $P < .01$

[a] Mean squares

Table 3. Relative Area[a] of Fibers, Endomysium and Perimysium

Treatment[b]	% Fibers		% Endomysium		% Perimysium	
	Maturity[c] A	Maturity[c] E	Maturity A	Maturity E	Maturity A	Maturity E
1 (-48%)	79.24	79.81	13.52	11.73	7.38	8.47
2 (-36%)	79.00	79.05	13.17	12.21	7.69	8.74
3 (-24%)	78.40	77.73	13.51	13.19	8.09	9.08
4 (-12%)	78.02	77.51	13.53	13.16	8.45	9.34
5 (+12%)	77.12	76.50	14.04	14.16	8.74	9.34
6 (+24%)	76.31	76.26	14.76	14.26	8.94	9.65
7 (+36%)	75.89	75.57	15.31	15.16	8.80	9.27
8 (+48%)	74.61	74.26	16.11	16.33	9.28	9.58

[a]Relative area given as percentage of total of three components.

[b]Semitendinosus portions (excised 45 min. post-mortem) were permitted to contract to specific percentages of pre-excised lengths as shown in treatments 1-4; similar portions were stretched-restrained to specific percentages of pre-excised lengths as shown in treatments 5-8.

[c]According to USDA Beef Grading Standards, 1965.

Table 4. Analysis of Variance for Muscle Components

Source	df	% Fibers[a]	% Endomysium[a]	% Perimysium[a]
Age	1	1.81	5.57	13.89**
Treatment	7	36.25**	19.23**	3.29**
A X T	7	.52	1.13	.254
Error	80	3.71	3.05	1.136

** P< .01

[a] Mean squares

TABLE 5. Shear force and panel tenderness score as affected by state of post-mortem contraction and post-mortem aging.

Treatment[a]	Shear Force[b]						Panel Score[c]					
	Maturity A			Maturity E			Maturity A			Maturity E		
	48[d]	120	240	48	120	240	48	120	240	48	120	240
1 (-48%)	13.3	11.3	9.2	15.1	14.4	12.0	2.3	3.2	3.5	1.8	1.9	2.3
2 (-36%)	11.0	10.0	7.7	14.9	11.8	10.8	2.6	3.5	4.3	2.0	2.2	2.9
3 (-24%)	9.2	8.4	6.9	13.5	10.9	10.2	3.3	3.9	4.1	2.2	2.7	2.9
4 (-12%)	7.7	6.5	5.5	10.9	9.6	9.2	4.4	5.8	5.7	2.7	2.9	3.5
5 (+12%)	6.7	5.3	5.3	9.2	7.2	6.9	5.0	6.0	6.0	3.1	4.3	4.7
6 (+24%)	6.8	5.3	5.2	8.6	6.8	6.5	4.9	5.7	6.5	3.8	5.0	5.1
7 (+36%)	6.6	5.2	4.8	8.1	6.5	6.0	4.8	6.0	6.2	4.1	5.1	5.3
8 (+48%)	6.4	5.1	4.9	8.8	6.7	6.2	5.2	6.2	5.9	4.3	4.9	4.7

[a]Semitendinosus portions (excised 45 min. post-mortem were permitted to contract to specific percentages of pre-excised lengths as shown in treatment 1-4; similar portions were stretched-restrained to specific percentages of pre-excised lengths as shown in treatments 5-8.

[b]Shear force in kg. on 1.27 cm. core.

[c]Panel score based on Hedonic scale, 1 very tough to 9 very tender.

[d]Post-mortem aging in hours (4° C.).

[e]According to USDA Beef Grading Standards 1965 (Six animals per maturity group).

Table 6. Analysis of Variance for Shear Force and Panel Tenderness Score

Source	df	Shear force[a]	Panel tenderness score[a]
Age (A)	1	410.68**	118.66**
Aging period (B)	2	154.90**	27.98**
Treatment (T)	7	216.27**	45.87**
A x B	2	2.45	1.65*
A x T	7	6.18	1.70*
A x B x T	14	.676	.261
Error	254	3.298	.707

* $P < .05$

** $P < .01$

[a] Mean squares

Explanation of Figures

Fig. 1 Apparatus for restraining muscle portions.

Fig. 2 Enlarged view of clamp employed in apparatus for restraining muscle portions.

Fig. 3 Effect of sarcomere length on fiber diameter. Scatter diagram showing each maturity group with regression lines drawn from regression equations. Regression equations:

$$\text{A maturity, } Y = 1047.74 - 1608.20\,X + 971.17\,X^2 - 258.44\,X^3 + 25.40\,X^4 \quad (R = .95)$$

$$\text{E maturity, } Y = 137.15 - 51.79\,X + 7.037\,X^2 \quad (R = .87)$$

Fig. 4 Effect of fiber diameter on shear force (48 hr. postmortem). Scatter diagram showing each maturity group with regression lines drawn from regression equations. Regression equations:

$$\text{A maturity, } Y = .238\,X - 4.101 \quad r = .82$$

$$\text{E maturity, } Y = .229\,X - 2.596 \quad r = .87$$

Fig. 5 Effect of sarcomere length on shear force (48 hr. postmortem). Scatter diagram showing each maturity group with lines drawn from regression equations. Regression equations:

$$\text{A maturity, } Y = 329.06 - 5.03.06\,X + 294.14\,X^2 - 76.08\,X^3 + 7.33\,X^4 \quad R = .90$$

$$\text{E maturity, } Y = 33.03 - 16.14\,X + 2.618\,X^2 \quad R = .75$$