Labile Collagen from Epimysial and Intramuscular Connective Tissue as Related to Warner-Bratzler Shear Values

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Steaks from the *M. longissimus* and *M. biceps femoris* were obtained from aged Holstein Friesian cows grading Cutter and Canner. Steaks were roasted and shear values were obtained with the Warner-Bratzler shear. The yield of heat labile collagen from the toughest and most tender muscles was studied. The effects of epimysial contraction and heating temperature on yield of labile collagen were determined. Heat labile collagen from both intramuscular and epimysial tissue was higher for tender than for tough muscles. As the amount of contraction during heating of epimysial tissue increased, the yield of labile collagen decreased. The greater amount of shortening upon heating was associated with higher shear values. Results suggest that tenderness increased as the yield of labile collagen increased.

The stability and number of cross-links of the collagen molecule and their relationship to muscle tenderness is presently an area of very active research. One measure of cross-linking which is thought to be related to tenderness is yield of heat labile collagen.

The total amount of hydroxyproline released from connective tissue during thermal contraction was first measured by Meyer and Verzar (1959). Verzar (1964) later reviewed the release of hydroxyproline during thermal contraction. He concluded that the quantity of hydroxyproline released declined with increasing biological age. Tail tendon of young rats released 75 to 90% of the total hydroxyproline on heating at 65° C. for 50 minutes, whereas, the tendon from 30 to 36 month old rats lost about 25% of the hydroxyproline after similar treatment. Verzar (1964) called the fraction, which is solubilized upon heating in Ringers solution or water, labile collagen. He suggested that the decrease of labile collagen with age can be explained on the basis of increased cross-linking of collagen during aging.

Goll *et al.* (1964a,b) found that the labile intramuscular collagen varied from 42% at 40 to 49 days of age to as low as 2% at 10 years of age in the bovine. They then suggested that the number and strength of the cross-links in collagen may play an important role in meat tenderness. More recent studies by Hill (1966) and Vognarova *et al.* (1968) have confirmed that less collagen is solubilized during cooking of meat from old than from young animals. The hypothesis that decreased yields of labile collagen are correlated with increased toughness in cooked meat from animals of a widely different age range has been confirmed by Herring *et al.* (1967).

It is evident that labile collagen decreases with increased biological age. In addition, the literature clearly indicates that over a wide age range, tenderness decreases as age increases. Nevertheless, within maturity groups there is a great deal of variation in muscle tenderness (Palmer, 1963). Information explaining the relationship between tenderness variability within maturity groups and yield of labile collagen is lacking. The purpose of this study was to obtain this information using epimysial and intramuscular connective tissue from two different muscles.

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EXPERIMENTAL

The *M. longissimus* (L) and *biceps femoris* (BF) muscles were obtained approximately 48 hours postmortem from 40 Holstein Friesian cow carcasses of the "E" maturity group which graded Cutter or Canner (USDA, 1965). Steaks from the L muscle were cut from the 11th and 12th rib region and steaks from the BF muscle were removed midway between the proximal and distal ends. Each steak (3 cm. thick) was roasted in a 163° C. oven to an internal temperature of 71° C. Three 1.27-cm. diameter cores cut parallel with the muscle fibers were taken from the lateral, central, and medial positions of the cooked L or BF muscles, and each core was sheared 3 times. Epimysial and intramuscular connective tissue was obtained from steaks adjacent to the 11 L and BF muscles with the lowest average shear values and the 11 L and BF muscles with the highest average shear values.

Epimysial tissue from the L and BF muscles was removed and dissected free of muscle and adipose tissue and frozen up to 5 weeks before labile collagen or shear values of the epimysium were determined. Frozen L and BF muscles freed from epimysial tissue were powdered as described by Borchert and Briskey (1965). The powdered muscle and the epimysial tissue were dried at 4° C. overnight in an evacuated desiccator containing calcium chloride before determining the labile collagen. Approximately 2 grams of the dried powdered muscle or 0.5 gram of the dried epimysium was placed in 50-ml. centrifuge tubes, and 15 ml. of 0.9% NaCl in distilled water were added. The samples were allowed to stand for 2 hours at room temperature to rehydrate before heating in a water bath at 71° C. for 1 hour. The contents, which required 10 minutes to reach 71° C., were stirred occasionally during heating. At the end of the 1-hour heating period, the tubes were centrifuged at 3000 × G. After centrifugation and removal of the supernatant, the residue in the tubes was washed with 10 ml. of distilled water and centrifuged again. The supernatants and residues were hydrolyzed in 6*N* HCl for 12 hours at approximately 120° C. The samples were cooled and the powdered muscle samples were decolorized with activated carbon (Norit) to remove the humin. This step was not necessary for the epimysial samples because no appreciable amount of humin was formed. All samples were neutralized to pH 7 with NaOH, after which dilutions were made and hydroxyproline determined according to the procedure of Woessner (1961). Hydroxyproline values were converted to collagen by multiplying by 7.14.

Percent moisture gained, contraction measured as a percent

Table I. Characteristics of Intramuscular and Epimysial Connective Tissue from Tough and Tender Muscles of Aged Cows

	Tough (N = 11)		Tender (N = 11)	
	Mean	S.D.	Mean	S.D.
M. longissimus				
Warner-Bratzler shear, kg./cm. 2	6.41[a]	1.65	3.16[b]	0.46
Intramuscular				
Collagen content[c]	18.46	5.18	18.87	3.73
Labile collagen, %	5.35[a]	1.39	6.52[b]	1.11
Epimysial				
Warner-Bratzler shear, kg./cm.^{2}	21.12[a]	7.68	14.35[b]	8.63
Collagen content[c]	705.75	61.51	708.53	30.19
Labile collagen, %	1.33[a]	0.91	2.68[b]	1.96
Moisture gained, %[d]	175.09	17.06	181.63	33.84
Contraction, %[e]	49.73[a]	6.31	43.36[b]	7.44
M. biceps femoris				
Warner-Bratzler shear, shear, kg./cm.^{2}	11.99[a]	1.13	7.03[b]	0.73
Intramuscular				
Collagen content[c]	46.03	13.36	39.31	4.25
Labile collagen, %	3.17	1.31	4.01	1.52
Epimysial				
Warner-Bratzler shear, kg./cm.^{2}	48.53[a]	11.63	31.59[b]	11.75
Collagen content[c]	623.29	39.71	653.21	47.59
Labile collagen, %	0.84	0.82	1.17	0.95
Moisture gained, %[d]	145.18	17.84	156.54	19.72
Contraction, %[e]	48.55	7.80	47.18	7.57

[a,b] Means between tough and tender muscles differ significantly ($P < 0.05$).
[c] Mg./gram dry tissue.
[d] (Cooked weight/fresh weight) × 100.
[e] (Initial length minus heated length/initial length) × 100.

Table II. Characteristics of Intramuscular and Epimysial Connective Tissue from the *M. longissimus* and *M. biceps femoris* of Aged Cows

	L (N = 22)		BF (N = 22)	
	Mean	S.D.	Mean	S.D.
Warner-Bratzler shear, kg./cm.^{2}	4.78[a]	2.04	9.51[b]	2.70
Intramuscular				
Collagen content[c]	18.67[a]	4.41	42.67[b]	10.27
Labile collagen, %	5.94[a]	1.52	3.59[b]	1.44
Epimysial				
Warner-Bratzler shear, kg./cm.^{2}	17.74	8.17	40.06[b]	12.08
Collagen content[c]	707.14[a]	47.31	638.25[b]	48.41
Labile collagen, %	2.01[a]	1.64	1.00[b]	0.88
Moisture gained, %[d]	178.36[a]	26.37	150.86[b]	19.25
Contraction, %[e]	46.55	7.48	47.87	7.53

[a,b] Means between L and BF muscles differ significantly ($P < 0.05$).
[c] Mg./gram dry tissue.
[d] (Cooked weight/fresh weight) × 100.
[e] (Initial length minus heated length/initial length) × 100.

of fresh length, and Warner-Bratzler shear values were determined on fresh epimysial samples from the L and BF muscles. The samples, which weighed approximately 2 grams and were 3.8 cm. long, were heated 2 hours at 71° C. in 25 ml. of 0.9% NaCl. The epimysial tissue was chilled at 4° C. for 12 to 14 hours in the cooking fluid before weight, contracted length, and shear values were determined.

Warner-Bratzler shear values were obtained on cross-sectional areas of longitudinal sections approximately 0.6 cm.^{2} in area. Cross-sectional areas slightly less than 0.6 cm.^{2} were made whenever the 23-kg. capacity of the Warner-Bratzler shear was exceeded. All cooked and chilled epimysial tissue was cut to the desired cross-sectional area with a scalpel. Care was taken to cut parallel to epimysial tissue fibers. Cross-sectional areas were obtained by pressing the sheared epimysial tissue on an ink pad and making an imprint on graph paper marked off in areas of 0.06 cm^2. Six shear readings were obtained for epimysial tissue from each muscle of each animal. Coefficients of variation for shear values within muscles and animals ranged from 5 to 10%. Shear values were also recorded for some epimysial tissue which was heated for 1 or 4 hours at 71° C. in order to demonstrate the effect of heating time on shear values of the epimysium.

Epimysial and intramuscular connective tissue from 10 Yorkshire pigs approximately 5 months old was used to study the effect of heating temperature on yield of labile collagen. The tissue was heated at 60 or 80° C. for 1 hour.

RESULTS AND DISCUSSION

Means and standard deviations for some characteristics of intramuscular and epimysial connective tissue from tough and tender L and BF muscles of "E" maturity Cutter and Canner cows are presented in Table I. Warner-Bratzler shear values for the cooked muscle were obtained on 1.27-cm. diameter cores but are expressed here as kg. per cm.^{2} area so that the values obtained can be compared directly with those obtained on the cooked epimysial tissues. Of the 40 cooked L muscles from "E" maturity cows, only 3 had average shear values of less than 2.8 kg. per cm.^{2} core. Muscles that have an average shear force value of 2.8 kg. or less per cm.^{2} are acceptable in tenderness according to Field *et al.* (1967). None of the BF muscles had shear values which even approached this low figure (Table I). Shear values of the cooked epimysial tissues were higher ($P < 0.05$) on the tough L and BF muscles than on the tender muscles. The high standard deviations show that there was a great deal of variability in epimysial shear force values within tough and tender muscles. However, standard deviations for the other variables in Table I were also high indicating that biological variability in connective tissue did exist within tough and tender muscles.

The collagen content of muscle or of epimysial tissue did not vary significantly in tough *vs.* tender muscles. However, the yield of labile collagen was higher ($P < 0.05$) in the tender L muscles than in the tough L muscles for both intramuscular and epimysial tissue.

Moisture gained during heating of the fresh epimysial tissue was not significantly different between tough and tender muscles but percent contraction of the epimysium was higher ($P < 0.05$) for tough than for tender L muscles (Table I).

In Table II characteristics of intramuscular and epimysial connective tissue from L and BF muscles of aged cows are compared. Shear values of muscle and of epimysial tissue were approximately twice as high in the BF as they were in the L. Shear values for epimysial tissue in Tables I and II were obtained after cooking 2 hours at 71° C. Epimysial tissue was first heated 1 hour at 71° C. so the shear data would be more comparable to the yield of labile collagen obtained after 1 hour. However, after the 1-hour heating period many BF epimysial tissues of only 0.3 cm.^{2} in cross-sectional area sheared over 23 kg. (capacity of the shear), so the heating time was increased in order to decrease the shear values. Increasing the heating time from 1 to 2 hours decreased the shear values for 12 BF epimysial samples 13.9 kg. per cm.^{2} area.

Five epimysial samples from old cows sheared 39.6 kg. per cm.^{2} after 2 hours heating but were reduced to 26.3 kg. per cm.^{2} after 4 hours at 71° C. The L epimysial tissue

Table III. Simple Correlations between Warner-Bratzler Shear Values and Characteristics of Connective Tissue within Muscles of Aged Cows

	Shear of Muscle		Shear of Epimysium	
	L	BF	L	BF
Intramuscular				
Labile collagen, %	−0.37	−0.34	−0.41	−0.52
Epimysial				
Labile collagen, %	−0.39	−0.24	−0.69	−0.74
Moisture gained, %[a]	0.05	−0.34	−0.52	−0.56
Contraction, %[b]	0.40	0.27	0.66	0.57

[a] (Cooked weight/fresh weight) × 100.
[b] (Initial length minus heated length/initial length) × 100. $r = 0.41$ for $P < 0.05$, $r = 0.53$ for $P < 0.01$.

Table IV. Effect of Temperature and Connective Tissue Source on Yield of Labile Collagen from Porcine *M. longissimus*

	Heating Temperature			
	60° C. ($N = 5$)		80° C. ($N = 5$)	
	Mean	S.D.	Mean	S.D.
Tissue source				
Epimysium				
Collagen content[c]	632.50	63.80	625.00	55.97
Labile collagen, %	34.68[a]	4.45	54.33[b]	6.08
Intramuscular				
Collagen content[c]	...	...	12.18	0.58
Labile collagen, %	...	...	13.10	2.17

[a,b] Means between 60 and 80° C. differ significantly ($P < 0.05$).
[c] Mg./gram dry tissue.

from five yearling bulls heated for 1 hour at 71° C. had average shear values of 2.2 kg. per cm.^{2} area compared to 17.7 kg. per cm.^{2} for tissue from aged cows (Table II). Thus, youthful epimysial tissue yielded 21.66% of labile collagen compared to 2.01% of labile collagen for L tissue from the aged cows. Yield of labile collagen was significantly higher in the L intramuscular and epimysial tissue than in the corresponding tissues from the BF (Table II). The L epimysium also absorbed more water during heating than the BF epimysium.

Simple correlations between shear values of muscle and characteristics of connective tissue within L and BF muscles given in Table III are not significant ($P < 0.05$). Nevertheless, correlations between shear values for the L muscle and percent labile collagen or percent contraction approached significance ($r = -0.39$ and 0.40, respectively). All correlations listed between shear of the L or BF epimysium and connective tissue characteristics are significant. Percent labile collagen in the BF or L epimysium accounts for approximately 50% of the variation in shear values of these tissues. Greater amounts of epimysial contraction are associated with higher epimysial shear values ($r = 0.66$ and 0.57 for L and BF, respectively).

Correlations not shown in the tables between percent labile collagen and percent contraction were −0.83 and −0.71 for the L and BF epimysial tissue, respectively. Therefore, as amount of contraction increased the tissue yielded less labile collagen, and these conditions were associated with higher shear values in epimysial tissue from "E" maturity cows. Simple correlations ($n = 22$) between percent intramuscular labile collagen and percent epimysial labile collagen were 0.41 and 0.43 in the L and BF muscles, respectively.

Correlations between the characteristics of L and BF muscles from the same cows ($N = 12$) indicated that the percent of labile collagen in the L epimysium was closely associated with labile collagen in the BF epimysium ($r = 0.93$). Percent contraction of L and BF epimysial tissue was also closely related ($r = 0.84$). This indicated that epimysial tissue from different muscles ages in the same manner. However, it appears that BF epimysial collagen may be more highly cross-linked than L epimysial collagen, since the yield of labile collagen is lower in the BF (Table II). The correlation for labile intramuscular collagen from the L *vs.* the BF muscles was also significant ($r = 0.58$).

Effect of heating temperature on yield of labile collagen from the L epimysium of 5-month old pigs is shown in Table IV. As temperature was increased from 60 to 80° C. the amount of labile collagen in the epimysium increased from 34.68 to 54.33%, respectively. At 80° C. yields of labile collagen from intramuscular connective tissue were 13.1% compared to 54.3% from epimysial tissue. Therefore, epimysial tissue may be less highly cross-linked than intramuscular connective tissue in 5-month old pigs. However, the data for "E" maturity cows showed the opposite effect. Intramuscular bovine collagen was 5.94% soluble and epimysial bovine collagen was 2.01% soluble in old cows (Table II). Schaub (1963) indicated that from the third to the twentieth month of age, rat muscle has a fairly constant amount of collagen. Then an increase begins, and in very old animals the amount of collagen is about double that found in the muscles of younger adults. Verzar (1964) stated that in 30- to 40-month old rats, there is 3 to 5% labile collagen in muscle connective tissue, while less than 1% of the collagen is labile in the corium. He explained the difference by stating that in old animals new collagen is being formed at a faster rate in muscle than in the corium. Perhaps this is also the case for intramuscular collagen when compared to epimysial collagen of aged cows.

Labile epimysial collagen appears to follow the same pattern as corium collagen. It is very labile in the young but practically insoluble in the old animals. The yield of labile epimysial collagen for 5-month old pigs, yearling bulls and aged cows was 43.88, 21.66, and 2.34%, respectively, after 1 hour heating at 71° C.

The data in this study show that in young animals the yield of labile collagen is relatively high and under these conditions shear force readings on epimysial tissue are low. Yields of labile collagen can be increased by higher cooking temperatures. Therefore, in muscle from young animals where high internal temperatures are reached, connective tissue would not be expected to offer any appreciable resistance to the shear. As a result, correlations between shear and yield of labile collagen in young animals would be expected to be low. The work of Smith *et al.* (1968) supports this conclusion. They found little relationship between labile collagen and tenderness in lamb muscle that was heated at 77° C. for 65 minutes.

The conditions in this study of 1 hour at 71° C. for epimysial tissue allowed enough variability in labile collagen to create high correlations with shear of epimysium from aged cows. These high correlations would not be expected with shear of cooked muscle since many factors in addition to connective tissue have been implicated in tenderness (Bailey, 1964). Correlation coefficients of −0.37 and −0.34 between shear of muscle and yield of labile collagen from intramuscular connective tissue approached significance (Table III). Since shear force of epimysial tissue decreased as yield of labile collagen increased, it seems safe to conclude that within limits labile collagen is related to tenderness. However, labile collagen yields in the range of 20 to 50% were characterized by very low epimysial shear force values indicating that within this range increased yields of labile collagen have little relationship to tenderness.

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