

Structural Changes in Intramuscular Connective Tissue During the Fattening of Japanese Black Cattle: Effect of Marbling on Beef Tenderization¹

T. Nishimura², A. Hattori, and K. Takahashi

Meat Science Laboratory, Department of Animal Science, Faculty of Agriculture,
Hokkaido University, Sapporo 060, Japan

ABSTRACT: We investigated changes in structures and mechanical properties of the intramuscular connective tissue during the fattening of Japanese Black steers, using the cell maceration method for scanning electron microscopy. During the early fattening period, from 9 to 20 mo of age, collagen fibrils of the endomysium in longissimus muscle associated more closely with each other, and collagen fibers in the perimysium increased in thickness and their wavy pattern became more regular. These changes were closely related to the increase in mechanical strength of the intramuscular connective tissue and resulted in a toughening of the beef during the period. The shear force value of longissimus muscle decreased after 20 mo of age, concomitantly with the rapid increase in the crude fat content. Scanning electron micrographs

of the longissimus muscle dissected from 32-mo-old steers clearly showed that the adipose tissues were formed between muscle fiber bundles, that the honeycomb structure of endomysia was partially broken, and that the perimysium separated into thinner collagen fibers. In semitendinosus muscle, in which the crude fat content was lower ($P < .05$) than that in longissimus muscle, the structure of the intramuscular connective tissue remained rigid at 32 mo of age. The shear force value of the muscle increased even in the late fattening period, from 20 to 32 mo of age. Thus, the development of adipose tissues in longissimus muscle appears to disorganize the structure of the intramuscular connective tissue and contributes to tenderization of highly marbled beef from Japanese Black cattle during the late fattening period.

Key Words: Finishing, Fat, Connective Tissue, Tenderness, Beef, Meat Characteristics

©1999 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 1999. 77:93–104

Introduction

Texture is one of the most important factors in determining the eating quality of meat from the consumer's point of view (Dransfield et al., 1984). The intramuscular connective tissue plays a significant part in determining meat texture, and its mechanical stability increases with animal growth (Bailey and Light, 1989, McCormick, 1994). Collagen in the intramuscular connective tissue becomes progressively tougher, more rigid and resistant, and less easily denatured (Shimokomaki et al., 1972; Tanzer, 1973).

The arrangement of collagen fibrils and fibers in the intramuscular connective tissue becomes more regular during development of bovine semitendinosus muscle (Nishimura et al., 1996). These changes in collagen and collagen fibrils could be related to the toughening of meat during growth. However, Avery et al. (1996) reported that the total collagen content or the nature of collagen intermolecular cross-links are unrelated to texture of longissimus lumborum in pigs of the same age. There must be some other factors that determine meat texture.

Marbling is one of the main factors used to determine beef quality grade in Japan (JMGA, 1988) and also one of factors used in the United States (USDA, 1989). Japanese Black cattle are characterized by a unique ability to deposit a large amount of intramuscular fat (Zembayashi et al., 1988; Cameron et al., 1994). The finishing of Japanese Black cattle typically begins at about 10 mo of age and continues for about 20 mo, and so the fattening is completed at about 30 mo of age. Most earlier studies have shown that marbling degree accounts for only 3 to 10% of the

¹We thank T. Ito of the Electron Microscope Laboratory, Faculty of Agriculture, Hokkaido University, for his technical assistance in taking electron micrographs and Y. Hongo of Hokkaido Prefectural Takikawa Animal Husbandry Exp. Sta. in the Sampling of bovine muscle.

²To whom correspondence should be addressed: phone: 81-11-706-3881; fax: 81-11-716-0879.

Received October 27, 1997.

Accepted August 21, 1998.

variation in texture of beef (Campion et al., 1975; Tatum et al., 1980). However, the crude fat content of the muscles used in these earlier studies was relatively low, and the age of cattle was younger than that of fattened Japanese Black cattle (Zembayashi et al., 1988; Zembayashi, 1994). Little is known about relationships between intramuscular fat and texture of beef in highly marbled beef from breeds such as Japanese Black cattle and about structural changes in the intramuscular connective tissue induced by fattening. We report here changes in structures and mechanical properties of the intramuscular connective tissue during fattening of beef cattle and the tenderization mechanism of highly marbled beef.

Materials and Methods

Thirty-two purebred and 19 crossbred Japanese Black steers, from 9 to 32 mo of age, were used. They were weaned at approximately 6 mo of age, placed on a growing diet until 9 mo of age, and given free access to a finishing diet (concentrate diet consisting of 30% flaked corn, 45% steam-rolled barley, 20% wheat bran, and 5% soybean meal, with corn silage and orchard-grass hay) during a fattening period up to 32 mo of age. The animals were slaughtered humanely at 9 ($n = 6$), 12 ($n = 6$), 14 ($n = 6$), 18 ($n = 6$), 20 ($n = 6$), 24 ($n = 7$), 30 ($n = 7$), and 32 mo ($n = 7$) of age, and dressed as normally practiced. Four purebred and two or three crossbred steers were slaughtered at each time. Semitendinosus muscle and a portion of longissimus muscle (between the 7th and 12th ribs) were dissected from the right side of carcasses at 48 h postmortem and were stored at 4°C until sampled.

Shear Force Value of Raw and Cooked Beef. The shear-force value of raw and cooked beef was measured according to the method of Nishimura et al. (1998). Samples (10 × 10 × 20 mm) for shear force of raw beef were cut from longissimus and semitendinosus muscles at 72 h postmortem, and subsequently their shear force was determined. Samples (25 × 25 × 40 mm) for shear force of cooked beef were cut from these muscles at 72 h postmortem and frozen at -80°C, before thawing and cooking for shear force measurement. Frozen samples were thawed at 2°C for 24 h, wrapped in polyethylene bags, and heated for 60 min in a water bath maintained at 80°C. After heating, each sample was cooled to room temperature and cut into smaller pieces (10 × 10 × 20 mm). The peak shear force value perpendicular to the axis of muscle fibers was measured for raw and cooked samples (10 samples of each) using a rheometer (NRM-2002J, Fudo Industry Co., Tokyo, Japan), with a straight-edged blade (.35-mm thickness) and a crosshead speed of 6 cm/min.

Crude Fat Content of Muscle. Duplicate tissue samples (2 g) from each muscle were weighed and dried at 105°C for 12 h. The dried samples were then

extracted for 16 h using ether in a Soxhlet extractor. The extracted samples were dried and weighed to determine the amount of crude fat, which was expressed as a percentage of wet weight of the muscle.

Total Amount and Heat Solubility of Collagen. Total amount of collagen was determined with the following procedures. Frozen powdered samples (100 mg) of longissimus and semitendinosus muscles were hydrolyzed in 6 N HCl for 24 h at 110°C. After removing the HCl, the amount of hydroxyproline was determined with the procedure of Bergman and Loxley (1963). The hydroxyproline content was converted to collagen content with a factor of 7.25 (Goll et al., 1963). Heat solubility of collagen was determined by Hill's method (1966) with slight modifications. Two grams of the sample was homogenized for 1 min at 10,000 rpm with a fourfold volume of ¼-strength Ringer's solution containing .86% NaCl, .03% KCl, and .033% CaCl₂ using a homogenizer (Virtis Co., Gardiner, NY). The resulting homogenate was heated for 70 min at 77°C and then centrifuged for 30 min at 1,000 × *g*. The supernatant solution was decanted, and the pellet was suspended in the same solution and recentrifuged. The supernatant solutions were combined, and the amount of collagen was determined as described above. The amount of heat-soluble collagen was expressed as a percentage of the total amount of collagen.

Procedure of Intramuscular Connective Tissue Preparation. The intramuscular connective tissue preparation (IMCT preparation) was prepared with our method (Nishimura et al., 1996) with slight modifications. Small pieces of muscle (10 × 10 × 15 mm) were dissected from the midregion of longissimus and semitendinosus muscles 72 h postmortem. They were fixed for 3 d with 2.5% glutaraldehyde in a .1 M phosphate buffer solution, pH 7.3, immersed in a 10% aqueous solution of sodium hydroxide for 4 d, and then rinsed in distilled water for 5 d at room temperature and water changed twice daily. They were treated with ethanol to remove triacylglyceride and then rinsed in distilled water. The samples were immersed for 3 h in an acrylamide solution containing 7.5% acrylamide and 1.5 mg/mL ammonium persulfate at room temperature. Immediately after the incubation, the acrylamide solution was polymerized completely by the addition of N,N,N',N'-tetramethylethylenediamine (.75 μL/mL).

Shear Force Value of IMCT Preparations. The shear force value of IMCT preparations embedded in acrylamide gels was measured according to the method of Nishimura et al. (1998) for 10 samples prepared from longissimus and semitendinosus muscles of each animal using a rheometer (NRM-2002J, Fudo, Tokyo, Japan) with a straight-edged blade (.35-mm thickness) and a crosshead speed of 6 cm/min.

Low-Vacuum Scanning Electron Microscopy. Small pieces of muscle (5 × 5 × 5 mm) were cut from longissimus muscles of 32-mo-old steers ($n = 7$) and wiped with filter paper. The intact muscle tissues

were observed under a low-vacuum scanning electron microscope (JSM-5310LV, Japan Electric, Tokyo, Japan) with an accelerating voltage of 15 kV.

Cell Maceration/Scanning Electron Microscopy. According to the cell maceration method of Ohtani et al. (1988), small pieces of muscle ($10 \times 10 \times 15$ mm) were cut from longissimus and semitendinosus muscles 72 h postmortem. They were fixed for 3 d with 2.5% glutaraldehyde in a .1 M phosphate buffer solution, pH 7.3, immersed in a 10% aqueous solution of sodium hydroxide for 5 d, and then rinsed in distilled water for 5 d at room temperature with water changed twice daily. They were put in 1% tannic acid for 3 h, rinsed in distilled water for several hours, and postfixed in 1% osmium tetroxide for 1 h. The samples were dehydrated in a series of graded concentrations of ethanol, freeze-fractured with a razor blade in liquid nitrogen, and dried with the t-butyl alcohol freeze-drying method (Inoue and Osatake, 1988). The dried samples were coated with gold and observed under a scanning electron microscope (S-800, Hitachi, Tokyo) with an accelerating voltage of 10 kV.

Sensory Panel Assessment. For sensory panel evaluation, longissimus and semitendinosus muscles from all cattle after 20 mo of age ($n = 24$) were used. The beef samples were thawed and cooked in the same manner as for the shear force determination of cooked samples and served warm to seven trained panelists. Samples were evaluated for overall tenderness on an 8-point scale: 1 was extremely tough and 8 was extremely tender. The mean score for each sample was determined and taken as an indicator for sensory texture.

Statistical Analysis. Data were analyzed with the analysis of variance procedure of JMP (1994), using Tukey's multiple range test to detect differences between means. A simple correlation between the crude fat content and shear force value were calculated.

Results

Figure 1 shows changes in the shear force value of raw beef during the fattening period of Japanese Black steers. The shear force value of longissimus muscle increased during the early fattening period from 9 to 20 mo of age, and decreased thereafter during the late fattening period, until 32 mo of age. The shear force values of the muscle dissected from 20-mo-old steers was higher ($P < .05$) than those dissected from 9-mo-old steers and 32-mo-old steers. However, the shear force value of semitendinosus muscle increased rapidly ($P < .05$) from 9 to 24 mo of age and gradually thereafter. Figure 2 shows changes in the shear force value of cooked beef during the fattening period of Japanese Black steers. The shear force value of longissimus muscle increased ($P < .05$)

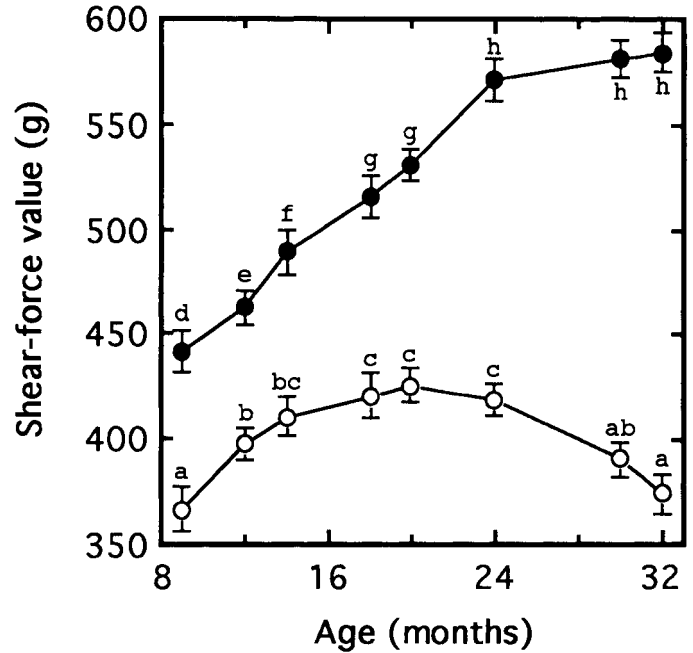


Figure 1. Changes in shear force value of raw beef during the fattening period of Japanese Black steers. The shear force value of longissimus and semitendinosus muscles perpendicular to the axis of muscle fibers was measured for 10 samples of each animal with a rheometer. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

from 9 to 20 mo of age and decreased thereafter up to 32 mo of age. The shear force value of the muscle dissected from 32-mo-old steers was lower ($P < .05$) than that from 20-mo-old steers. In contrast, the shear force value of semitendinosus muscle increased ($P < .05$) from 9 to 32 mo of age.

The crude fat content of longissimus muscle increased gradually up to 20 mo of age and rapidly thereafter (Figure 3). The crude fat content of the muscle dissected from 32-mo-old steers was about 18% of wet weight of the muscle, fourfold that from 20-mo-old steers ($P < .01$). The crude fat content of semitendinosus muscle increased ($P < .05$) gradually during the fattening period. The collagen content of longissimus muscle decreased ($P < .05$) from 12 to 32-mo of age (Figure 4). In semitendinosus muscle, the collagen content decreased gradually during the fattening period, although no significant difference was detected between 9 and 32-mo-old steers. The heat solubility of collagen in longissimus muscle decreased ($P < .05$) up to 24 mo of age and then increased gradually until 32 mo of age, although no significant difference was detected between 24- and 32-mo-old steers (Figure 5). In semitendinosus muscle, the heat solubility of collagen decreased ($P < .05$) with chronological age of cattle.

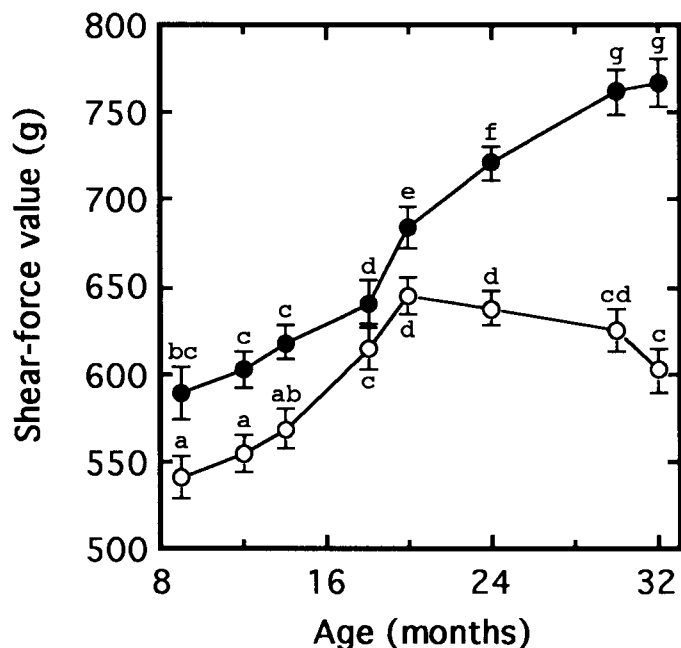


Figure 2. Changes in shear force value of cooked beef during the fattening period of Japanese Black steers. The shear force value of longissimus and semitendinosus muscles perpendicular to the axis of muscle fibers was measured for 10 samples of each animal with a rheometer. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

The mechanical strength of the intramuscular connective tissue was examined using the IMCT preparation, which is composed of collagen fibrils and fibers that maintained the organization of the endomysium and perimysium and is useful to measure the mechanical strength of the intramuscular connective tissue (Nishimura et al., 1996). The shear force value of the IMCT preparation from longissimus muscle increased ($P < .05$) up to 24 mo of age, and then decreased ($P < .05$) gradually until 32 mo of age (Figure 6). The shear force value of the IMCT preparation from semitendinosus muscle increased ($P < .05$) with age during the fattening period.

Because all samples taken from different animals of the same age were comparable, we here show typical electron micrographs of the muscle from a 9- (Figure 7), a 20- (Figure 8), and a 32-mo-old steer (Figures 9 and 10). Marbling was rarely visible on the cross section of longissimus muscle at the 7th rib of a 9-mo-old steer (Figure 7a). The intramuscular connective tissue was clearly observed by cell maceration/scanning electron microscopy; skeletal muscle fiber elements were completely removed, and collagen fibrils and fibers were exposed (Figure 7b to e). The endomysium displayed a honeycomb structure in

longissimus muscle (Figure 7b). A group of endomysial sheaths was surrounded by the perimysium, which consisted of several layers of sheets referred to as collagen fibers. The endomysial sheaths were membranous and constructed from a loose network of collagen fibrils (Figure 7c and d). The perimysium was constructed from collagen fibers of about 10 μm in diameter that displayed gently wavy patterns (Figure 7e). A small amount of marbling was visible on the cross section of the longissimus muscle at the 7th rib of a 20-mo-old steer (Figure 8a). The endomysium displayed a honeycomb structure (Figure 8b). Endomysial sheaths were tightly connected with adjacent ones (Figure 8c) and were membranous structures that consisted of a dense network of collagen fibrils (Figure 8d). The perimysium consisted of collagen fibers in a wavy pattern (Figure 8e).

A large amount of marbling was visible on the cross section of longissimus muscle at the 7th rib of a 32-mo-old steer (Figure 9a). To observe adipose tissues in the muscle closely, we examined intact (raw) longissimus muscle from 32-mo-old steers by low-vacuum scanning electron microscopy. Figure 9b shows a low-vacuum scanning electron micrograph of the same muscle as is shown in Figure 9a. The adipose tissues were formed between muscle fiber bundles (i.e., at the perimysium) and composed of several compartments of approximately 100 μm in diameter.

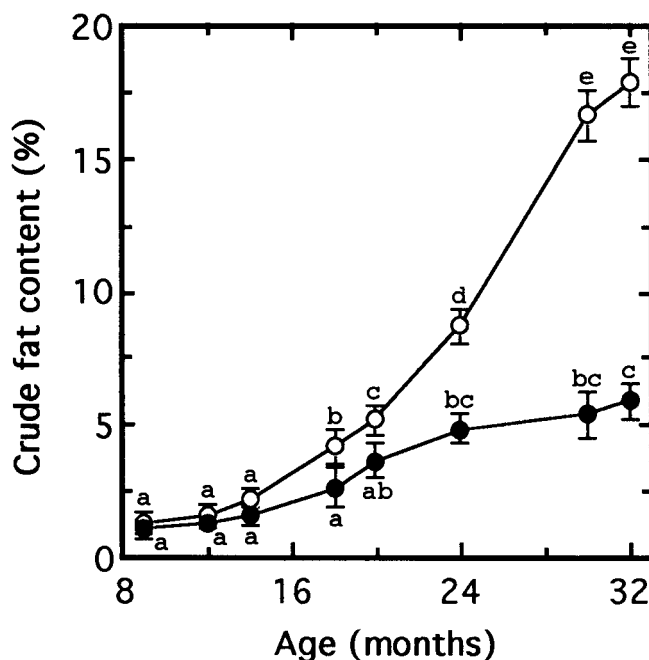


Figure 3. Changes in crude fat content of longissimus and semitendinosus muscles during the fattening period of Japanese Black steers. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

Gaps between muscle fibers and the intramuscular fat were caused by shrinkage of the muscle sample in the sample chamber of a low-vacuum scanning electron microscope. Figure 9c to f show scanning electron micrographs of the cell-macerated longissimus muscle, in which muscle fiber elements and fat globules were eliminated. The honeycomb structure of endomysia was partially broken (Figure 9c). Structures composed of several compartments were observed between groups of endomysia. Large gaps between the structure and the endomysia could be artifact resulting from the maceration of muscle fibers and(or) extraction of fat during sample preparation. Each compartment of the structure was membranous and approximately 100 μm in diameter (Figure 9d) and constructed from a loose network of collagen fibrils. The membranous structures would be connective tissue surrounding fat cells of approximately 100 μm in diameter, determined by comparison of scanning electron micrographs of cell-macerated muscles (Figure 9c) with low-vacuum scanning electron micrographs of intact ones (Figure 9b). Ribbonlike structures were observed between the endomysium and the connective tissue that surrounds fat cells (Figure 9e), and they were built up of collagen fibers of approximately 5 μm in thickness (Figure 9f). In semitendinosus muscle of a 32-mo-old steer, the honeycomb structure of endomysia was clearly observed (Figure

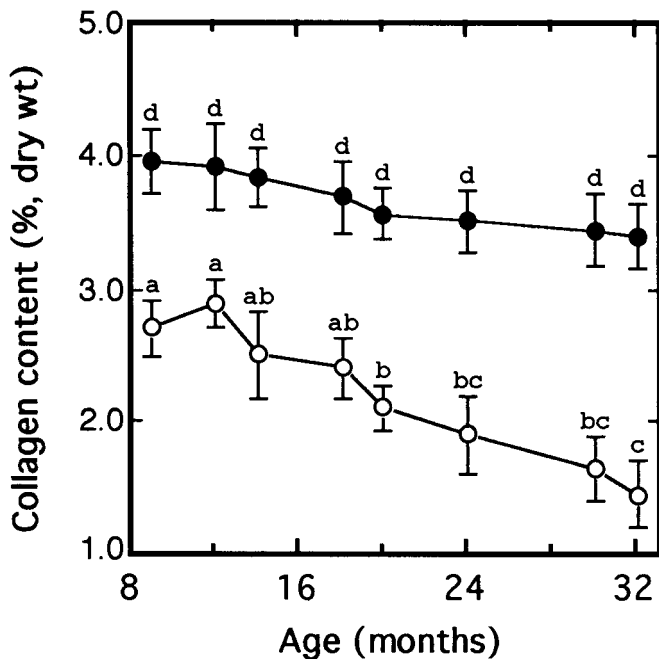


Figure 4. Changes in collagen content during the fattening period of Japanese Black steers. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

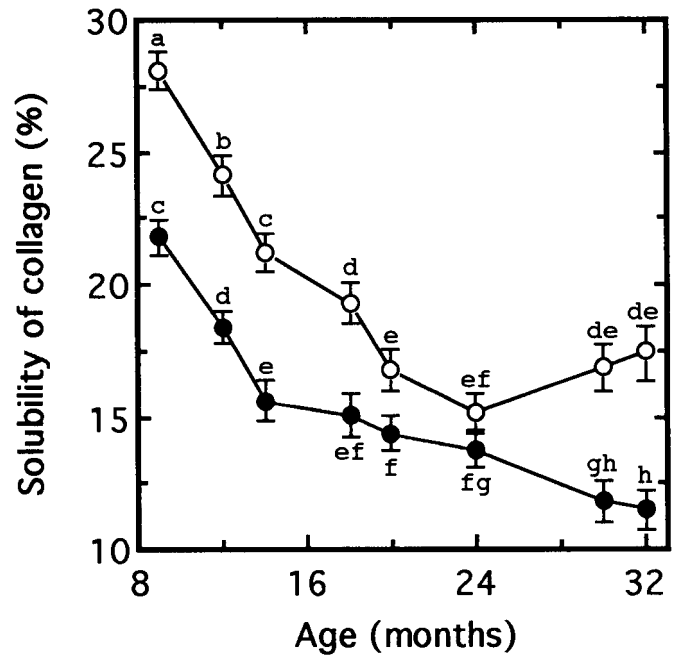


Figure 5. Changes in heat solubility of collagen during the fattening period of Japanese Black steers. The amount of heat-soluble collagen was expressed as a percentage of the total amount of collagen in muscle. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

10a). A group of endomysial sheaths was surrounded by the perimysium, which was composed of several layers of thick sheets. The endomysial sheaths were membranous and tightly connected with adjacent ones (Figure 10b). They consisted of a tightly woven network of collagen fibrils (Figure 10c). The perimysium consisted of collagen fibers in an extremely regular wavy pattern (Figure 10d).

Discussion

Reducible crosslinks of collagen are transformed into more stable nonreducible compounds with chronological age of cattle (Bailey and Shimokomaki, 1971; Robins et al., 1973; McCormick, 1994). This brings about a decrease in collagen solubility, resulting in tougher beef (Hill, 1966; Dikeman et al., 1971). The decrease in heat solubility of collagen in longissimus muscle from 9 to 24 mo of age (Figure 5) is certainly due to the increase in nonreducible cross-links of collagen (Shimokomaki et al., 1972), and it seems to be closely related to the increase in mechanical strength of the intramuscular connective tissue. The mechanical stability of the intramuscular connective tissue depends not only on intermolecular cross-links

of collagen but also on the size and arrangement of collagen fibrils (Rowe, 1981). We have shown that collagen fibrils in the endomysium of bovine semitendinosus muscle associate more closely with each other, collagen fibers in the perimysium of the muscle increase in thickness, and the wavy pattern of collagen fibers become more regular during growth of beef cattle (Nishimura et al., 1996). Structural changes in the intramuscular connective tissues of longissimus muscle during the early fattening period are similar to those of semitendinosus muscle. All of these changes bring about strengthening of the intramuscular connective tissue, and they may contribute to the increase in toughness of beef until 20 mo of age for longissimus muscle and 32 mo of age for semitendinosus muscle.

The shear force value of raw longissimus muscle decreased after 20 mo of age, concomitantly with the rapid increase in the crude fat content in the muscle. However, the shear force value of semitendinosus muscle, in which the content of the intramuscular fat was lower ($P < .01$) than that of longissimus muscle after 20 mo of age, increased linearly during the

fattening period up to 32 mo of age. We measured the shear force value of raw beef to investigate changes in mechanical strength of intact muscles without denaturing and shrinkage of the intramuscular collagen, from a physiological point of view. However, because meat is mostly eaten after cooking, we also measured the shear force value of cooked beef, in practical terms. The decrease in mechanical strength of cooked beef after 20 mo of age was also detectable in longissimus muscle, but not in semitendinosus muscle. There was a demonstrable relationship ($r = .627$) between the shear force values measured with our method (Nishimura et al., 1998) and the sensory panel scores (Figure 11). Thus, these results suggest that a large amount of deposited fat in muscle participates in the improvement of beef toughness during the late fattening period of Japanese Black cattle.

To observe where and how intramuscular fat is deposited in highly marbled beef, we examined intact longissimus muscles from 32-mo-old steers by low-vacuum scanning electron microscopy. The intramuscular fat was deposited mainly between bundles of muscle fibers, within the perimysium. Furthermore, we observed the cell-macerated longissimus muscle of a 32-mo-old steer under a scanning electron microscope to determine the effect of deposited fat on the structure of the intramuscular connective tissue. Typical structures of the perimysium as shown in longissimus muscle of a 20-mo-old steer and in semitendinosus muscle of a 32-mo-old steer were not observed in longissimus muscle of a 32-mo-old steer. The ribbonlike structure observed between the endomysium and the connective tissue surrounding fat cells could be the disorganized perimysium caused by the development of fat cells. The disorganization of the perimysium seems to cause the partial breakdown of the honeycomb structure of endomysia, which are bundled by the perimysium. These structural changes in the intramuscular connective tissue of longissimus muscle are consistent with a decrease in mechanical strength of the intramuscular connective tissue of the muscle during the late fattening period of Japanese Black cattle. In contrast, in semitendinosus muscle of a 32-mo-old steer, such structural changes as observed in longissimus muscle were not detected. The mechanical strength of the intramuscular connective tissue increased continuously up to 32 mo of age. These results suggest that highly developed adipose tissues in longissimus muscle disorganize the structure of the intramuscular connective tissue and bring about a weakening of the intramuscular connective tissue, contributing to tenderization of highly marbled beef during the late fattening period. A smaller degree of marbling (less than 8% of crude fat content) is also observed in longissimus muscle of steers before 24 mo of age and in semitendinosus muscle of 32-mo-old steers. However, the structures of the intramuscular

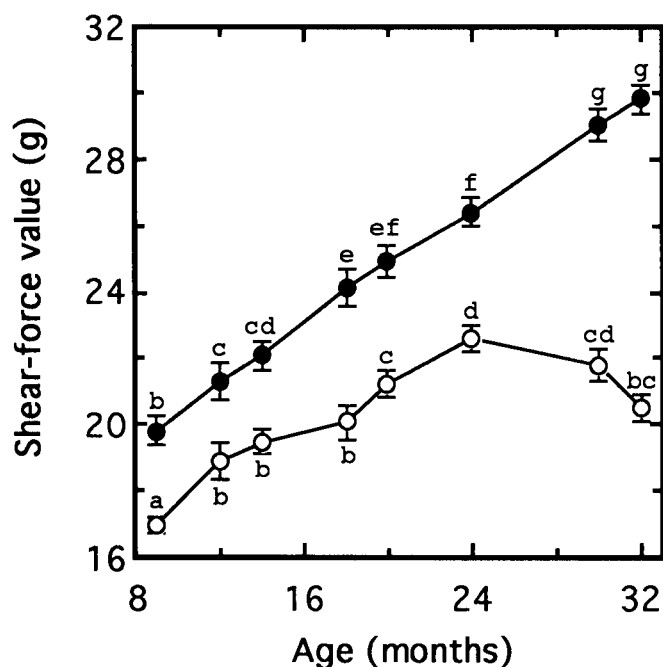


Figure 6. Changes in mechanical strength of the intramuscular connective tissue during the fattening period of Japanese Black steers. The shear force value of the intramuscular connective tissue preparation perpendicular to the axis of muscle fibers was measured for 10 samples of each animal with a rheometer. Open and closed symbols represent the mean value of longissimus and semitendinosus muscle, respectively, from six to seven animals, and the error bars show the standard error. Means without common superscript letters differ ($P < .05$).

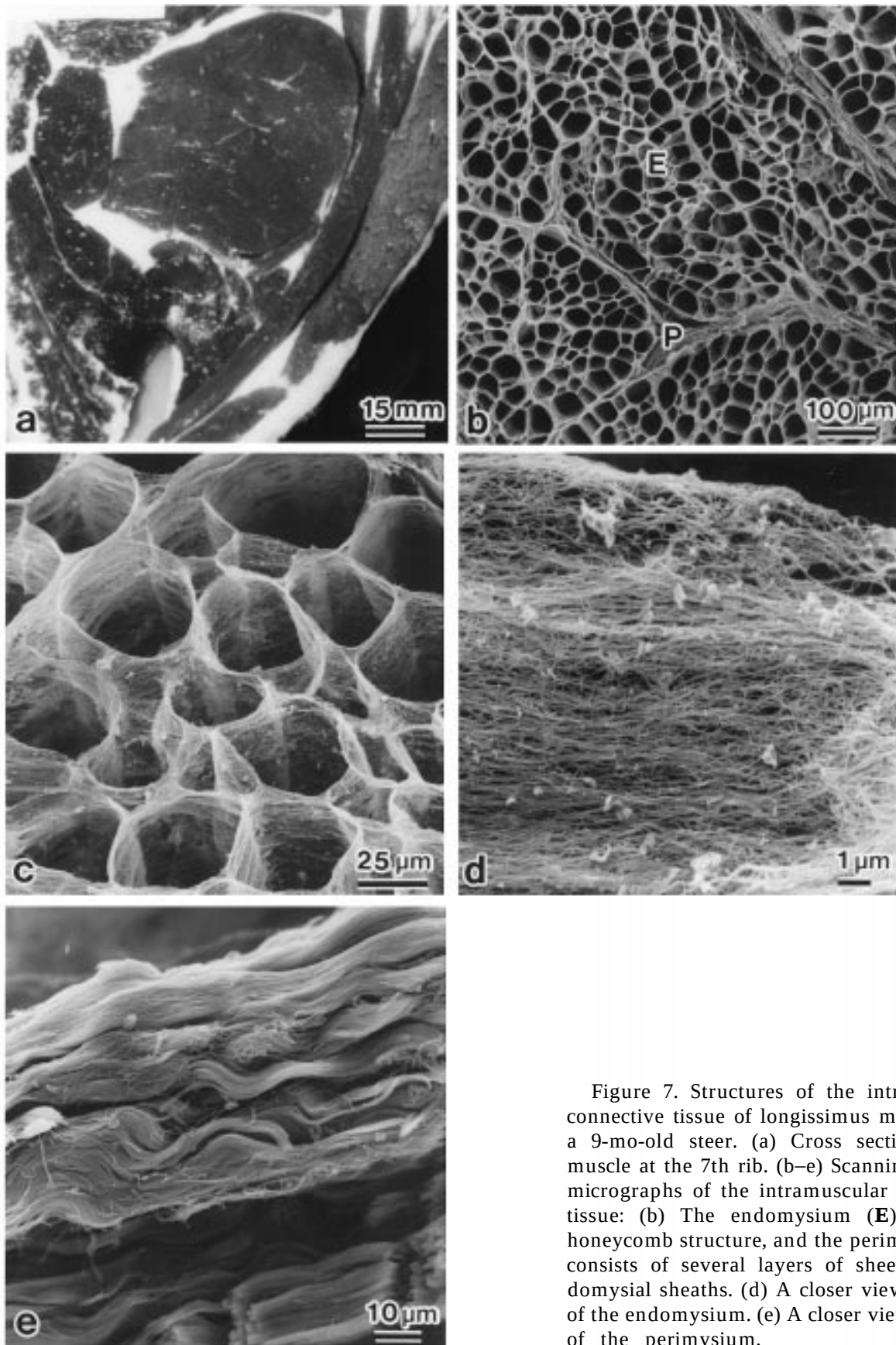


Figure 7. Structures of the intramuscular connective tissue of longissimus muscle from a 9-mo-old steer. (a) Cross section of the muscle at the 7th rib. (b–e) Scanning electron micrographs of the intramuscular connective tissue: (b) The endomysium (**E**) shows a honeycomb structure, and the perimysium (**P**) consists of several layers of sheets. (c) Endomysial sheaths. (d) A closer view of a part of the endomysium. (e) A closer view of a part of the perimysium.

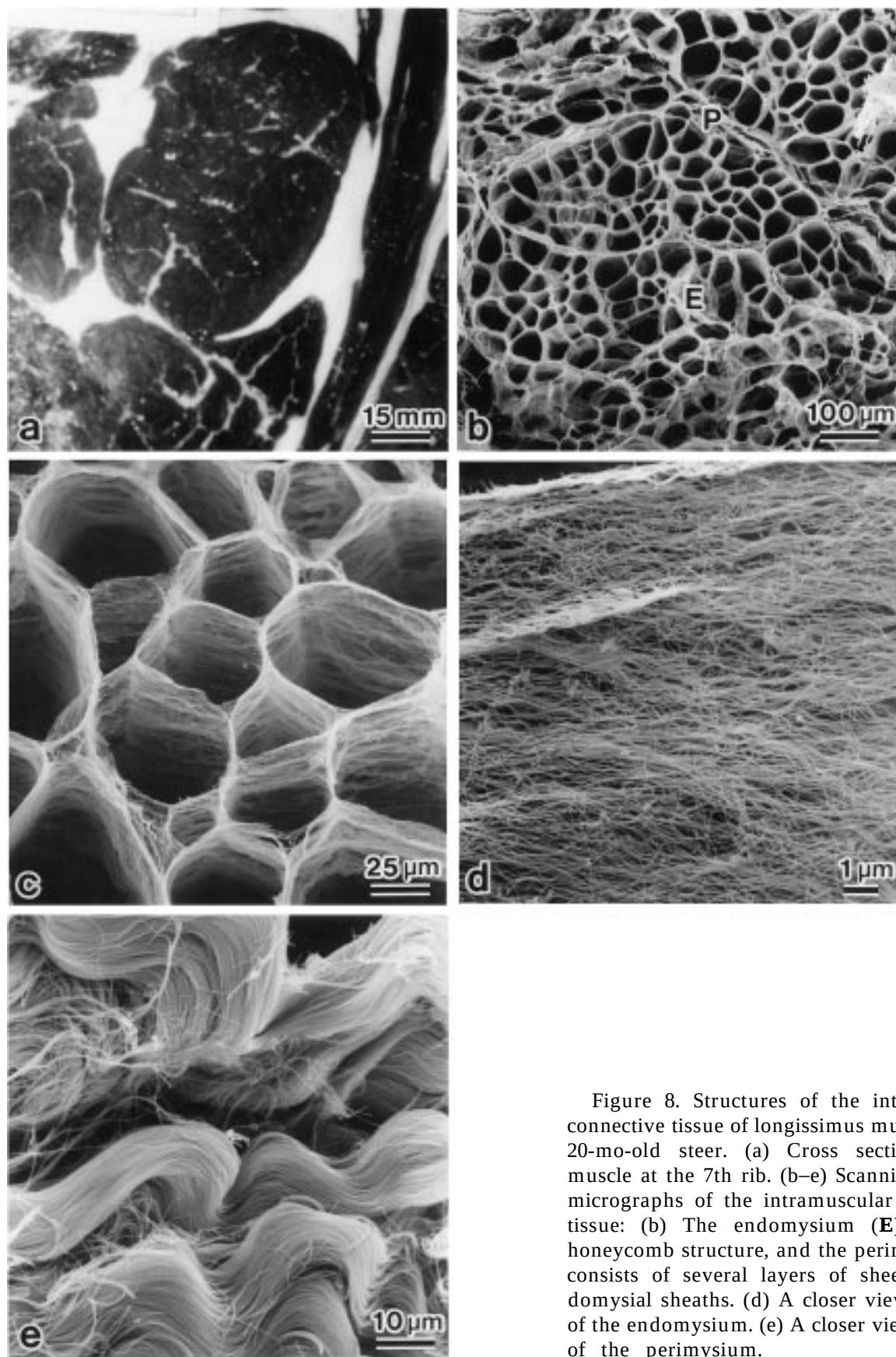


Figure 8. Structures of the intramuscular connective tissue of longissimus muscle from a 20-mo-old steer. (a) Cross section of the muscle at the 7th rib. (b–e) Scanning electron micrographs of the intramuscular connective tissue: (b) The endomysium (**E**) shows a honeycomb structure, and the perimysium (**P**) consists of several layers of sheets. (c) Endomysial sheaths. (d) A closer view of a part of the endomysium. (e) A closer view of a part of the perimysium.

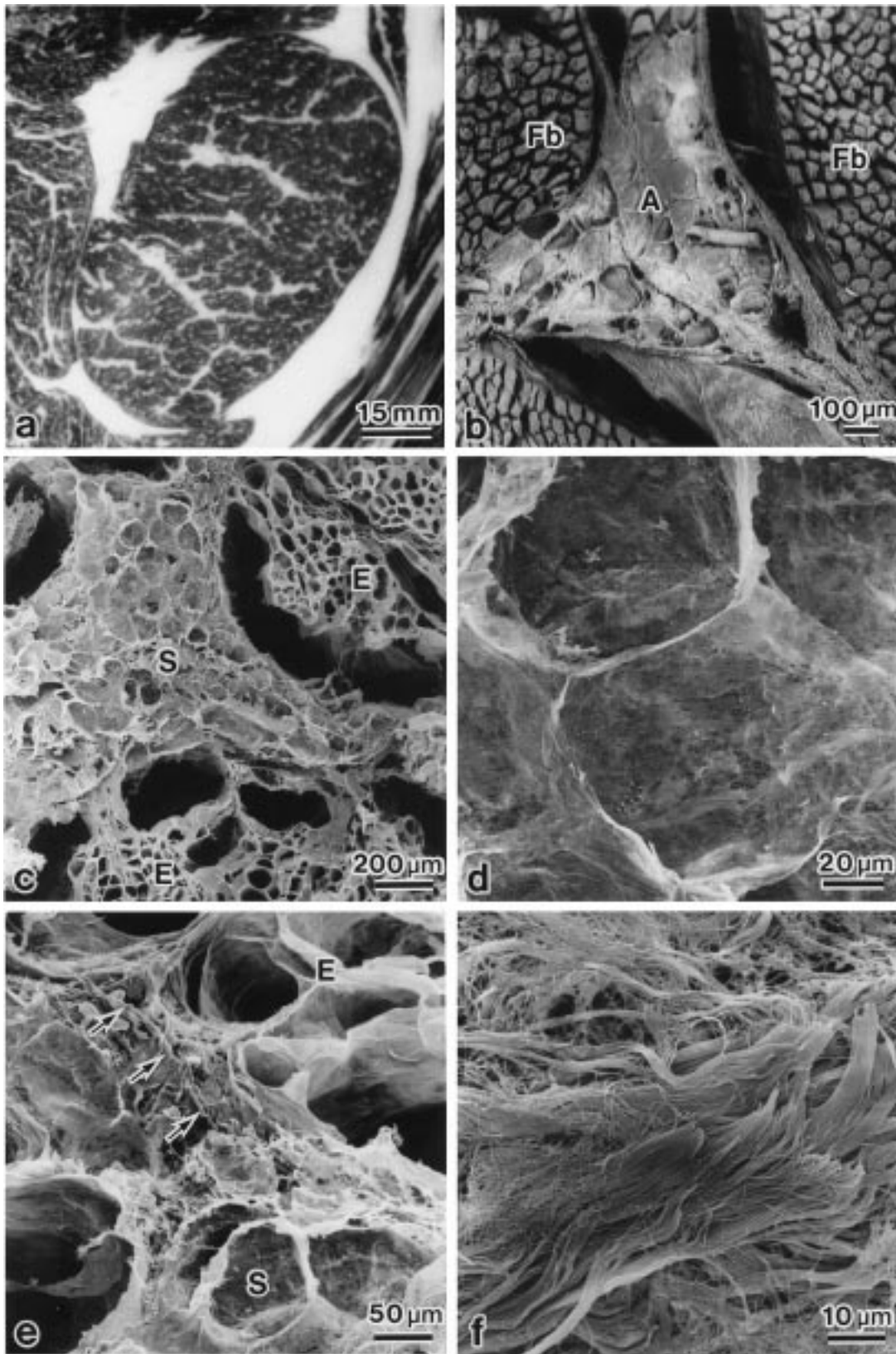


Figure 9. Structures of the intramuscular connective tissue of longissimus muscle from a 32-mo-old steer. (a) Cross section of the muscle at the 7th rib. (b) A low-vacuum scanning electron micrograph of the muscle. The adipose tissue (**A**) composed of several compartments of approximately 100 μ m in diameter is observed between muscle fiber bundles (**Fb**). (c–f) Scanning electron micrographs of the intramuscular connective tissue: (c) Large gaps and structures (**S**) composed of several compartments are observable between endomysia (**E**). (d) A closer view of the compartment. (e) Ribbon like structures (arrows) between endomysia (**E**) and the structure (**S**). (f) A closer view of a part of the ribbon like structures.

connective tissue do not disintegrate so markedly as to induce the decrease in the mechanical strength of the intramuscular connective tissue.

The mechanical strength of semitendinosus muscle increases through the fattening period, whereas total collagen content in the muscle does not increase. In longissimus muscle, the mechanical strength of raw and cooked muscle increases during the early fattening period up to 20 mo of age, whereas total collagen content in the muscle decreases during the period. These results suggest that changes in total collagen content with age do not directly contribute to tenderization. However, the decrease in total collagen content after 20 mo of age may participate in tenderization. The slight increase in heat solubility of collagen after 24 mo of age may be due to a relative increase in

reducible cross-links of collagen, newly synthesized to surround fat cells, and may also participate in decrease in mechanical strength of the muscle during the late fattening period. Tenderization of highly marbled beef in the late fattening period seems to be caused by not only intramuscular fat content, but also by other factors (i.e., collagen content, nature of collagen cross-links, and diameter of collagen fibrils), that relate to texture of meat (Dransfield, 1977; Light et al., 1985; Bailey and Light, 1989). However, Avery et al. (1996) reported that the total collagen content or the nature of collagen intermolecular cross-links are unrelated to texture of longissimus lumborum in pigs of the same age. They also pointed out the overestimation for collagen content in meat obtained with the colorimetric method. Further investigations

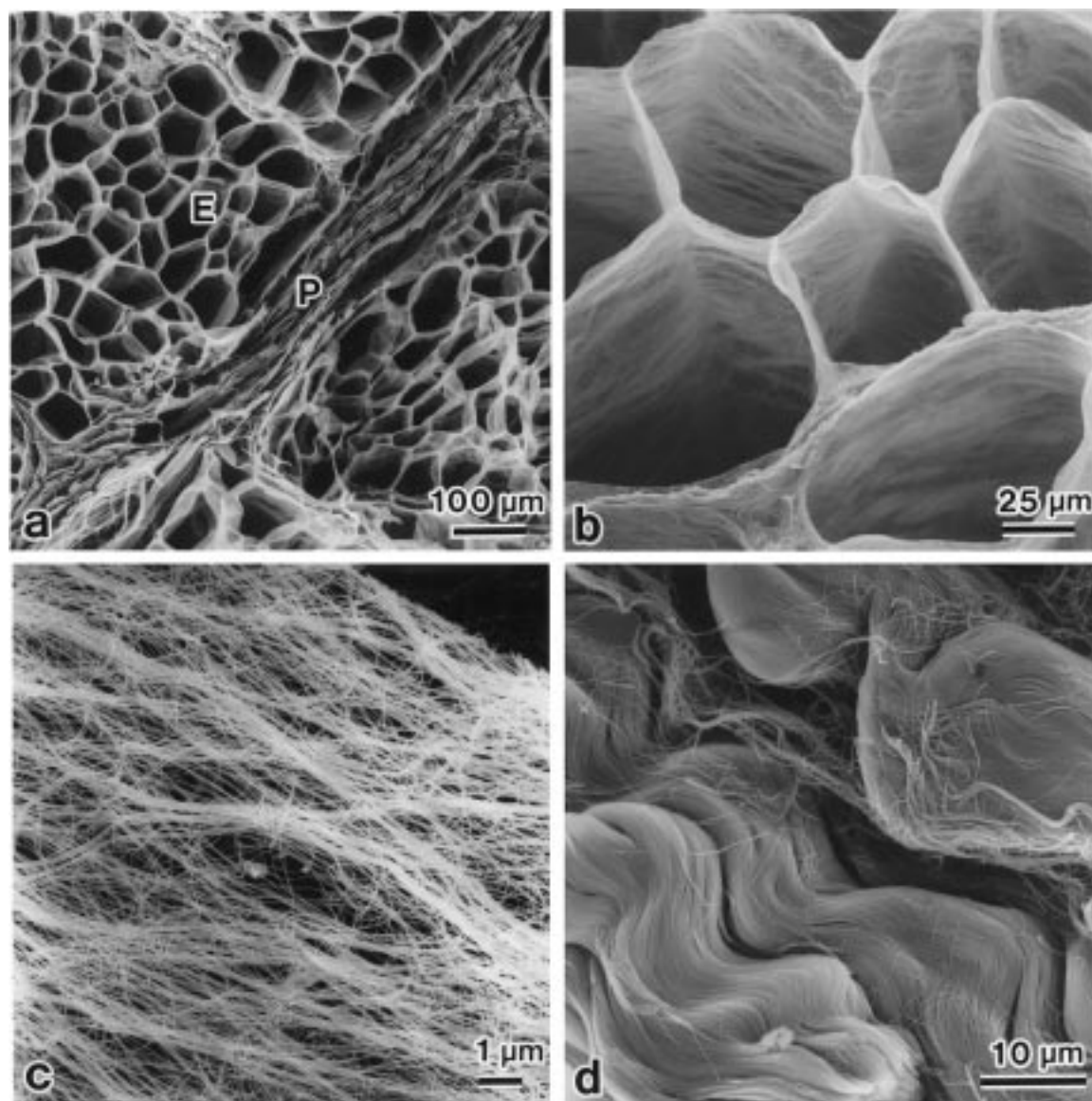


Figure 10. Structures of the intramuscular connective tissue of semitendinosus muscle from a 32-mo-old steer. (a) The endomysium (**E**) shows a honeycomb structure, and the perimysium (**P**) consists of several layers of sheets. (b) Endomysial sheaths. (c) A closer view of a part of the endomysium. (d) A closer view of a part of the perimysium.

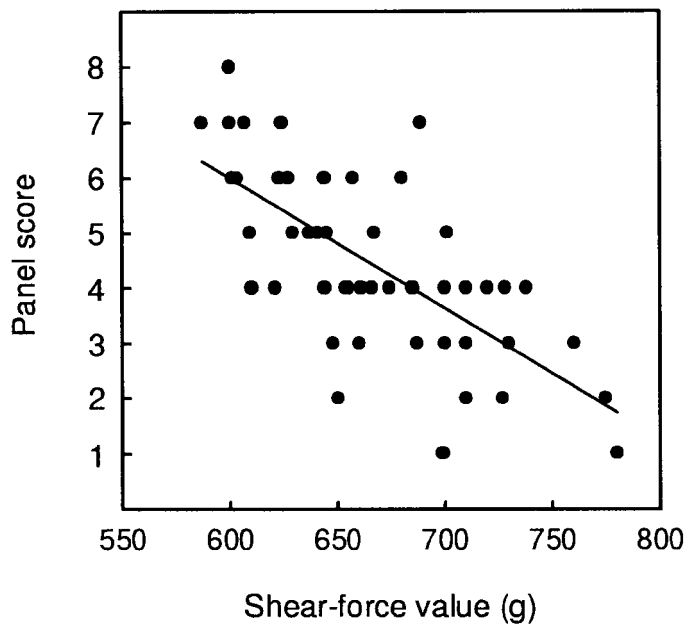


Figure 11. Relationship between the taste panel scores and the shear force value of cooked beef from cattle after 20 mo of age ($r = .627$); 1 was extremely tough and 8 was extremely tender.

are needed to clarify changes in collagen content and cross-links of collagen during the fattening period of cattle and its effects on beef texture using a more accurate method, such as the HPLC method proposed by Avery et al. (1996).

Most of the earlier studies on the effect that marbling has on meat tenderness have demonstrated small positive relationships between marbling degree and sensory tenderness, and a small inverse relationship with shear force value of cooked beef (Pearson, 1966; Smith et al., 1988). Marbling degree accounts for only 3 to 10% of the variation in sensory tenderness of beef (Campion et al., 1975; Crouse et al., 1978; Tatum et al., 1980). However, May et al. (1992) have shown that marbling score is moderately related to sensory panel tenderness ($r = .51$) and shear force ($r = -.61$) in Angus $\times$ Hereford steers, which are known for a relatively high ability to marble. Japanese Black cattle are characterized by the ability to deposit very large amounts of marbling (Lunt et al., 1992; Zembayashi, 1994). The crude fat content of longissimus muscle from steers after 24 mo of age is much greater (8.7 to 20.3% of crude fat) than that used in the earlier studies. A simple correlation coefficient between the crude fat content and the shear force value of raw longissimus muscle was small ($r = .18$) for all steers used in this study ($n = 51$, 9 to 32 mo of age), but high and inverse ($r = -.76$) for those after 20 mo of age ($n = 26$). It seems likely that a higher level of marbling (above 8% of intramuscular fat) is closely related to the tenderness of beef from Japanese Black cattle. Further studies of Japanese

Black cattle are needed to elucidate the relationship between marbling and tenderness of beef. It is well-known that the degree of marbling depends on the breed of cattle (Zembayashi et al., 1995; Herring et al., 1996), the sire (Vieselmeyer et al., 1996), and the finishing system (Zembayashi, 1994; Allen et al., 1996). The effects of these factors on the properties of the intramuscular connective tissue will be the subjects of further investigations by the authors.

Implications

As cattle mature, the mechanical strength of the intramuscular connective tissue increases, resulting in tougher beef. However, in highly marbled beef from well-fattened Japanese Black cattle, the adipose tissue became highly developed between muscle fiber bundles during the late fattening period. The developed adipose tissue weakened structures of the intramuscular connective tissue and contributed to tenderization of beef. This tenderization effect would be applicable only to some breeds of cattle that are capable of depositing large amounts of intramuscular fat: no less than 8%, which corresponds to a moderate marbling score or greater in USDA quality grades or Beef Marbling Score 2 or greater in Japanese quality grades.

Literature Cited

- Allen, V. G., J. P. Fontenot, R. F. Kelly, and D. R. Notter. 1996. Forage systems for beef production from conception to slaughter: III. Finishing systems. *J. Anim. Sci.* 74:625-638.
- Avery, N. C., T. J. Sims, C. Warkup, and A. J. Bailey. 1996. Collagen cross-linking in porcine *M. longissimus lumborum*: Absence of a relationship with variation in texture at pork weight. *Meat Sci.* 42:355-369.
- Bailey, A. J., and N. D. Light. 1989. *Connective Tissue in Meat and Meat Products*. Elsevier Applied Science, London and New York.
- Bailey, A. J., and M. S. Shimokomaki. 1971. Age related changes in the reducible cross-links of collagen. *FEBS Lett.* 16:86-88.
- Bergman, I., and R. Loxley. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* 35:1961-1965.
- Cameron, P. J., M. Zembayashi, D. K. Lunt, T. Mitsuhashi, M. Mitsumoto, S. Ozawa, and S. B. Smith. 1994. Relationship between Japanese beef marbling standard and intramuscular lipid in the *M. longissimus thoracis* of Japanese Black and American Wagyu cattle. *Meat Sci.* 38:361-364.
- Campion D. R., J. D. Crouse, and M. E. Dikeman. 1975. Predictive value of USDA beef quality grade factors for cooked meat palatability. *J. Food Sci.* 40:1225-1228.
- Crouse, J. D., G. M. Smith, and R. W. Mandigo. 1978. Relationship of selected beef carcass traits with meat palatability. *J. Food Sci.* 43:152-157.
- Dikeman, M. E., H. J. Tuma, and G. R. Beecher. 1971. Bovine muscle tenderness as related to protein solubility. *J. Food Sci.* 36:190-193.
- Dransfield, E. 1977. Intramuscular composition and texture of beef muscles. *J. Sci. Food Agric.* 28:833-842.

- Dransfield, E., G. R. Nute, T. A. Roberts, R. Boccard, C. Touraille, L. Buchter, M. Casteels, E. Cosentino, D. E. Hood, R. L. Joseph, J. Schon, and E. J. C. Paardekooper. 1984. Beef quality assessed at European research centres. *Meat Sci.* 10:1–20.
- Goll, D. E., R. W. Bray, and W. G. Hoekstra. 1963. Age-associated changes in muscle composition. The isolation and properties of a collagenous residues from bovine muscle. *J. Food Sci.* 28: 503–509.
- Herring, A. D., J. O. Sanders, R. E. Knutson, and D. K. Lunt. 1996. Evaluation of F₁ calves sired by Brahman, Boran, and Tuli bulls for birth, growth, size, and carcass characteristics. *J. Anim. Sci.* 74:955–964.
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. *J. Food Sci.* 31:161–166.
- Inoue, T., and H. Osatake. 1988. A new drying method of biological specimens for scanning electron microscopy: The t-butyl alcohol freeze-drying method. *Arch. Histol. Cytol.* 51:53–59.
- JMP. 1994. JMP Statistics and Graphics Guide. Version 3 of JMP. SAS Inst. Inc., Cary, NC.
- JMGA. 1988. New beef carcass grading standards. Japan Meat Grading Association, Tokyo, Japan.
- Light N., A. E. Champion, C. Voyle, and A. J. Bailey. 1985. The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. *Meat Sci.* 13:137–149.
- Lunt, D. K., R. R. Riley, and S. B. Smith. 1992. Growth and carcass characteristics of Angus and American Wagyu steers. *Meat Sci.* 34:327–334.
- May, S. G., H. G. Dolezal, D. R. Gill, F. K. Ray, and D. S. Buchanan. 1992. Effects of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. *J. Anim. Sci.* 70:444–453.
- McCormick, R. J. 1994. The flexibility of the collagen component of muscle. *Meat Sci.* 36:79–91.
- Nishimura, T., A. Liu, A. Hattori, and K. Takahashi. 1998. Changes in mechanical strength of intramuscular connective tissue during postmortem aging of beef. *J. Anim. Sci.* 76:528–532.
- Nishimura, T., K. Ojima, A. Liu, A. Hattori, and K. Takahashi. 1996. Structural changes in the intramuscular connective tissue during development of bovine semitendinosus muscle. *Tissue Cell* 28:527–536.
- Ohtani, O., T. Ushiki, T. Taguchi, and A. Kikuta. 1988. Collagen fibrillar networks as skeletal frameworks: A demonstration by the cell-maceration/scanning electron microscope method. *Arch. Histol. Cytol.* 51:249–261.
- Pearson, A. M. 1966. Desirability of beef—Its characteristics and their measurement. *J. Anim. Sci.* 25:843–854.
- Robins, S. P., M. Shimokomaki, and A. J. Bailey. 1973. The chemistry of the collagen cross-links. Age related changes in the reducible components of intact bovine collagen fibres. *Biochem. J.* 131:771–780.
- Rowe, R. W. D. 1981. Morphology of perimysial and endomysial connective tissue in skeletal muscle. *Tissue Cell* 13:681–690.
- Shimokomaki, M., D. F. Elsdon, and A. J. Bailey. 1972. Meat tenderness: Age related changes in bovine intramuscular collagen. *J. Food Sci.* 37:892–896.
- Smith, G. C., B. W. Berry, J. W. Savel, and H. R. Cross. 1988. USDA maturity indices and palatability of beef rib steaks. *J. Food Qual.* 11:1–13.
- Tanzer, M. L. 1973. Cross-linking of collagen. Endogenous aldehydes in collagen react in several ways to form a variety of unique covalent cross-links. *Science* 180:561–566.
- Tatum, J. D., G. C. Smith, B. W. Bery, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. 1980. Carcass characteristics, time on feed and cooked beef palatability attributes. *J. Anim. Sci.* 50:833–840.
- USDA. 1989. Official United States standards for grades of beef carcasses. Agric. Marketing Serv., USDA, Washington, DC.
- Vieselmeyer, B. A., R. J. Rasby, B. L. Gwartney, C. R. Calkins, R. A. Stock, and J. A. Gosey. 1996. Use of expected progeny differences for marbling in beef: I. Production traits. *J. Anim. Sci.* 74: 1009–1013.
- Zembayashi, M. 1994. Effects of nutritional planes and breeds on intramuscular-lipid deposition in *M. longissimus dorsi* of steers. *Meat Sci.* 38:367–374.
- Zembayashi, M., H. Nabeta, and T. Mototsuji. 1988. Effects of breeds and nutritional planes on intramuscular lipid deposition of fattening of steers. *Jpn. J. Zootech. Sci.* 59:39–48.
- Zembayashi, M., K. Nishimura, D. K. Lunt, and S. B. Smith. 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *J. Anim. Sci.* 73:3325–3332.