Intramuscular Connective Tissue Components Contributing to Raw Meat Toughness in Various Porcine Muscles

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Abstract Shear force value, contents of intramuscular collagen, elastin and proteoglycans and heat solubility of collagen were determined on eight different muscles from miniature pigs in order to confirm the relationship between raw meat toughness and intramuscular connective tissue components. *Semitendinosus* and *semimenbranosus* muscles were significantly tougher than any other muscles tested ($P < 0.05$). The strongest correlations ($P < 0.001$) were found between elastin content or heat solubility of collagen and raw meat toughness. No meaningful links could be found between textural quality of the raw meat and heat-labile collagen content or the proportion of ground substance relative to fibrous connective tissue ($P > 0.05$). Significant differences were seen, however, between the total collagen or proteoglycan content and the relative toughness of each muscles ($P < 0.01$, $P < 0.05$), respectively.

The results suggest that the maturity of intramuscular collagen with a stable intermolecular cross-linking and the accumulation of elastin may be directly contributing to meat toughness.

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Key words : raw meat toughness, connective tissue, collagen, elastin, proteoglycan

Among appearance, color, flavor, juiciness, taste, texture and so on, tenderness has been shown to be most important factor contributing to the acceptability of meat by consumers²⁾. The meat animal carcasses contains over 600 muscles which in general terms may be considered to be similar in composition. However, it is known that certain cuts of meat such as shin are tough, whilst others such as fillet steak are nearly always the most tender. Both so-called actomyosin toughness attributable to the myofibrillar contractile apparatus and so-called background toughness attributable to the connective tissue present, organizing into stable supramolecular structures in muscle are involved in meat toughness²²⁾.

Majority of the studies on meat texture have

been carried out in cooked meat because commonly eating and having a greater difference of toughness than the raw one. However, it is necessary to investigate the raw meat toughness for elucidations of relationships between the structural components and physiological properties of muscles and/or effects of the components on postmortem changes during conversion of muscle to meat. Thus, in the present study, the raw meat toughness is examined. HATAE *et al.*¹⁴⁾, determining both firmness of the raw and cooked muscles of five fish species, reported that the species with the softer raw meat textures gave a firmer on cooking. On the other hand, a positive relationship between the raw and cooked meat toughness is considered in beef¹⁷⁾.

Intramuscular connective tissues are the perimysium which surrounds the muscle fiber bundles and groups of bundles, and the endomysium which surrounds each muscle fiber as basement membrane. The main intramuscular connective tissue components are fibrous collagen and elastin and amorphous ground substances, such as proteoglycans and glycosaminoglycans. Studies on the effects of intramuscular connective tissue have been chiefly focused on collagen because of its abundant existence and structural firmness, but all the roles of connective tissue components in meat toughness have not been defined.

The objectives of this study were to determine the raw meat toughness of various porcine muscles, to determine the relative concentrations of collagen, elastin and proteoglycans in various porcine muscles, and to find out the direct or indirect relationships between the raw meat toughness and these connective tissue components in porcine muscle.

Materials and Methods

Muscles: Eight muscles were used to obtain a wide range of meat texture and connective tissue contents. The muscles were: *longissimus dorsi* (LD), *triceps brachii* (TB), *supraspinatus* (SS), *infraspinatus* (IS), *gluteus medius* (GM), *biceps femoris* (BF), *semitendinosus* (ST) and *semimembranosus* (SM).

Three 418-day-old Göttingen miniature pigs of the same litter were slaughtered by exsanguination. Following a 7-day conditioning period at 4°C, these muscles were excised from each carcass. The individual muscles were dissected and trimmed of all visible external fat and epimysium, partially frozen and cut into 10×10×30 mm³ pieces with the muscle fiber direction along the long axis. The pieces were used for texture measurements.

Texture measurements: Shear force values for the 10×10×30 mm³ pieces from eight muscles were determined using a Rheometer (R-DM-2, San-Kagaku Co., Tokyo) with a

knife-blade. A crosshead speed and full scale load of 40 mm/min and 2 kg, respectively were used. Pieces were placed with their long direction across the shear blade; the muscles fibers were cut at right angles by the blade. The cross-sectional area of each piece was 1 cm². Twenty shears per muscle per carcass were obtained (i.e. 60 shears per muscle). Peak shear force values were expressed in 'g/cm²'.

Chemical analyses: After the determination of shear force value, the muscle samples were minced three times through a meat chopper with 3 mm size holes. A part of the mince (approximately 20 g) was retained for elastin analysis, and the remainder was dried and defatted in chloroform-methanol mixture solution in a 2 : 1 ratio. The dried-defatted matter (DDM) was weighed to determine moisture and fat losses and then powdered in a grinder mill (ANM-150, Nitto-Kagaku Co., Tokyo). The DDM was used for chemical analyses of the intramuscular connective tissue components.

The powdered DDM was heated for 70 min at 77°C in distilled water and separated into heat-soluble and insoluble fractions by twice centrifugations following the procedure outlined by HILL¹⁵. The individual muscle fractions were then hydrolyzed in 6 N HCl for 24 h at 110°C and the hydroxyproline contents of the hydrolysates were determined¹¹. Hydroxyproline values were converted to insoluble collagen using a factor of 7.25¹³ and to soluble collagen using a factor of 7.52 based on absence of elastin⁹. The total concentration of intramuscular collagen for each sample was determined by adding the amounts of heat-soluble and insoluble collagen, and the percentage of soluble collagen in each muscle sample was calculated.

Elastin was isolated from each of the eight muscles using the procedure outlined by Cross *et al.*⁹. Briefly, the minced muscle samples were weighed and exhaustively extracted with 1.1 M KI in 0.1 M phosphate buffer, pH 7.4, to ensure complete removal of myofibrillar and

sarcoplasmic proteins. Following fat extraction with an excess of 2 : 1 chloroform-methanol and removal of the solvent, the residue was selectively hydrolyzed in 0.1 N NaOH for 50 min at 98°C to remove the collagen portion of the connective tissue fraction. The remaining residue was hydrolyzed in 6 N HCl and the hydroxyproline was quantitated as mentioned above. The concentration of intramuscular elastin was determined by multiplying the hydroxyproline content by 66.225 based on the report of the amino acid composition of the intramuscular elastin⁸⁾.

For the determination of uronic acid, as an index of proteoglycans, proteins contained in the muscles were digested with papain in advance according to the report of ALEXANDER *et al.*¹⁾. Following an activation by incubating the papain (type III, Sigma) with 0.1 M EDTA, the powdered DDM was extracted with the activated papain solution for 24 h at 65–70°C and the supernatant was collected by centrifugation. The residue washed with 0.1 M phosphate buffer, pH 6.4 containing 0.3 M NaCl was re-centrifuged for the collection of the supernatant. Uronic acid content in the both supernatant fractions was estimated using the procedure of BITTER and MUIR⁵⁾ with glucuronolactone as a standard.

Statistical analysis: Data was analysed by analysis of variance. Muscle group means were separated at 5% level of significance using the Tukey's Studentized multiple range test²⁴⁾. Linear correlation coefficients were computed between the shear force value, the contents of each of the intramuscular connective tissue components, heat solubility of collagen and the proportion of uronic acid relative to hydroxyproline.

Results and Discussion

Meat toughness: Textural difference of meat have been assessed either by subjective sensory panel or objective mechanical testing, with many arguments. In the present study, a Rheometer with a knife-blade was used for objective determination of raw meat toughness. As shown in Table 1, peak shear force values in both ST and SM were significantly higher than in the other six muscles ($P < 0.05$). This data supports the earlier findings in cooked meat¹⁷⁾ and in raw meat²⁰⁾, however, it is difficult to detect a textural difference in raw meat compared with cooked meat.

Chemical properties of intramuscular connective tissue components: As shown in Table 1, the total concentrations of intramuscular collagen varied among muscles from 4.34 ± 0.73 mg/

Table 1. Physical and chemical measurements of eight different muscles from miniature pigs¹

Muscles	Shear force vglue (g/cm ²)	Collagen content (mg/g)	Heat-labile collagen content (mg/g)	Elastin content (μg/g)	Uronic acid content (μg/g)	Heat solubility of collagen (%)	Uronic acid/ Hydro- xyproline
<i>semitendinosus</i>	558±51 ^a	8.62±1.49 ^{ab}	1.03±0.12 ^b	239±31 ^a	537±59 ^a	11.90	0.45
<i>semimembranosus</i>	507±51 ^a	6.61±1.30 ^{abc}	0.88±0.15 ^b	178±27 ^b	534±79 ^a	13.37	0.59
<i>supraspinatus</i>	444±51 ^b	7.76±0.91 ^{ab}	1.24±0.20 ^{ab}	163±22 ^{bc}	398±34 ^b	16.00	0.37
<i>biceps femoris</i>	441±78 ^b	9.33±0.90 ^a	1.62±0.22 ^a	159±26 ^{bc}	441±68 ^b	17.40	0.34
<i>triceps brachii</i>	440±76 ^b	6.23±1.06 ^{bc}	1.22±0.22 ^{ab}	126±30 ^{bc}	400±45 ^b	19.55	0.46
<i>infraspinatus</i>	416±56 ^b	6.26±0.94 ^{bc}	1.30±0.11 ^{ab}	144±27 ^{bc}	296±24 ^c	20.79	0.34
<i>gluteus medius</i>	413±74 ^b	4.34±0.73 ^c	0.98±0.08 ^b	101±20 ^c	482±51 ^{ab}	20.61	0.81
<i>longissimus dorsi</i>	384±54 ^b	4.91±0.53 ^c	1.07±0.15 ^b	114±25 ^c	485±97 ^{ab}	21.76	0.72

¹Results are expressed as mean±SD of 30 to 80 determinations. Both values of heat solubility of collagen and uronic acid/hydroxyproline are expressed as mean.

^{a, b, c}: Means in the same vertical row with different superscripts are significantly different ($P < 0.05$).

g wet muscle tissues in GM to 9.33 ± 0.90 mg/g in BF. The toughest ST had a significantly higher collagen concentration than the most tender LD ($P < 0.05$). However, no definite relationship between meat toughness and the total collagen content could be observed, because BF contained significantly more collagen in spite of showing relatively tender meat. Collagen, occupying about 25% of total protein in the mammalian body, is also a major constituent of intramuscular connective tissues such as perimysium and endomysium, but it quantitatively only comprises less than 1% of the total weight of wet muscle tissues.

Heat-labile collagen contents ranged from 0.88 ± 0.15 mg/g in SM to 1.62 ± 0.22 mg/g in BF. However, there was little variation in the heat-labile collagen content (Table 1). This finding indicates that heat-labile collagen content in muscles could not be applied as a parameter for the determination of textural quality of meat on animals of the same age.

It was seen that the lower heat solubility of intramuscular collagen (%), the tougher meat (Table 1). Collagen present in tender meat (LD, GM, IS) solubilized more than 20% by heating compared with 11.9% solubilization in the toughest meat (ST), which confirmed that there were more abundant heat-stable, matured crosslinks in tough meat collagen. It is apparent that collagens in intramuscular connective tissue have various types of intermolecular crosslinking²¹, in addition to the cystine disulfide linkage in types III and IV collagens^{6,12}. Also, a relationship between the

concentration of pyridinoline, one of matured crosslinks, and thermal stability of bovine intramuscular collagen has been reported²³, and that the proportion of matured crosslinking was higher in collagen-poor tissues, e.g. muscle, than in collagen-rich tissues, e.g. skin¹⁸. As shown in Table 2, significant correlations between total collagen content and heat-labile collagen content ($r = 0.521$, $P < 0.05$) and heat solubility of collagen ($r = -0.689$, $P < 0.01$) indicate that relatively collagen-rich muscle may have less matured crosslinks than in collagen-poor muscle in same animal. And also indicated are some contributions of proteoglycans and to stabilizing intramuscular collagen fibers or fibrils, from the findings (Table 2) of significantly negative correlations between uronic acid content and heat-labile collagen content ($r = -0.606$, $P < 0.01$) and heat solubility of collagen ($r = -0.440$, $P < 0.05$). However, little information is available concerning the interactions between intramuscular connective tissue components.

Correlations between meat toughness and intramuscular connective tissue components: Fig. 1 shows the correlations between shear force value and intramuscular connective tissue components of different porcine muscles. It was both heat solubility of collagen and elastin content that the best parameters had the strongest correlations with the shear force value of the raw meat ($r = -0.821$, $P < 0.001$; Fig. 1c, $r = 0.758$, $P < 0.001$; Fig. 1d, respectively). Significant difference in total collagen content and meat toughness was also seen ($P < 0.01$; Fig. 1

Table 2. Linear correlation coefficients between intramuscular connective tissue components

	Heat-labile collagen content	Elastin content	Uronic acid content	Heat solubility of collagen	Uronic acid/Hydroxyproline
Collagen content	0.521**	0.681**	0.005	-0.689**	-0.781***
Heat-labile collagen content		0.080	-0.606**	0.236	-0.707***
Elastin content			0.315	-0.655**	-0.417*
Uronic acid content				-0.440*	0.574**
Heat solubility of collagen					0.338

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

a), however, no meaningful links between textural quality of the meat and heat-labile collagen content or the proportion of uronic acid relative to hydroxyproline ($P > 0.05$; Fig 1 b

and 1 f). These findings indicate that the predominant factors in determining meat texture may be the maturity of the fibrous connective tissue components, *i.e.* the amount of elastin or

Fig. 1. Correlations between shear force value and total collagen content (a), heat-labile collagen content (b), heat solubility of collagen (c), elastin content (d), uronic acid content (e), and uronic acid/hydroxyproline (f) of eight different muscles from three miniature pigs.

the quality of collagen, rather than the quantity of collagen present in the muscles, although DRANSFIELD¹⁰⁾ reported that the meat toughness was mainly influenced by the amount of total collagen. Formations of various intermolecular crosslinkings in collagen are progressing with the increase of animal age because of a much slower turnover of collagen, so that the steady collagen fiber in muscle or meat tend not to solubilize easily and more on heating²¹⁾. On the other hand, as the turnover of elastin is slower than that of collagen, the heating treatment could not be denatured and solubilized the elastin at all¹⁸⁾. Therefore, elastin present in this porcine muscles in very small quantities may influence the meat toughness and, especially in both cases of some specific muscles such as bovine *M. semitendinosus* in which elastin occupies 40% of the intramuscular fibrous connective tissue³⁾ and cooked meat flowed out of a heat-denatured collagen, gelatin, it is expected that elastin could play an important role in determining meat texture.

A significant correlation was also seen between uronic acid content and meat shear force value ($r=0.494$, $P<0.05$; Fig. 1e); besides this, significant correlations were found between uronic acid content and heat-labile collagen content ($r=-0.606$, $P<0.01$), heat solubility of collagen ($r=-0.440$, $P<0.05$), and the proportion of uronic acid relative to hydroxyproline ($r=0.574$, $P<0.01$) in Table 2. These findings suggest that the intramuscular proteoglycans may not have direct effects on meat toughness but may influence the toughness indirectly by means of some action on the intramuscular collagen. Collagen fiber network is embedded in an amorphous, flexible, juicy, cement-like gel composed mainly of proteoglycans. Proteoglycans have been suggested as playing important physiological roles in water retention and electrolyte control of connective tissue⁷⁾ and control of cell growth and differentiation¹¹⁾, as well as a physical role in maintenance of the tissue configuration in fetal or

new-born tissues limited collagen fiber networks^{16,19)}. Stiffness and flexibility of meat, therefore, may be controlled by the proteoglycans by means of limiting a molecular diffusion in the whole connective tissue and making collagen fibers arrange precisely.

Relationships between shear force value and individual connective tissue component in various porcine muscles were investigated in order to determine the effects of intramuscular connective tissue components on meat toughness. The present results confirm that not the quantity but the quality of collagen, such as a collagen maturity, contributes to a delicate distinction in the relative toughness of each muscle. And also, the effects of the trace components, elastin and proteoglycans on meat toughness are suggested. Both direct and indirect roles of intramuscular connective tissue components in determining meat texture may, therefore, appear using various techniques for analysis of types and crosslinks of intramuscular collagen, observation of three-dimensional architecture of the collagen fiber network, and an elucidation of interactions among the connective tissue components.

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種々のブタ筋肉の硬さに対する筋肉内結合組織成分の寄与

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食肉の硬さに及ぼす筋肉内結合組織成分の影響を調べるために、ミニブタの8種の筋肉の剪断力値、筋肉内コラーゲン含量、エラスチン含量、プロテオグリカン含量、コラーゲンの加熱溶解性を測定し、筋肉の硬さと各筋肉内結合組織成分との相関関係を検討した。他の筋肉に比べ、半腱様筋および半膜様筋が有意に硬かった ($P < 0.05$)。筋肉の硬さと最も相関したのは、エラスチン含量とコラーゲンの加熱溶解性であった ($P < 0.001$)。筋肉の硬さと加熱溶解コラーゲン含量や結合組織線維成分に対する基質の割合との間には、全く相関がなかった ($P > 0.05$)。しかしながら、筋肉の硬さと総コラーゲン含量およびプロテオグリカン含量との間には、有意な相関が認められた (各々 $P < 0.01$, $P < 0.05$)。

これらの結果から、食肉の硬さにはコラーゲンの分子間架橋などによるコラーゲンの安定化ならびに筋肉内のエラスチンの蓄積が関与することが示唆された。

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