STRUCTURAL CHANGES IN THE INTRAMUSCULAR CONNECTIVE TISSUE INDUCED BY FATTENING OF BEEF CATTLE: RELATION TO TENDERIZATION OF BEEF

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Background

The intramuscular connective tissue plays a significant part in determining meat toughness (Bailey & Light, 1989). Meat becomes firm with chronological ageing concomitantly with the increase in intermolecular non-reducible crosslinks of collagen, and this process toughens meat (Bailey & Shimokomaki, 1971; Robins et al., 1973). The mechanical stability of the intramuscular connective tissue depends on not only intermolecular crosslinks of collagen but also the size and arrangement of collagen fibrils (Rowe, 1981). We have investigated structural changes in the intramuscular connective tissue during development of *bovine semitendinosus* muscle using the cell-maceration method for scanning electron microscopy, by which cellular elements are eliminated and collagen fibrils and fibres are exposed; collagen fibrils in the endomysium bind more closely with each other, collagen fibres in the perimysium increase in thickness and the wavy pattern of collagen fibres become more regular with growth of cattle (Nishimura et al., submitted to Tissue & Cell).

Fattening of cattle increases the degree of marbling and brings about tenderness of beef. In Japan, Wagyu such as Japanese Black cattle is fattened for a period of over 20 months after rering, so the age of finishing is over 30 months old. The degree of marbling remarkably increases in this period. However, little is known about the effect of fattening on tenderness of beef.

Objectives

The objective of this study was to investigate changes in structure and mechanical properties of the intramuscular connective tissue during fattening of beef cattle, and to clarify the tenderization mechanism of marbled beef.

Methods

Japanese Black and its cross-breed cattle (n = 53) up to 36 months old were used. *Longissimus thoracis (dorsi)* muscle was dissected from carcasses immediately post-mortem.

Shear-force value

Specimens (10 x 10 x 20 mm) were cut out from *longissimus thoracis (dorsi)* muscle. The shear-force value perpendicular to the axis of muscle fibres was measured for 8 -10 specimens with a rheometer (NRM-2002J, Fudo Industry Co., Tokyo).

Heat-solubility of collagen

Heat-solubility of collagen was determined by the method of Hill (1966) with slight modifications. Two g of samples were homogenized for 1 min at 10,000 rpm with a four-fold volume of 1/4 strength Ringer's solution in a homogenizer (The Virtis Co., New York). The resulting homogenate was heated for 70 min at 77°C, and then centrifuged for 30 min at 3,000 rpm. The supernatant was decanted, and the pellet was washed with the same solution, and recentrifuged. The both supernatants were combined, and the amount of hydroxyproline was determined by the procedure of Bergman and Loxley (1963). The amount of heat-soluble collagen was expressed as a percentage of the total amount of collagen.

Cell-maceration/scanning electron microscopy

According to the cell-maceration method of Ohtani et al. (1988), small pieces of muscle (10 x 10 x 15 mm) were cut out and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer solution, pH 7.3, for 1 day. The pieces were immersed in 10% NaOH for 7 days and then rinsed in distilled water for 5 days at room temperature. They were put in 1% tannic acid for 3 h, rinsed in distilled water for several hours, and post-fixed in 1% osmium tetroxide for 1 h. The specimens were dehydrated in a series of graded concentrations of

ethanol, freeze-fractured with a razor blade in liquid nitrogen, and dried by the t-butyl alcohol freeze-drying method. The dried specimens were coated with gold and observed under a scanning electron microscope (S-800, Hitachi, Tokyo) with an accelerating voltage of 10 kV.

Preparation of "IMCT model"

Small pieces of muscle (10 x 10 x 15 mm) were cut out from *longissimus thoracis (dorsi)* muscle, and macerated according to the method of Ohtani et al. (1988). They were immersed for a few hours in a acrylamide solution containing 7.5% acrylamide, 1.5 mg/ml ammonium persulfate at room temperature, then N,N,N',N'-tetramethylethylenediamine (0.75 μl/ml) was added. The acrylamide was polymerized completely at room temparature. We termed them "intramuscular connective tissue (IMCT) model" in this paper. The shear-force value perpendicular to the axis of muscle fibres was measured for ten samples with a rheometer (NRM-2002J, Fudo Industry Co., Tokyo).

Results and Discussion

The shear-force value of *longissimus thoracis (dorsi)* muscle increased during the early fattening period from 9 months old up to 20 months old, but decreased gradually thereafter until 36 months old. The crude fat content of *longissimus thoracis (dorsi)* muscle increased markedly during the latter period. The heat solubility of collagen decreased up to 24 months old, and increased gradually until 36 months old, indicating the weakening of mechanical properties of the intramuscular connective tissue.

The mechanical strength of the intramuscular connective tissue was examined using our "IMCT model." The IMCT model was composed of collagen fibrils and fibres which maintained the organization in the endomysium and perimysium. The shear-force value of the model increased linearly up to 24 months old, then decreased gradually until 36 months old.

Structural changes in the endomysium and perimysium in *longissimus thoracis (dorsi)* muscle were observed by the cell-maceration/SEM method. Collagen fibrils in the endomysium bound more closely with each other, collagen fibres in the perimysium increased in thickness and their wavy pattern became more regular with growth of cattle up to 20 months old. These changes in the arrangement of collagen fibrils and fibres seemed to be closely related to increase in toughness of beef during the early fattening period. In *longissimus thoracis (dorsi)* muscle from 32-month old steers, however, gaps of various sizes opened everywhere; the junction between the endomysium and perimysium disintegrated and large gaps opened in the perimysium, and membranous structures consisted of loose networks of collagen fibrils were visible between muscle fibre bundles. It was proved in comparison with electron micrographs of SEM and those of low-vacuum SEM that the gaps were due to a deposit of fat and the membranous structures were the connective tissue surrounding fat cells of about 120 μm in diameter. The development of adipose tissue in skeletal muscle is considered to disintegrate the structure of the perimysium.

Conclusions

Fattening of cattle increases fat deposition in *longissimus thoracis (dorsi)* muscle; numerous fat cells of about 120 μm in diameter appear at the junction between the endomysium and perimysium, and in the perimysium. The development of adipose tissue weakens the perimysium and brings about tenderization of beef.

Pertinent Literature

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