CHARACTERISTICS OF INTRAMUSCULAR CONNECTIVE TISSUES FROM DOUBLE-MUSCLED YOUNG BULLS

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Background

Double-muscled cattle have a greater muscle development and give 20% more beef on roughly the same food intake as ordinary cattle, and their meat is very tender. Double-muscling in cattle, such as Belgian Blue and Piedmontese, is caused by a mutation in myostatin gene that induces hyperplasia and hypertrophy in skeletal muscle (McPherron and Lee, 1997). Double-muscled cattle have a higher proportion of type IIB muscle fibres and a lower proportion of type I fibres than normal cattle (Bailey et al., 1982). In addition, their muscles have a lower collagen content (Boccard, 1982), and may have a higher collagen heat-solubility (Bailey et al., 1982), and less type III collagen (Listrat et al., 1999a). However, very little is known about structural properties of the intramuscular connective tissues (IMCT) in double-muscled beef. The IMCT provides a framework bundling muscle fibres and their structural properties are closely related to texture of meat (Nishimura et al., 1996).

Objectives

The aim of this study was to investigate characteristics of the IMCT in double-muscled young bulls in order to clarify the contribution of structural properties of the IMCT to the tenderness of double-muscled beef.

Materials and Methods

Animals and samples

The experiment was performed using six normal and six double-muscled Belgian Blue young growing bulls which were fed individually a solid diet composed of approximately 50% grass hay and 50% pelleted concentrated feed. Growth parameters and body composition at slaughter were described previously (Hocquette et al., 1999). Briefly, average daily gain did not significantly differ between the two groups of bulls, but double-muscled bulls exhibited a higher proportion of muscles (+22%) and a reduced proportion of fat (-49%) in the carcass at the time of slaughter. *Semitendinosus* (ST) muscle samples were taken at slaughter, frozen in liquid nitrogen in less than 30 min post-exsanguination and stored at –80°C until use.

Biochemical studies

Total hydroxyproline content was measured as previously described by Listrat et al. (1999b) to assess total collagen content. Collagen solubility was determined according to the procedure of Hill (1966) slightly modified by Listrat et al. (1999b). Anaerobic glycolytic metabolism was assessed by lactate dehydrogenase (LDH) activity which transforms pyruvate into lactate. Aerobic oxidative metabolism was assessed by isocitrate dehydrogenase (ICDH) activity. Enzyme activities were measured according to the method of Jurie et al (1995).

Picro-Sirius Red polarisation method

Transverse sections (6 µm in thickness) of the frozen muscle were cut in a cryostat and stained with Picro-Sirius Red solution containing 0.1% Sirius Red and saturated picric acid. Then, they were examined under a light microscope, and the areas of muscle fibres were measured on ten photographs obtained from at least two blocks from each animal.

Cell-maceration/scanning electron microscopy (SEM)

Small pieces of muscle were cut out, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, immersed in a 10% NaOH for 4 days, then rinsed in distilled water. They were put in a 1% aqueous solution of tannic acid, rinsed in distilled water, and post-fixed in a 1% OsO_4. The specimens were dehydrated in a series of graded concentrations of ethanol, freeze-fractured, and dried by the t-butyl alcohol freeze-drying method. The dried specimens were mounted on metal stubs, coated with gold and observed by SEM with an accelerating voltage of 10 kV.

Preparation of "IMCT model" and measurement of shear-force value

Small pieces of muscle were macerated with NaOH following rinsing with distilled water, and immersed for a few hours in an acrylamide solution containing 7.5% acrylamide, 1.5 mg/ml ammonium persulfate at room temperature. The solution was allowed to polymerize the acrylamide completely after adding TEMED. We termed them "IMCT model" in this paper. The shear-force value of IMCT models perpendicular to the axis of muscle fibres was measured with a rheometer.

Results and Discussion

The primary muscle fibre bundle of double-muscled bulls were significantly larger ($P<0.01$) than those of normal ones. The mean numbers of muscle fibres in the primary muscle fibre bundle were higher in double-muscled bulls than in normal ones. Furthermore, ICDH activity was significantly lower in ST from double-muscled bulls ($P<0.05$). All together, these results indicated hypertrophy, hyperplasia and a reduced oxidative metabolism in the double-muscled animals, which is consistent with the earlier reports (Bailey et al., 1982 ; Picard et al., 1994).

Collagen content was 34% lower in double-muscled animals ($P<0.001$), but collagen solubility did not significantly differ between the two groups. The shear-force value of double-muscled bulls was lower than that of normal ones, but there was no significant difference between them. The mechanical strength of the IMCT was examined using our "IMCT model", which composed of collagen fibrils and fibres. The shear-force value of the IMCT model from double-muscled bulls was significantly lower ($P<0.05$) than that of normal ones. This result demonstrates that the mechanical strength of the IMCT in double-muscled bulls might be weaker than that in normal ones. So, we investigated the ultrastructure of the IMCT in double-muscled animals by SEM. The honeycomb structures of the IMCT were clearly observed by the cell-maceration/SEM method. The sheath of endomysium was membranous and consisted of tightly arranged collagen fibrils. The perimysium was composed of collagen fibres made up of tightly bundled collagen fibrils. However, little different could be observed in the fine structure of the IMCTs between double-muscled animals and normal ones, although the thickness of the primary perimysium was somewhat thinner in double-muscled animals. The tenderness of double-muscled beef could be due to the lower amount of total collagen and of the IMCT per unit volume of the muscle than normal one.

Table 1. Muscle fibre sizes and numbers, and shear-force values of double-muscled and normal muscle.

	Size of fibre bundle (mm^2)	Numbers of muscle fibres	ICDH activity [2]	LDH activity [2]	Collagen		Shear-force value (g)	
					Content[1]	Solubility (%)	ST muscle	IMCT model
Double-muscled	0.213±0.149 [a]	76.4±42.3	0.10±0.01 [a]	155.6±8.0	3.37±0.34 [a]	22.82±2.25	606±21	451±21 [a]
Normal	0.139±0.117 [b]	68.1±37.1	0.14±0.06 [b]	146.1±16.5	5.11±0.56 [b]	24.55±4.30	660±42	507±24 [b]

[1] Results are in µg hydroxyproline per mg dry matter, [2] Results are in nkat per g wet tissue. Results are means ± standard deviations. Different superscripts, within a raw means significant differences ($P<0.05$)

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