ELASTIN IN SEMITENDINOSUS MUSCLE OF DOUBLE-MUSCLED BELGIAN BLUE CATTLE

Tania Ngapo[1], Philippe Berge[1], Jean-François Martin[1], Joseph Culioli[1] and Stefaan De Smet[2]

[1]Station de Recherches sur la Viande, INRA, Theix, 63122 St-Genès-Champanelle, France
[2] Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

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Background

In 1967, Bendall showed that most muscles contain less than 0.2% elastin on a fat free dry matter basis, which is less than 5% of connective tissue. However, in some muscles, such as St, at least 35% of the connective tissue is elastin. Elastin does not undergo a thermal transition below 100°C and, therefore, is unlikely to be weakened by cooking (Rowe, 1986). Histochemically, muscles with high elastin contents contain coarse elastic fibres in the perimysium, not observed in other muscles (Bendall, 1967; Rowe, 1986; Nishiumi, 1999). While the significance of elastin in the context of meat toughness is unclear, its contribution has been considered of minor importance compared to that of collagen, as bovine muscles with different elastin contents yield beef of similar toughness (Bendall, 1967; Dransfield, 1977; Cross et al., 1973). However, considering its thermal and organisational properties, elastin may play an important role in determining the toughness of cooked meat in muscle containing considerable amounts of this protein.

Recently, shear force of cooked *M. gluteobiceps* was found significantly less in meat from double muscled (DM) than normal cattle, whereas using *M. semitendinosus* (St), no differences were observed (Ngapo et al., 2001a). These muscles express similar mass increases with the DM condition relative to normal (Boccard, 1981) and had similar collagen concentrations, being significantly less in DM than normal animals (Ngapo et al., 2001a). Therefore, neither of these two factors provide a likely explanation for the difference in shear force trends. The difference in elastin content between these two muscles, however, deserves investigation.

Objective

The aim of this study was to investigate the content of the elastin crosslinks, desmosine and isodesmosine, as an estimate of elastin content in *M. semitendinosus* from Belgian Blue DM, heterozygous and normal bulls, and their relationships with tenderness.

Materials and methods

Thirty-one Belgian Blue bulls of known ages, from different farms and fattened on high-concentrate diets, were slaughtered at the Ghent University abattoir. Dressed carcasses were hung by Achilles tendon suspension in a chiller at 2°C. At 24 h post mortem *M. semitendinosus* (St) was removed and standardised sections (approx. 500 g) taken from the centre of the muscles were vacuum packaged and transported chilled arriving at INRA within 24 h. The muscle sections were aged for 4 weeks at 2°C prior to crosslink analyses and frozen storage (-18°C) before total collagen analyses. The remainder of the muscle was stored at 2°C for 11 days then frozen at -18°C and later used for shear force (SF) measurements. The animals sampled in this study were part of a larger trial, of which some meat quality data have been published (De Smet et al., 2000).

All methods and HPLC instrumentation are described in detail by Ngapo et al. (2001a). All chemicals and reagents used were at least analytical grade and water was deionised. Double-muscling classification was undertaken by genotyping for the mutation nt821 (del 11) in the mysotatin gene according to the method of Grobet et al. (1998; DM, mh/mh; heterozygous, mh/+; normal, +/+). Preparation of intramuscular connective tissue (IMCT) was undertaken using the method of Light and Champion (1984) for extraction of perimysium. The method of reduction of IMCT at pH 7.5 with sodium borohydride was adapted from the method of Robins et al. (1973). Hydrolysis and cellulose chromatography procedures were based on the methods of Black et al. (1988). The HPLC analyses was based on the procedure for elution of amino acids described by Horgan et al. (1991) using a 0.1 M ninhydrin solution (Moore, 1968). Quantification of desmosine and isodesmosine were obtained using a colour yield relative to leucine of 3.4, and elastin was estimated assuming the sum of the desmosines to be 2.7% of the weight of elastin (Sims and Bailey, 1992) and having a molecular weight of 879 (Fasman, 1988). Hydroxyproline analyses of total collagen in whole meat samples were determined using the colorimetric method of Bergman and Loxley (1963). Collagen content was calculated from five sample repetitions assuming collagen weighed 7.14 times the hydroxyproline weight and had a molecular weight of 300 000.

Steaks of 2.5 cm thickness were thawed overnight (4°C) and cooked (a) in open polyethylene bags hanging in a water bath at 75°C for 1h (WB75) and, (b) grilled to an internal temperature of 80°C (WBgrill). Samples were cooled (30 min in tap water for the WB75 samples) before taking cores (∅ 1.27 cm). Warner Bratzler shear force was measured with a Lloyd TA 500 Texture Analyser. The average maximum force of 15-30 measurements per sample was taken as the shear force of the sample.

The effects of animal type (DM, heterozygous and normal) were analysed by analysis of variance (ANOVA) and detected by least square means test for multiple means comparison using the General Linear Models (GLM) procedure of SAS (1996). Age was used as a covariant. Pearson correlation coefficients were calculated (SAS, 1996).

Results and Discussion

Means and standard deviations (SD) of the elastin crosslinks and total collagen concentrations are presented in Table 1 for the different animal groups. The collagen results which have been presented in more detailed papers (Ngapo et al., 2001a,b), are included here for comparison. The ages of the animals ranged from 16 to 25 months. The analysis of variance (ANOVA) using age as a covariant showed that there was no significant effect of age on the crosslink, collagen or estimated elastin concentrations. With increasing age it has been shown that elastin becomes less elastic (Bailey and Light, 1989). This has been suggested a result of excessive crosslinking, but it was noted that no change in the quantity of known elastin crosslinks had been reported following animal maturation despite obvious changes in physical properties.

Table 1. Elastin and its crosslinks, and collagen concentrations and shear force values in St muscle of Belgian Blue bulls.

Double-muscling genotype	Normal		Heterozygous		DM		Animal Type Significance[2]
	Mean	SD	Mean	SD	Mean	SD	
Isodesmosine (nmol/g wet meat)	103	58	111	69	52	43	
Desmosine (nmol/g wet meat)	152[a]	75	170[a]	55	94[b]	49	*
Elastin estimate (mg/g wet meat)	8.3[a]	4.3	9.2[a]	4.0	4.8[b]	2.9	*
Collagen (nmol/g wet meat)	38[a]	6	34[a]	4	24[b]	2	***
Collagen (mg/g wet meat)	11.3[a]	1.8	10.1[a]	1.3	7.3[b]	0.6	***
Elastin/collagen	0.23	0.12	0.27	0.12	0.19	0.11	
Wbgrill (N)	34	3	33	3	31	4	
WB75 (N)	34	4	33	2	30	4	

[1]Means with differing superscripts are significantly different at $p = 0.05$, compared across a line only.
[2]Where * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

In the present work, the ANOVA did show that the animal type significantly affected desmosine, estimated elastin and total collagen concentrations, but not the isodesmosine concentration or the elastin/collagen ratio. Examination of the least square means for animal type shows that desmosine, estimated elastin and collagen in the DM animals were significantly less than in the normal or heterozygous animals. A large difference in isodesmosine concentrations was also observed between DM and normal or heterozygous animals, however this difference was not significant because of the large variation in the measures of the elastin crosslinks; coefficients of variation ranged from 56 to 83% and 32 to 52% for the isodesmosine and desmosine concentrations, respectively. This variability could be an inherent characteristic of this muscle in this particular breed or perhaps a result of the preparative and analytical procedures used. A large variation in concentrations of elastin was also observed by Cross et al. (1973) who directly measured elastin weight in meat from Hereford cows. The lack of difference in the elastin/collagen ratio indicates that these connective tissue proteins are in lower concentrations of the same order in the DM animals compared to normal animals.

Neither WBgrill, nor WB75 shear force were significantly affected by animal type. Furthermore, neither of the shear force measures was significantly correlated with the collagen or elastin measures. Differences were observed in both the levels of collagen content and some collagen crosslinks in St (Ngapo et al., 2001b). However, as no differences were found in the cooked shear force measures of St from DM, normal and heterozygous animals, no significant and strong correlations between either the collagen content or its crosslinks and the shear force values were observed (Ngapo et al., 2001a,b).

It was suggested that elastin, because of its relatively high concentrations in St, may play a direct role in the tenderness of this muscle when cooked (Ngapo et al., 2001a). A lack of differences in both shear force and elastin contents with animal type would warrant further investigation of relationships between these factors. However, the significant effect of animal type on elastin content leads to the conclusion that a direct relationship between elastin content and shear force of cooked St was not found. Therefore no evidence is provided to suggest that elastin is directly responsible for the lack of tenderness differences in cooked St from DM, heterozygous and normal Belgian Blue cattle.

Conclusions

Relationships between elastin content and shear force of cooked St were not observed. Therefore, no evidence is provided to suggest that elastin content is responsible for the lack of cooked meat tenderness differences in St from DM, heterozygous and normal Belgian Blue cattle.

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