Biochemistry and morphological characterization of Intra Muscular Connective Tissue of two contrasting bovine muscles

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Abstract— **Beef meat tenderness depends on biochemistry factors as collagen and cross-link contents and muscle fibres characteristics. Tenderness could also depend on structural characteristics of intramuscular connective tissue (perimysium and endomysium). This study aims to develop an image analysis method able to measure some intramuscular connective tissue characteristics that reflect the specificity of two contrasting muscles. We used *Longissimus thoracis* (LT) and *Biceps femoris* (BF) muscle from 42 young bulls. Picro-Sirius red method applied on histological muscle cryosections stained intramuscular connective tissue in red and muscle fibres in yellow. Our image analysis procedure was based on this contrast. The computer algorithm (Visilog 6.7, Noesis, France) calculated automatically the intramuscular connective tissue area, length, width, optical density and connection points. Collagen (total and insoluble) and cross-link contents were determined, respectively by hydroxy-proline and pyridinoline measurements, on muscle powder. Statistical correlations between morphological and biochemical parameters were evidenced. Area and optical density of BF intramuscular connective tissue were higher than in LT. BF connective tissue network was longer and wider and was more ramified. Total and insoluble collagen contents and cross-link level were higher in BF than in LT. This confirms that morphological parameters are different from muscle to another and that our algorithm is able to highlight them. BF collagen content and solubility were positively correlated with endomysium characteristics. No correlation between morphological and biochemical parameters appeared in LT muscle, neither between perimysium characteristics and biochemical characteristics. BF and LT show different relationships between biochemistry and morphological characteristics.**

Keywords— **Intramuscular Connective Tissue, muscle, beef**

I. INTRODUCTION

After ageing of meat, toughness is mainly due to intramuscular connective tissue (IMCT). It's composed of two structures: perimysium which surrounds individually fibre bundles and endomysium which surrounds muscle fibre. A lot of studies have shown that quantity of connective tissue (estimated by total collagen assay) and degree of cross-linking play a role in meat toughness. But few authors have taken account perimysium and endomysium.

In order to study the role of IMCT in tenderness, we had chosen *Biceps femoris* (BF) and *Longissimus thoracis* (LT), two muscles contrasting in texture (LT is more tender than BF [1]) and in their total collagen content.

The aim of the study was to develop a method of image acquisition and analysis able to measure perimysium and endomysium characteristics on the same histological muscle section. In addition we've measure total and insoluble collagen contents and cross-link content.

II. MATERIALS AND METHODS

Longissimus thoracis and *Biceps femoris* were removed from 42 young bulls aged of 16-17 months at slaughtered. Muscles were taken just after slaughter. For histology, samples of 1 cm^3 were snap-frozen by immersion in isopentane cooled by liquid nitrogen in order to preserve IMCT and then stored at -80°C until analysis. For biochemistry parameters, around 200 g of muscle were taken, cut into pieces of 2 cm^3 and then stored at -20°C under vacuum until lyophilisation.

Morphologic IMCT parameters were determined with histological sections of muscle associated with image analysis. Histological cryosections of 10 µm were stained according the Picro-Sirius red method [2] which stained perimysium and endomysium in red and muscle fibres in yellow. Image analysis procedure was based on this contrast. For perimysium study, staining sections were acquired by optical density scanner (EPSON 10000XL

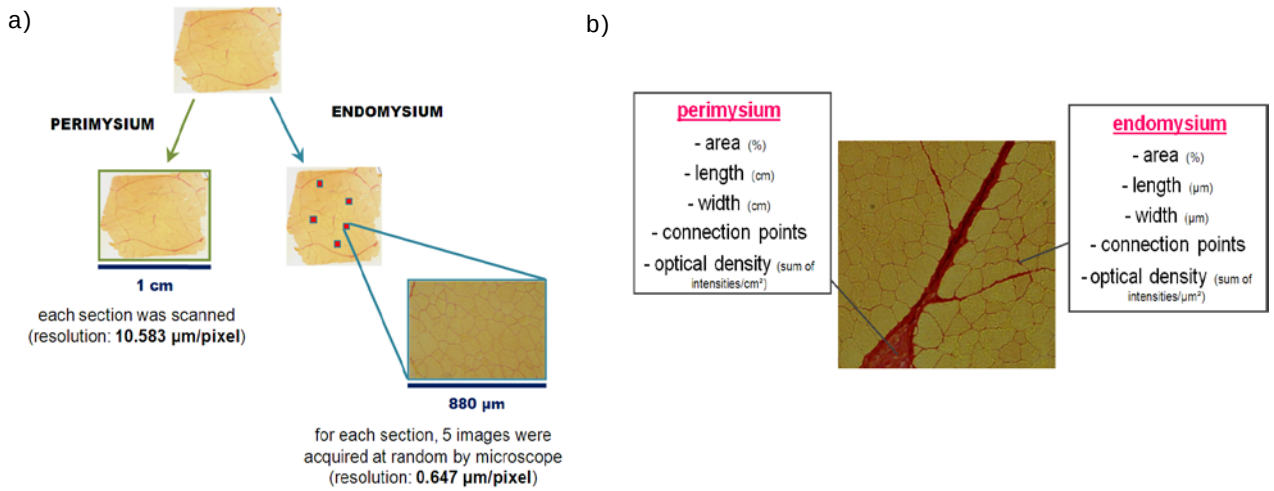

Fig. 1: Image analysis method to study perimysium and endomysium. For perimysium, histological staining section was scanned. For endomysium, 5 images were acquired by microscope (a). Images were analyzed with our home-made computer algorithm and each structure was characterized by area, length, width, connection points and optical density (b).

PRO) and for endomysium, staining sections were acquired by microscope (Olympus BX 51). Images were analysed with home-made computer algorithm [3], developed under Visilog 6.7 Professional Software (Noesis, France).
Figure 1 summarizes acquisition of images (fig. 1a) and extracted parameters for perimysium and endomysium (fig. 1b). Perimysium and endomysium were characterized by their area (expressed as % of total area of image), optical density (expressed as sum of intensities per cm² for perimysium or per µm² for endomysium), length (expressed in cm for perimysium and µm for endomysium), width (expressed in µm for perimysium and endomysium) and connection points (expressed as a number). This last parameter indicated the degree of ramification of perimysium and endomysium network.

Total and insoluble collagen contents were measured as described by Listrat et al [4]. The data were expressed in mg of hydroxy-proline (OH-pro) per g of dry matter of muscle.

Cross-link content was measured by ELISA method (MicroVue PYD EIA Kit) used to study the urinary excretion of pyridinolic links in bone resorption and adapted in the laboratory to study the pyridinolic links in bovine muscle. The data were expressed in nM of pyridinoline per g of dry matter of muscle. Pyridinoline is a stable cross-link reflecting the solubility of collagen in association with insoluble collagen content.

Data were analysed using GLM procedure of Statistical Analysis System Institute (SAS) to find out differences within studied parameters. Results were presented as means ± standard error of the mean (SEM). Differences were claimed significant for a risk of error $\alpha = 0.05$.

In order to study relationships between parameters for each muscle, we were calculated Pearson's correlation coefficient based on values of observations and we made Pearson's test to know if dependence was significant (dependence was significant for a risk of error $\alpha = 0.05$).

III. RESULTS

Table 1 summarizes results for IMCT (perimysium and endomysium), collagen (total and insoluble) and cross-link contents.

BF and LT were significantly different regarding their morphological and biochemical parameters. *Biceps femoris* IMCT (perimysium and endomysium) had greater area, was denser, longer, wider and more ramified than *Longissimus thoracis* IMCT.

BF muscle contained more collagen than LT muscle and more insoluble collagen and cross-link contents.

In a second time, we had compared parameters from perimysium and endomysium obtained using image analysis with parameters from collagen and cross-link by Pearson's correlation coefficient (data not shown).

In LT muscle, morphological parameters were independents from biochemical parameters.
Perimysium variables were correlated each other. The same observation was made for endomysium and collagen variables.

Table 1: Morphological and biochemical parameters of intramuscular connective tissue

Descriptive parameters	LT[1]	BF[1]	SEM[1]	P - value
PERIMYSIUM				
area, %	6.10 [b]	9.40 [a]	0.20	< 0.001
optical density, sum of intensities/cm² (x10^6)	3.45 [b]	5.66 [a]	0.14	< 0.001
length, cm	15.38 [b]	20.84 [a]	0.43	< 0.001
width, µm	39.40 [b]	44.87 [a]	0.35	< 0.001
connection points, number	517 [b]	949 [a]	43	< 0.001
ENDOMYSIUM				
area, %	4.80 [b]	6.50 [a]	0.10	< 0.001
optical density, sum of intensities/µm²	1 412 [b]	2 000 [a]	38	< 0.001
length, µm	2.90 [b]	3.03 [a]	0.03	0.001
width, µm	1.65 [b]	2.13 [a]	0.03	< 0.001
connection points, number	328 [b]	366 [a]	10	0.001
CROSS-LINK				
pyridinoline, nM/g DM	18.97 [b]	37.62 [a]	0.96	< 0.001
COLLAGEN				
total collagen content, mg OH-pro/g DM	3.79 [b]	6.79 [a]	0.13	< 0.001
insoluble collagen content, mg OH-pro/g DM	2.79 [b]	4.38 [a]	0.11	< 0.001

[a-b] within a row, means without a common superscript differ (P < 0.05)
1: LT = *Longissimus thoracis,* BF = *Biceps femoris,* SEM = Standard Error of the Mean

In BF muscle, total collagen content was positively dependent from endomysium area and optical density (correlation coefficients of 0.38 and 0.33). The same observation was made for cross-link content (correlation coefficients of 0.48 and 0.44). Cross-link content was also dependent from endomysium width (correlation coefficient of 0.37). Insoluble collagen content was positively correlated with endomysium variables excepted with connection points (correlation coefficients comprised between 0.31 and 0.49).
So collagen quantity and quality in BF muscle are dependent from endomysium characteristics; if endomysium is more developed, muscle will contain more collagen which will be less soluble (because of insoluble collagen and cross-link contents more important).
We were observed in a second time that area, optical density and length of perimysium and endomysium were positively dependent each other.

Whatever the muscle we're considering, there was no correlation between the perimysium parameters and biochemical parameters.

IV. DISCUSSION

Results showed that BF muscle had got IMCT more developed than LT muscle.

Morphological parameters were in accordance with results obtained by Sifre-Maunier in 2006 [5]. But this author worked only on area, length and width of perimysium. Then our image analysis method provides additional information about perimysium (its optical density and its degree of ramification). Moreover, we were studied endomysium contrary to Sifre-Maunier who have studied only perimysium. The endomysium study on the same muscle section that perimysuim study is a newly approach, which permits to study 2 levels of IMCT: perimysium in millimetric level and endomysium in micrometric level. In

addition, this method seems promising since it allows separating the two muscles.

Results obtained regarding collagen content were in accordance with results of Torrescano et al [6]. These authors showed that BF muscle had greater collagen content (total and insoluble) than LT muscle. We used this result to biologically validate our image analysis method.

Results obtained for morphological and biochemical parameters were in accordance with the fact that muscle with little developed IMCT network and low collagen content (collagen which is more soluble) are more tender [7].

The study of perimysium and endomysium by image analysis brings additional information about IMCT properties. This information could help to explain the observed variability in tenderness of a muscle to another or within the same muscle.

The study of correlations between parameters showed that dependences are different from muscle to another. So, according to the muscle type, morphology and biochemistry of IMCT are different.

Correlations had showed that in BF muscle, biochemistry parameters are dependent from endomysium characteristics. That means that modifications of IMCT quantity are due to modifications in endomysium network which represents only 10% of total IMCT network (perimysial frame represents 90% of total IMCT network). So endomysium will be more sensitive than perimysium.

V. CONCLUSION

Image analysis method developed in the laboratory permitted us to study objectively perimysium and endomysium on the same histological section, and to discriminate two muscles contrasting in collagen content and in tenderness.

Results obtained are in accordance with collagen measurements (quantity and quality) and with bibliography knowledge about IMCT characteristics and tenderness.

Perimysium and endomysium study by image analysis brings complementary information about IMCT, especially about its structure.

It will be interesting to study relationships between morphological and biochemical parameters and meat tenderness measurements in order to try to explain tenderness variability.

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