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CONNECTIVE TISSUE: CONTENT AND ARRANGEMENT OF PERIMYSIUM IN BOVINE MUSCLES WITH SPECIAL EMPHASIS ON ELASTIN.

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INTRODUCTION

Each living muscle is adapted to its own specific and complex movements. This is reflected by great variations in the composition of both muscle fibers and connective tissue.

The basic organization of the connective tissue in skeletal muscle consists of a perimysium that connects the tough connective tissue cover of the muscle (epimysium) to the thin envelops (endomysium) which surround the individual muscle fiber. Collagen and elastic fibers are the major components of the connective tissue and form a network that is responsible for the transmission of tension in the muscle. In meat, both the collagen and elastin influence the texture (Bendall 1967, Sims and Bailey 1980, Offer 1984). It has proved difficult to assess this influence by direct biochemical methods. This is mainly because such methods do not reveal the distribution of collagen and elastin within the connective tissue at each level of organization in various muscles.

When meat is chewed, it is fractured into smaller pieces. When forces are applied perpendicularly to the fiber direction the fracture zone usually lies within the boundaries between the muscle bundles i.e. the perimysium (Purslow 1985, for review see Offer 1984). Tenderness of meat therefore depend on the strength of the perimysium or the perimysium/muscle fiber bundle interface.

In the present work the content, composition and arrangement of the perimysial connective tissue were by means of morphometric techniques on histologically stained sections. Knowledge of the arrangement of the elastic fibers within the connective tissue may provide insight both to how the network functions in the living muscle and give a better understanding of its contribution to meat texture.

MATERIALS AND METHODS

M. semitendinosus (ST), m. semimembranosus (SM), m. vastus lateralis (VL), m. glutaeus (GM) and m. psoas major (PM) were removed from two year old Norwegian bulls (NRF) half an hour after slaughter. Within 1 h after slaughter 15 pieces (each 1 cm^3) from various parts of three different cross- sections (proximal/distal or cranial/caudal) of each muscle were frozen in liquid propane precooled in liquid nitrogen. The frozen muscle pieces were then transferred to a cryostat or stored in liquid nitrogen. Frozen sections, 4-10 µm thick, were allowed to dry on glass slides and stained with Mallory-aniline-blue (Mallory 1936) for determination of volume fraction of perimysium. Collagen and elastin were specifically stained with Sirius red (Sweat et al. 1964) and orcein (Romeis 1948) or Verhoff elastin stain (Mallory 1944), respectively. Sections were also stained for myofibrillar adenosine triphosphatase activity (ATPase) after both alkaline (pH 10.3) and acid (pH 4.3 and 4.6) preincubation (Brooke and Kaiser 1970) for determination of muscle fiber types. Photomicrographs were taken of all sections. A minimum of 200 fibers were counted from each section.

Measurements of connective tissue components.

The volume fraction occupied by the perimysium and the ratio of elastin to collagen were measured by morphometric techniques that allow accurate quantitation. In the present procedure, pictures of the sections were examined by means of a computerized digital image analyzer. The black and white images sensing various gray levels were converted to binary image after selecting a threshold value (Risk 1976, Uitto et al. 1983). The binary image were digitized and the data analyzed by a computer program designed toexpress the properties of the image, thus allowing determination of the volume fraction occupied by the component studied. The thickness of the perimysium were measured with a morphometric analyzer (MOP-AM03, Kontron) from lines, 1 cm apart, drawn perpendicular to the fiber direction. The area of the primary muscle bundles were also measured and the mean area calculated.

RESULTS

The perimysium which interconnect the epi- and endomysium penetrate into the muscle, separating bundles of muscle fibers (Fig. 1). Each primary muscle bundle may also be divided by thinner septa of perimysium into smaller fiber bundles (i.e. secondary muscle bundles) The size of the primary muscle bundles can be ranked in the following descending order: PM>GM>VL>ST>SM. The perimysium is a connective tissue matrix which interconnect the muscle bundles. The perimysium varies both in volume fraction (%) and thickness among the examined muscles. The volume fraction occupied by the perimysium can be ranked in the following descending order: SM>VL>ST>GM>PM (8.6% in SM and 3.4% in PM). The thickness of the perimysium followed the same order.

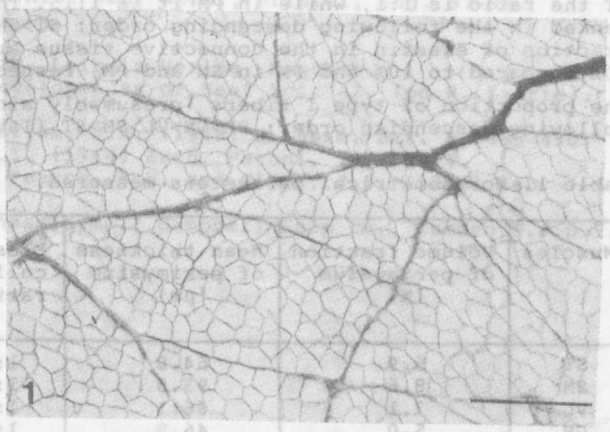
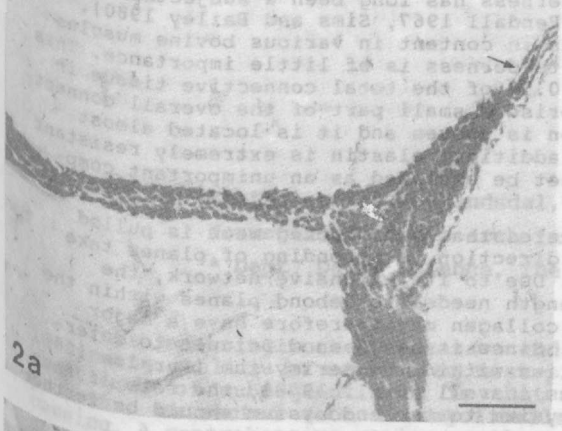


Fig.1. Cross-section of m. semimembranosus to show the perimysial connective tissue which separate bundles of muscle fibers. Mallory-anailine-blue. Scale bar: 500 µm.

The collagen fibers of the perimysium in SM,VL and ST consist of thick, wavy bundles. In GM and PM these are smaller in diameter indicating a more flexible network. The elastic fibers intermingle with the collagen fibers and are mainly located in the epi- and perimysium. Occasionally, small elastic fibers are found scattered in the endomysium. Most of these pierce from the perimysium into the endomysium of the fibers in the periphery of the muscle bundles. The elastin has generally a wavy appearance that disappears when the muscles are streched. The elastin fibers are extensively crosslinked forming a continuous three-dimensional network. In ST and SM, bundles of elastic fibers with a large diameter (ø 7-8 µm) are found (Fig. 2 a,b). In ST these bundles form several layers (Fig. 2 a) and are spirally arranged around each primary muscle bundle. In SM the bundles are small and located at the at the junction between the epi- and perimysium (Fig. 2 b). In VL, GM and PM the elastic fibers (ø 1-2 µm) are



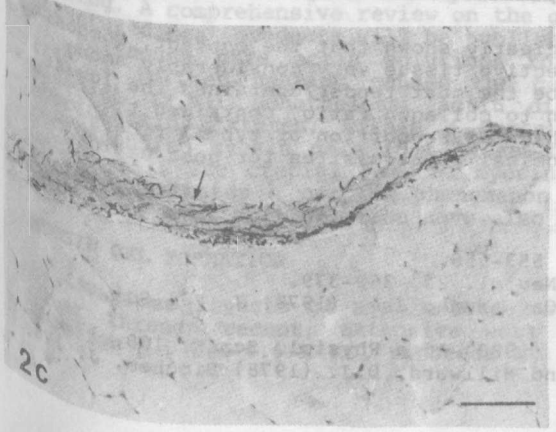


Fig. 2a. Elastic fibers (densely coloured) in the perimysium of m. semitendinosus. Note that the fibers forms distinct layers (arrow) and have large diameter.Verhoff elastin stain. Scale bar: 100µm

Fig. 2b. Bundles of elastic fibers aggregated at the junction between the epi- and perimysium (E/P) in m. semimembranosus. Verhoff elastin stain. Scale bar: 100 µm

Fig. 2c. Thin elastic fibers evenly distributed in the perimysium of m.psoas major (arrow). Verhoff elastin stain. Scale bar: 100 µm

evenly distributed in the perimysium (Fig. 2 c).

The elastin to collagen ratio varies considerably among the examined muscles. In ST the ratio is 1:1, while in PM it is 1:15. The elastin to collagen ratio can be ranked in the following descending order: ST>SM>GM>VL>PM (Table 1). The volume fraction of elastin in the connective tissue area of ST is in other words about 50% compared to 10% and 6% in SM and PM, respectively.

The proportion of type I fibers (presumably slow oxidative) can be ranked in the following descending order: PM>GM>VL>SM>ST (Table 1).

Table 1. Morphometrical parameters measured.

Muscles	Volume fraction of perimysium (%)	Mean thickness of perimysium (μm)	Elastin: collagen ratio	Mean size of primary muscle bundle (μm^2 x 1000)	Proportion of type I fibers (%)
ST	5.9	54.8	1:1	154	18.3
SM	8.6	93.5	1:9	105	20.6
VL	7.1	66.1	1:14	329	26.9
GM	5.0	46.9	1:11	385	36.4
PM	3.4	31.3	1:15	534	44.7

DISCUSSION

Other investigators (Laurent et al. 1978, Kovanen et al. 1980) have shown that the amount of collagen measured by biochemical methods is higher in slow than in fast contracting muscles. Slow contracting muscles are also characterized by a high content of type I fibers (slow oxidative fibers). Our results indicate an inverse relationship between the volume fraction of perimysium and the content of type I fibers. The higher content of collagen reported from slow contracting muscles may therefore primarily be due to a higher collagen content in the endomysium. A high content of endomysial connective tissue may be required to support the extensive capillary network demanded by the type I fibers.

The contribution elastin makes to meat tenderness has long been a subject to controversy (Standine et al. 1949, Vanable 1963, Bendall 1967, Sims and Bailey 1980). Bendall (1967) who determined the total elastin content in various bovine muscles claims that elastin's contribution to meat tenderness is of little importance. This is partly because it only contribute about 0.5% of the total connective tissue in most muscles. Even though elastin only comprise a small part of the overall connective tissue in most muscles, its distribution is uneven and it is located almost exclusively to the epi- and perimysium. In addition, elastin is extremely resistant to degradation. The elastin can therefore not be excluded as an unimportant component of the connective tissue of meat.

Studies of fracture behaviour of meat indicates that when cooked meat is pulled apart by forces perpendicular to the fiber direction, a debonding of planes take place within the perimysium (Purslow 1985). Due to its extensive network, the elastin is well suited to increase the strength needed to debond planes within the connective tissue. The ratio of elastin to collagen may therefore have a major influence on the tenderness of cooked meat. Since it has been difficult to determine accurately whether the fracture zone lies within the perimysium (Purslow 1985) or between the perimysium and the endomysium (Caroll et al. 1978), the role of the observed elastin protrusions from the perimysium to the endomysium should be further investigated.

The results obtained from our studies have clearly shown that the content, composition and arrangement of the perimysial connective tissue vary considerably among the examined muscles. The muscles known to be the most tender contained the lowest amount of perimysium, had the lowest elastin to collagen ratio, contained the largest primary muscle bundles and had the highest proportion of type I fibers. These characteristics may therefore be anatomical prerequisites for good eating quality.

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