DETERMINATION OF CONTRACTILE AND METABOLIC CHARACTERISTICS OF THE LAMB SEMITENDINOSUS MUSCLE : RELATIONSHIP BETWEEN THREE METHODS

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SUMMARY

The present study intends to define and compare different variables enabling an accurate muscle typing assessment. Metabolic and contractile characteristics in three locations of the middle cross-sectional area of the semitendinosus muscle from an homogeneous group of lambs were studied. The contractile properties were studied by the analysis of myosin heavy chain isoforms (MHC) performed on electrophoresis. The glycolytic property was measured by immunochemical quantitation of the lactate dehydrogenase isoform M4. These variables were compared to the muscle fibre type determined histochemically. Type I and muscle heavy chain isoforms (MHCI and MHCIIa) fluctuated in the same way within the muscle. In contrast type IIB, MHCIIb and the level in M4 had an opposite distribution. No significant difference of the IIA and IIB oxydative fibre distribution was observed within the muscle. The studied variables discriminated the muscle heterogeneity with comparable and complementary efficiency.

INTRODUCTION

Mammalian skeletal muscles are heterogeneous, with regard to their fibre types and their metabolic and contractile characteristics. Development of histochemical analysis for specifically staining myosin ATPases and metabolic enzymes has established that physiological properties of muscles depend on biochemical characteristics of myofibres. On the basis of these characteristics, muscles fibres have been classified into three main categories : slow twitch oxidative (SO=type I), fast twitch oxidative and glycolytic (FOG=type IIA) and fast twitch glycolytic (FG=type IIB) (Peter et al., 1972 ; Brooke and Kaiser, 1970). Additional intermediate categories have been described.

The different ATPases activities are represented by the myosin molecule. This hexamere contains two heavy chains (HC) and four light chains (LC). The HC contains actin binding sites and sites for ATPase hydrolysis. Three major histochemically distinguishable fibre types can be delineated which correlate with the expression of the three major myosin HC isoforms MHCI, MHCIIa and MHCIIb (Staron and Pette, 1986).

The polymorphism of the glycolytic enzyme lactate dehydrogenase (LDH) is also of great interest. Five isoforms have been described : H4, H3M, H2M2, HM3 and M4. These isoforms result from the combination of the H (heart) and M (muscle) monomer. The H4 isoform predominates in aerobic muscle, while the M4 isoform predominates in anaerobic muscles (Petter et al., 1971).

Fibre type proportions are known to vary widely between muscles and within individual animal muscles according to their functional properties (Ariano et al., 1973 ; Hunt et al., 1977 ; Lopez-Rivero et al., 1992 ; Petter et al., 1993). The fibre type composition of the semitendinosus muscle varies extensively in domestic mammals, especially as a function of depth (Totland et al., 1988). The superficial portion (close to the carcass surface) is light, and the deep portion (close to the bone) is dark.

Fibre type composition of muscles affects different traits of meat quality. This connection between meat quality characteristics and the muscle composition has been established by several authors (Cassens and Cooper, 1971 ; Ashmore, 1974 ; Valin et al., 1982 ; Totland et al., 1988). In order to make comparisons, any study of meat quality traits will required, standardized procedures of muscle typing, which should be achieved rapidly.

To enable an accurate muscle typing assessment, the present study intends to test different variables. The semitendinosus muscle is considered as a model for its heterogeneity in fibre type composition. Contractile properties will be studied by myosin heavy chain electrophoresis. The glycolytic property will be measured by

immunochemical quantitation of the M4. These results will be compared to the muscle typing obtained by histochemical.

MATERIAL AND METHODS

Animals and muscles

9 cross breed male lambs (Romanov*Berrichon) were obtained from the experimental herd of the Genetic Research Center of Langlade (INRA-Toulouse). Animals which had similar weight at birth and similar growth rate, were sacrified at the same weight of 41 kg (about 4 months).

Left semitendinosus muscles were excised just after slaughter and trimmed of fat and connective tissue before sampling.

Sample preparation

A 3 cm thick slice was cut transversely to the muscle fibre direction, in the midbelly region of the muscle. From each of the slices, 3 sticks were removed from superficial central and deep location of the muscle, in points 1, 2 and 3, respectively. Each of the 3 sticks was subdivided perpendicular to the muscle fibre direction, in 3 cubes (1cm3) which were analysed according to the 3 methods.

Histochemistry

Samples were immediately frozen in liquid nitrogen in a slightly stretched position and stored at -80°C. These blocks were further sectioned using a Frigocut microtome at -20°C. The transverse frozen sections (10mm), after drying, were stained for succinic dehydrogenase (SDH) to determine the oxydative capacity of the various fibres types. Sections were stained too, for the determination of myofibrillar ATPase activity, after preincubation at pH 4.40 (Brooke and Kaiser, 1970).

Photomicrographs were taken of each section and the fibre types identified on the basis of acid preincubation at pH 4.40 for myosin ATPase.

The relative frequency of various fibre types (I, IIA, IIB) was determined from an average of 300.

The presence of IIC fibres was inconsistent and included in the percentage of IIB fibres (Essen-Gustavson and Lindholm, 1985). Type IIB fibres were then subdivided into 2 subgroups according to the extend of their staining for SDH. Fibres with low intensity SDH staining were considered as IIB non-oxidative fibres, and the remaining type IIB fibres were classified as type IIB oxidative (Lopez-Rivero et al. 1992).

A minimum of 100 diameters were measured from each sample. Fibre diameters were obtained by the mean of the major and the minor diameters of fibre. Mean areas of each type were then calculated. The area proportion of the different fibre types was calculated according to the proportional number of each type and the mean area of each fibre type in each section.

Electrophoresis

Samples were extracted according to Bar and Pette (1988). Protein concentration were determinated using the method of Bradford (1976). The extracts were incubated 10 min at about 60°C in a sample buffer containing a final concentration of 30 % (v/v) glycerol, 5 % (v/v) ß-mercaptoethanol, 2.3 % (v/v) SDS, 62.5 mM Tris-HCL (pH 6.8) and 0.05 % (w/v) bromophenol blue.

Electrophoresis was loaded with 10mg of proteins in each well. SDS-PAGE was performed on plates of 16cm*19.5cm*0.8mm according to Laemli (1970) . 5-8 % polyacrylamide gradient was used for the separating gel (Bar and Pette, 1988) and the stacking gel was at 3.5 %. The separating gel also contained a 30-40 % glycerol gradient and the stacking gel was at 35 % (v/v) glycerol (Sugiura and Murakami 1990).

The running conditions were 150 V for 24 h at 5°C. To aid in identification of separated proteins, molecular weight standards (57-212 kDa PHARMACIA) were electrophoresed. The gel was stained in a solution of R250 Coomassie Blue. The percentage distribution of MHC isoforms was calculated by a densitometer SHIMADZU, CS9000.

Single Radial Immunodiffusion

Crude extracts were prepared by homogenizing the muscle 1:10 (v/v) in a 20 mM Tris-HCL (pH 8.5) buffer. The homogenates were centrifuged at 20 000g. 3ml of the supernatant fraction filtered through Glass Woll were applied in each wells.

The Mancini et al. (1965) method was performed to evaluate the M4 level. The used rabbit anti M4 bovin serum was produced by the Immunochemistry Laboratory of the S.R.V. (INRA-Theix). A standard used to evaluate the M4 level was obtained by mixing two muscles : the vastus intermedius (VI) poor in M4 isoform and the tensor fasciae latae (TFL) rich in M4 isoform (VI 100 %, VI 75 % + TFL 25 %, VI 50 % + TFL 50 %, VI 25 % + TFL 75 % , TFL 100 %). The ring diameter of VI muscle was defined as the minimum value (0) of the standard and the ring diameter of TFL muscle was defined as the maximun value (10) of the standard.

Statistics

Means and standard deviations were calculated from individual values by standard procedures. One way analysis of variance (ANOVA-SAS) to compare multiple samples was used to test the hypothesis that no difference is present in fibre type proportion or in HC type proportion or in M4 level between the different depths of the muscles (points 1, 2 and 3). The null hypothesis was rejected at the 0.05 level of significance. The Waller-Duncan t test was performed to compare group means 2*2.

Results

Histochemistry

The three main fibre types (I, IIA, and IIB) and the two subgroups (IIB non-oxidative and IIB oxidative) were present in all 3 parts of the muscle.

From the SDH stain, the tetrazolium deposits in IIA fibres were heavier than in I fibres. The intensity of the enzyme activity in the sheep semitendinosus was found to be more important in IIA fibres than in I fibres (Suzuki, 1970).

Significant differences in muscle fibre type composition were recorded specially between the superficial and the deep location. Differences between the central (point 2) and the extreme locations were not significant (table 1).

The more superficial portion (point 1) contained fewer I fibres (about 7 %) which increased in the deep portion (point 3) to about 15 %. The IIB non oxidative fibres were distributed within the muscle opposite to the type I fibres, about 46 % towards the superficial portion and 36 % towards the deepest. Relative frequencies of the IIA and IIB oxidative fibres were approximately similar within the different depths, differences between the 2 extreme locations were not significant.

Electrophoresis

In the three parts of the semitendinosus muscle, the MHC isoforms were separated in three types on the 200 kDa region. The electrophoresis profiles showed two slow migrating bands and one fast migrating band. In the three sample locations, the first slow migrating band was the most important and the fast migrating band the less important on the electrophoretic profile.

Lamb semitendinosus is classified as a fast white muscle with a high amount of type II fibres (Suzuki, 1975 ; Briand et al., 1981). According to the histochemical results of this study, the overall order of migration by MHC type was I>IIa>IIb. There was no change in percentages of MHCI and MHCIIa between the points 1 and 2 (table 2). But MHCI and MHCIIa increased significantly ($P<0.05$) from these two points to the deep location (point 3). Contrasting with this two MHC isoforms, the MHCIIb decreased significantly ($P<0.05$) between the two points (1-2) and the point 3. No significant difference was observed between the superficial and the central locations (points 1 and 2).

Single Radial Immunodiffusion

M4 levels in the 3 sample locations were high and near the TFL level. The M4 level increased significantly ($P<0.05$) from the deep to the superficial location. The central value was not significantly different from the deep value (table 2).

DISCUSSION

The central values of the variables I, IIB, MHCI, MHCIIa, MHCIIb and M4 level were always intermediate between those of the superficial and the deep points, it may suggest a gradient of contractibility and metabolism from superficial to deep locations in the muscle.

Histochemical and electrophoresis studies have shown comparable contractibility evolution from the superficial to the deep locations of the semitendinosus lamb muscle.

Percentages of type I fibres and MHCI isoform are higher in the deep location than in the superficial. In contrast, percentages of type IIB fibres and MHCIIb isoform are higher in the superficial location than the deep. These results are in accordance with those of Gunn (1978) and Totland et al., (1988) on the fibre type repartition in the semitendinosus muscle.

Histochemical and immunodiffusion studies have shown the oxidative and the glycolytic metabolism, respectively. The SDH stain revealed oxidative fibres (types I, IIA and IIB oxidative). Only the percentage of type I varied. Therefore the change of oxidative metabolism within the muscle resulted from the change in the distribution of type I. The M4 variation was opposite to the type I variation.

According to the studies of Briand et al., (1981 a, b) a relationship between the glycolytic capacity (M4 level) and the fast contractibility (type IIB and HCIIB percentages) was found.

There was no variation in the IIA and the IIB oxidative fibre distribution within the muscle. By contrast, the MHCIIa isoform percentage increased from the superficial to the deep location of the muscle. Differences between the results of the two methods can probably reflect MHC heterogeneity within the fibre. Billeter et al. (1981, 1982) revealed by single fibre analysis that the three major histochemically distinguishable fibre types I, IIA and IIB express only MHCI, MHCIIa and MHCIIb, respectively. However the muscle also contains "hybrid" fibers in which two MHC isoforms are expressed in different proportions (Staron et al., 1986, 1992). The histochemical ATPase staining conditions, with only one preincubating pH, were not accurate to reveal the intermediate fibre types of the three main types.

The present study led to the conclusion that the MHC isoforms analysed by electrophoresis and immunochemical quantitation of M4 by immunodiffusion may be accurate methods to discriminate the contractibility and glycolytic metabolism heterogeneity of the muscle. However the histochemical technic remains necessary to visualize the fibres, their types and their organization inside the bundles.

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