

Pattern of fibre type distribution within muscle fascicles of pigs (*Sus scrofa domestica*)

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ABSTRACT: In order to determine breed differences in the fascicle structure of skeletal muscles, longissimus dorsi muscles (LD) and semimembranosus muscles (SM), Duroc (D), Large White (LW), German Landrace (GL) and Swedish Landrace (SL) boars were analyzed. Fibre type delineation was based on the method of myofibrillar ATPase staining. The fibres in a fascicle were divided into layers according to their relation to the perimysium. In each layer, the proportions of fibre types I, IIA and IIB were determined and normalized according to the fibre type proportion in the whole fascicle. Generally, a high proportion of IIB fibres on the periphery, a prevalence of type I and IIA in the layer below, and altered proportions of type I, IIA and IIB in layer 3 were found to be the main characteristics of the distribution of fibres in a porcine fascicle. Breed dissimilarity was established in the arrangement of fibre types, particularly in the first and third layer. The breeds that showed the highest proportions of type I and IIB fibres in LD and SM muscles were GL and SL boars.

Keywords: pig; skeletal muscle; fascicle; muscle fibre types; spatial distribution

The skeletal muscle is composed of muscle fibres that differ in their speed of contraction and resistance to fatigue. From the histochemical aspect, muscle fibres can be classified into two main types: slow-twitch (type I) fibres display a low myofibrillar ATPase (mATP) activity under alkaline conditions and a high activity under acid conditions, whereas fast-twitch (type II) fibres exhibit the opposite. A more detailed study of the acid stability of mATP activity indicated a delineation of fast fibre subtypes, called type IIA and type IIB. The analysis of single muscle fibres demonstrated that specific myosin heavy chains I, IIA and IIB correspond to histochemically defined fibre types I, IIA, and IIB, respectively (Staron and Pette, 1986; Termin et al., 1989). An additional myosin heavy chain IID was electrophoretically identified and immunohistochemically characterized as type IID/2X (Bär and Pette, 1988; Termin et al., 1989; LaFramboise et al., 1990). Fibre types differ not only in their my-

ofibrillar protein isoform pattern but also in their metabolic profiles (for review see Pette and Staron, 1990).

In a normal muscle tissue, the fibres of a single motor unit are of one histochemical type and they are intermixed with fibres of other motor units (Lester et al., 1983). Most mammalian skeletal muscles exhibit a random model of the spatial distribution of fibre types. However, it is well known that pigs have a unique pattern of the spatial arrangement of fibre types within the skeletal muscle fascicles. It was observed in the longissimus (Essén-Gustavsson and Lindholm, 1984), psoas major (Iwamoto et al., 1983) and semitendinosus muscle (Beermann et al., 1978; Dwyer and Stickland, 1991) of pigs that type I fibres within fascicles had a characteristic central position and were surrounded by type II fibres. Moreover, type IIA fibres were contiguous to type I fibres, pure IIX fibres were in the direct vicinity of type I and IIA fibres, and hybrid IIX/IIB fibres

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were located mostly between the islets of type I fibres (Lefaucheur et al., 1998). With the exception of porcine muscles, the specific fibre type grouping has been linked to myopathies, diseases of the lower motor neurone, and other neuromuscular disorders in muscles. In addition, the type grouping observed in porcine skeletal muscles is not a result of multifibre innervation by subterminal axons, but it may rather be the manifestation of normal unique motor unit topography (Beermann and Cassens, 1977).

In the early 1980s, the first quantitative fascicle structure analysis was applied to compare type I fibre distribution in the peripheral and central parts of porcine muscle fascicles (Iwamoto et al., 1983). Using James' index of segregation, a distinct arrangement of type I fibres was estimated in the peripheral and central parts of the psoas major fascicles. Pigs of Berkshire and Large White breeds had higher index values in comparison with Duroc and Landrace pigs. These breeds had a stronger tendency for type I fibres to aggregate in the central region of the fascicles. In view of these findings, there arises a question whether the fibre type arrangement in layers within the fascicles of muscles with different locomotion functions follows a similar or different pattern. Fiedler (1988) described the arrangement of fibre types based on the NADH dehydrogenase reaction in different porcine skeletal muscles.

To our knowledge, no systematic study has been published on differences in the arrangement of type I fibres, type IIA fibres and type IIB fibres in layers within fascicles in porcine skeletal muscles.

The purpose of the present study is to use the histochemical fibre typing scheme to extend the observations done by Iwamoto et al. (1983), i.e. to determine the normalized proportions of fibre type I, IIA and IIB within layers of fascicles in the *longissimus dorsi* muscle and *semimembranosus* muscle. In order to correlate the fibre type pattern in the fascicle with the breed effect, we studied changes in fascicle layer levels in muscles of Duroc, Large White, German Landrace and Swedish Landrace pig breeds.

MATERIAL AND METHODS

Animals and muscles

The experiments were performed on adult male pigs of Duroc (D), Large White (LW), German

Landrace (GL) and Swedish Landrace (SL) breeds. One hundred boars belonging to four distinct breeds were included in this study. Twenty-five boars of each breed were used. The animals were housed in individual boxes and they were allowed access to food and water *ad libitum*. Boars were killed at an average live weight of 100 kg. Samples of the longissimus dorsi muscle (LD) and semimembranosus muscle (SM) were excised and immersed in liquid nitrogen. The LD muscle samples were obtained behind the last rib from the centre of the transverse muscle section and SM muscle samples were taken approximately 5 cm from the pubic bone.

Histochemical analysis

Cross-sections (10 µm thick) were cut on a microtome in a cryostat at –25°C. Serial 10 µm thick cross-sections of LD and SM muscles were air dried and subsequently stained for myofibrillar actomyosin ATPase (mATPase). The method was based on a modification of the histochemical staining for mATPase activity, originally described by Padykula and Herman (1955) and Guth and Samaha (1969). After preincubation steps at pH 9.4, 4.55, 4.50, 4.45, 4.42, 4.40 and 4.35, muscle fibres were classified as type I, type IIA and type IIB according to Brokke and Kaiser (1970). Fibre type I was stained lightly after alkaline preincubation (pH 9.4) and deeply after acid preincubations. Fibre type IIA was stained deeply after alkaline preincubation and lightly after acid preincubation. Type IIB fibres were stained deeply after alkaline preincubation with intermediate intensity following preincubations at pH 4.55 and 4.50, and lightly after preincubation at pH 4.45, 4.42, 4.40 and 4.35. In each area, a fascicle well-defined by its perimysium was photographed with an Opton photomicroscope at magnification 115×.

Analysis of fascicles

Stained sections were photographed with an Opton photomicroscope at magnification 115× and the arrangement of fibre types within fascicles was analysed from photos by a semi-automatic method. Three well-distinguished muscle fascicles were randomly chosen on each sample. A total of 600 fascicles and an average of 67 muscle fibres within each fascicle were analysed. The apparatusive setup

Table 1. Number of muscle fibres within fascicles of longissimus dorsi muscles (LD) and semimembranous muscles (SM) in different pig breeds ($n = 25$)

	Duroc	Large White	German Landrace	Swedish Landrace
LD	70.30 ± 3.13 ^a	53.10 ± 3.17 ^b	67.65 ± 3.10 ^a	75.63 ± 3.08 ^a
SM	41.42 ± 3.13 ^{ab}	39.73 ± 3.17 ^b	49.19 ± 3.10 ^a	49.74 ± 3.08 ^a

Data are LSM ± SE; values are compared between columns; values with different superscripts differ significantly ($P < 0.05$)

and procedure for a quantitative analysis of fascicle structure were previously described in detail (Pernuš and Eržen, 1991). In short, each muscle fascicle was decomposed into N layers and the proportion of types I, IIA and IIB was determined in layer 1, 2 and 3. The fibres in contact with the perimysium were defined as the first layer, the fibres adjacent to this layer constituted the second layer, and those bordering the second layer composed the third layer.

For fibres of each type t ($t = I, IIA, IIB$) and for each layer u ($u = 1, 2, 3$), the normalized fibre type proportion $x(t, u)$ was calculated using the equation:

$$x(t, u) = p(t, u)/P(t)$$

where:

$p(t, u)$ = the proportion of type t fibres in layer u

$P(t)$ = the proportion of type t fibres in the whole fascicle

Normalized proportions were used to compare fascicles with different frequency distributions of fibre types. The value higher than 1 indicates a higher fibre type proportion in a layer than in the whole fascicle and the value lower than 1 indicates a lower proportion in a layer than in the whole fascicle.

Statistical analysis

All results are given as least-squares means ± SE. The results were analyzed for variance using the general linear models (GLM) procedure (SAS/STAT, 1990) to examine the influence of breed on normalized proportions of fibre types in layers within fascicles. This procedure was applied because an unequal number of observations (muscle fibres) was analysed in the particular muscles. To test which breed differed significantly ($P < 0.05$), Duncan's multiple range analysis test was applied.

RESULTS

Data on the LSM number of fibres per fascicle are presented in Table 1. Muscle fibre numbers within fascicles differed significantly between pig breeds as well as between LD and SM muscles. Muscle fascicles of Duroc, Large White, German Landrace and Swedish Landrace breed were composed of approximately 40 to 80 fibres. The pigs of Large White breed displayed the significantly lowest number of fibres within fascicles of both

Table 2. Normalized fibre type proportions in layers within fascicles of longissimus dorsi muscles ($n = 25$)

	Duroc	Large White	German Landrace	Swedish Landrace
Layer 1				
I	0.10 ± 0.04 ^{ab}	0.05 ± 0.04 ^a	0.15 ± 0.04 ^b	0.11 ± 0.04 ^{ab}
IIA	0.28 ± 0.26 ^a	0.29 ± 0.26 ^a	0.32 ± 0.26 ^a	0.30 ± 0.26 ^a
IIB	1.18 ± 0.06 ^a	1.20 ± 0.06 ^a	1.18 ± 0.06 ^a	1.17 ± 0.06 ^a
Layer 2				
I	1.36 ± 0.10 ^a	1.38 ± 0.10 ^a	1.24 ± 0.10 ^a	1.26 ± 0.10 ^a
IIA	1.49 ± 0.10 ^a	1.44 ± 0.09 ^a	1.40 ± 0.09 ^a	1.31 ± 0.09 ^a
IIB	0.86 ± 0.04 ^a	0.92 ± 0.04 ^a	0.94 ± 0.04 ^a	1.05 ± 0.04 ^b
Layer 3				
I	2.67 ± 0.42 ^{ab}	3.14 ± 0.42 ^{ab}	3.61 ± 0.41 ^a	2.39 ± 0.40 ^b
IIA	1.71 ± 0.37 ^a	2.91 ± 0.37 ^{ab}	1.90 ± 0.36 ^b	2.36 ± 0.35 ^{ab}
IIB	0.64 ± 0.04 ^a	0.62 ± 0.04 ^a	0.68 ± 0.04 ^{ab}	0.75 ± 0.04 ^b

Data are LSM ± SE; values are compared between columns; values with different superscripts differ significantly ($P < 0.05$)

Table 3. Normalized fibre type proportions in layers within fascicles of semimembranous muscles ($n = 25$)

	Duroc	Large White	German Landrace	Swedish Landrace
Layer 1				
I	0.14 ± 0.04 ^a	0.14 ± 0.04 ^a	0.18 ± 0.04 ^a	0.11 ± 0.04 ^a
IIA	0.26 ± 0.17 ^b	0.27 ± 0.16 ^b	0.12 ± 0.06 ^a	0.24 ± 0.16 ^b
IIB	1.14 ± 0.06 ^a	1.18 ± 0.06 ^{ab}	1.32 ± 0.06 ^b	1.15 ± 0.06 ^{ac}
Layer 2				
I	1.49 ± 0.10 ^a	1.45 ± 0.10 ^a	1.31 ± 0.10 ^a	1.30 ± 0.10 ^a
IIA	1.44 ± 0.10 ^a	1.55 ± 0.10 ^a	1.52 ± 0.09 ^a	1.64 ± 0.10 ^a
IIB	0.87 ± 0.04 ^a	0.91 ± 0.04 ^a	0.92 ± 0.04 ^a	0.94 ± 0.04 ^a
Layer 3				
I	3.00 ± 0.42 ^a	3.76 ± 0.42 ^a	3.89 ± 0.41 ^a	3.78 ± 0.40 ^a
IIA	3.20 ± 0.37 ^a	2.57 ± 0.37 ^a	2.39 ± 0.36 ^a	2.41 ± 0.35 ^a
IIB	0.50 ± 0.04 ^a	0.59 ± 0.04 ^{ab}	0.70 ± 0.04 ^b	0.62 ± 0.04 ^{ab}

Data are LSM ± SE; values are compared between columns; values with different superscripts differ significantly ($P < 0.05$)

muscles. The lowest number of fibres per fascicle was observed in SM muscles, whereas LM muscles contained a larger population of fibres within the muscle fascicle.

Normalized proportions of type I, IIA and IIB are given as LSM ± SE and they are presented in Tables 2 and 3. Normalized proportions of fibre types differed markedly between layers within fascicles as well as between pig breeds. In layer 1 the relation between normalized fibre type proportions was as follows:

$$x(I, 1) < x(IIA, 1) < x(IIB, 1)$$

The proportion of type IIB fibres was on average 19% higher than in the whole fascicle, whereas the proportion of type I fibres was approximately 88% lower and that of type IIA fibres was 80% lower than in the whole fascicle. In layer 1, the muscles with the highest normalized proportion of type I fibres were LD (0.15) and SM (0.18) of the German Landrace breed. In this breed, the highest normalized proportion of type IIA fibres was contained in LD muscle. However, the differences between breeds were not statistically significant (Table 2). In layer 1, SM (1.32) muscles of German Landrace comprised the highest normalized proportion of type IIB fibres (1.32) in comparison with the other three breeds (Table 3). In layer 2, the following pattern of relationships prevailed:

$$x(IIA, 2) > x(I, 2) > x(IIB, 2)$$

Compared to layer 1, the proportions of fibre types I and IIA were markedly elevated in layer 2.

On average, the normalized proportion of type I was higher by 35% than in the whole fascicle, and the type IIA proportion was higher by 47% than in the whole fascicle. In layer 2, the normalized proportion of type IIB was reduced and this reduction continued with increasing populations of type I and IIA fibres. The proportion of type IIB fibres was lower by 8% than in the whole fascicle. In layer 2, increases in normalized proportions of type I and IIA fibres and decreases in normalized proportions of IIB fibres generally occurred in both muscles. Thus, fibre types I and IIA constituted the highest portion of the fibre population in layer 2. No significant difference was established between breeds in normalized proportions of type I and IIA fibres (Tables 2 and 3). The pronouncedly largest normalized proportion of type IIB fibres in layer 2 was contained in LD of the Swedish Landrace breed.

Compared to layer 2, the normalized proportions of type I, IIA and IIB were altered in layer 3. In LD and SM muscles, the proportion of type IIB fibres was reduced by approximately 36% compared with the whole fascicle, but this decrease was compensated by a corresponding increase in type I (2.3-fold) and IIA fibres (1.4-fold) (Tables 2 and 3). In layer 3, changes in the normalized proportion of type I fibres were induced by a breed effect. This was reflected by an increase in the normalized proportion of type I fibres in LD, as well as by a decrease in the normalized proportion of type IIB fibres in SM muscles of the German Landrace breed (Tables 2 and 3).

DISCUSSION

Muscle quantity is defined by muscle fibre number and fibre size. An increase in muscle mass can be achieved by increasing either the number of fibres or the average fibre area (Ashmore et al., 1973). No report has been found on the true total number of fibres in LD and SM muscles of adult pigs. A probable reason for this may be that the size of the whole muscle has rendered conventional histochemical preparative procedures inappropriate. It is impossible to prepare a cross-section of the whole muscle by using usual cryo-microtomes. Therefore, the smallest still separable muscle fascicle was chosen as a research unit in our study. The total number of fibres within the fascicle of LM and SM differed considerably between pigs breeds. Boars of Duroc, Large White, German Landrace and Swedish Landrace breed had 40 to 76 fibres distributed in three to four layers of the fascicle (Table 1). The present results agree with previous data on the muscle fibre number in porcine fascicles. The number of fibres in the smallest, still separable fascicles of LD muscle in pigs of Duroc, German Landrace and Swedish Landrace corresponds to the average of 73.4 fibres found by Miller et al. (1975). The lowest number of fibres in LD was reported for White Zlotnicka boars, only 33.8 fibres (Sosnicki, 1987). For SM it was established that fascicles of wild pigs were composed of approximately 50 to 75 muscle fibres (Solomon and Eastridge, 1987). The present study demonstrated a lower number of fibres within SM fascicles in pigs of Large White and Duroc breeds in comparison with wild pigs.

However, to our knowledge no study has directly assessed the muscle fibre number within a fascicle as a factor of meat quality alteration. Lengerken et al. (1994) demonstrated that the number of muscle fibres per unit area declined with increasing lean meat percentage, and at the same time the fibre diameter increased. Moreover, support to this finding came from an observation that the total number of fibres in the semitendinosus muscle had not altered during the postnatal growth and ageing of pigs (Fiedler et al., 1987; Rehfeldt et al., 1987). The underlying mechanism(s) of hypertrophy and possible postnatal hyperplasia in skeletal muscles of pigs remains unclear at the present time. Further studies will be necessary to find out whether hyperplasia can be combined with high meat yield and meat quality (Lengerken et al., 1994).

The normal muscle tissue has a mosaic distribution of different muscle fibre types (Lester et al., 1983). Generally, a fibre type is randomly dispersed among other types in the muscle fascicle (Lester et al., 1983; Venema, 1988). However, the first quantitative examination of the fascicle structure showed that the peripheral part of fascicles in the extensor digitorum longus muscles of rat, cat and rabbit were predominantly composed of type II fibres (James, 1971). A more precise analysis indicated that type IIB fibres were characteristically the prevailing fibre type in the first layer of fascicles below the perimysium in semimembranous muscles, gastrocnemius muscles and psoas major muscles of Norwegian bulls (Grotmol et al., 1988) and in superficial and deep portions of semitendinosus and gluteus muscles of standard bred horses (Grotmol et al., 2002). It was also demonstrated in the human vastus lateralis of young adults (Pernuš and Eržen, 1991; Čebašek et al., 1996), children (Snoj-Cvetko and Eržen, 1992) and human quadriceps muscles of males and females (Manta et al., 1995). The present results of our fascicle studies confirm and extend previous observations on the arrangement of fibre types in mammalian skeletal muscle fascicles. The observed accumulation of type IIB fibres in the periphery and of type I and IIA fibres in more central parts of fascicles in LD and SM muscles is influenced by a pig breed.

To our knowledge, the present study provides for the first time data on breed differences in the muscle fascicle geometry in skeletal muscles of domestic pigs. Our data indicated that layer 1 was composed of a low number of type I and IIA fibres. The significance of the observed fibre type distribution within this layer of the fascicle is not entirely clear. It may be associated with the ontogenetic development of mammalian skeletal muscles. A probable explanation is that during muscle growth some secondary fibres were transformed from fast-twitch to slow-twitch fibres, or that this arrangement may be a result of the phasic muscle formation (Ashmore et al., 1973; Lukáš et al., 1982) or a combination of both. However, muscle fibres are developed prenatally as two distinct populations: primary fibres are the progeny of type I fibres and serve as a structural framework for the formation of secondary fibres. This finding has been proved by research on large skeletal muscles. In addition, recent data have suggested that secondary fibres are used as a scaffold for the formation of a third generation of fibres (Draeger et al., 1987; Maier et al., 1992; Mascarello et al., 1992;

Wilson et al., 1992; Lefacheur et al., 1995; Picard et al., 2002). Therefore, the normal occurrence of type I, IIA and IIB fibres in layer 1 could be a result of phasic development of muscle fibre types from a few generations of myotubes, and the normal concomitant process of fibre type transformation which is present during muscle growth. The fibre type pattern may be influenced by the innervation of the fibres or by the formation of the capillary network (Beermann and Cassens, 1977; Sjöstrom et al., 1987). Type IIB fibres are probably the last to appear in myogenesis and show the highest glycolytic capacity. It is therefore tempting to speculate that type IIB fibres in the first layer are probably a consequence of muscle fibre adaptation to the rough perimysium, which has interrupted the supply of oxygen to the muscle fibres, thus causing poorer capillarization under the perimysium than inside the fascicle.

Despite differences in the normalized proportions of fibre types, the same general pattern of spatial distribution appeared in both muscles. The analysis confirmed that there was a higher proportion of type IIB fibres on the periphery than within the fascicles, and because the proportions of type I and IIA fibres and IIB fibres changed in the opposite direction, the percentage of type I and IIA fibres was markedly altered in the central part of the fascicles.

In summary, the results of this study demonstrate that porcine LD and SM muscles are predominantly composed of type IIB fibres. However, changes in the proportion of type I, IIA, and IIB occur in a fibre type specific manner. Type I and IIA fibres were primarily located in the deep muscle fascicle parts, and type IIB ones were predominant in the superficial muscle fascicle parts. Several spatial patterns of fibre type distribution in layers within the fascicles emerged. Breed dissimilarity was established in the arrangement of fibre types, particularly in the first and third layer.

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