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Original article

Fibre type differentiation during postnatal development of miniature pig skeletal muscles

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Summary — Histochemical differentiation of 12 skeletal muscles with a different fibre type composition was studied in miniature pigs from 80 d of gestation to 1 year of age. Two fetal myofibre types were distinguished at 100 d of gestation by the mATPase reaction after acid preincubation. The staining for oxidative enzyme activities showed no conspicuous differences between fibres up to the 6th day after birth. Starting from this age it was possible to distinguish 3 fibre categories: SO (slow-twitch oxidative); FOG (fast-twitch oxidative-glycolytic); and FG (fast-twitch glycolytic). A characteristic cluster distribution of the 3 fibre types was observed in all studied muscles with the exception of the masseter muscle which consisted only of the type SO and FOG fibres with a mosaic arrangement. The frequencies of both SO and FG fibre types increased and the proportion of type FOG fibres decreased during the postnatal period. These changes could be explained by developmental transformations among the individual fibre types. The type FOG fibres converted preferably to the fibre type (SO or FG) that prevailed in the muscles of adult animals.

muscle fibre type / differentiation / pig

Résumé — Différenciation des types de fibres pendant le développement postnatal des muscles squelettiques chez les porcs miniatures. *La différenciation histochimique de 12 muscles squelettiques avec constitution différente des types des fibres depuis 80 j de gestation jusqu'à l'âge de 1 an est étudiée chez les porcs miniatures. À 100 j de gestation, les 2 types de myofibres fœtales sont discernés à l'aide de la réaction mATPase après la préincubation acide. L'activité des enzymes oxidatives ne présente aucune différence significative entre les fibres pendant le développement embryonnaire et la première semaine de développement postembryonnaire. À partir du 6e jour après la naissance, on peut discerner 3 catégories de fibres : SO* (slow-twitch oxidative), *FOG* (fast-twitch oxidative-glycolytic) *et FG* (fast-twitch glycolytic). *L'organisation caractéristique de 3 types de fibres en agrégats est observée dans tous les muscles étudiés à l'exception du masséter, qui est formé seulement par les fibres SO et FOG avec une organisation en mosaïque. Les fréquences des 2 types de fibres SO et FG s'élèvent et la proportion de fibres de type FOG diminue pendant la période postnatale dans tous les muscles étudiés. Ces changements pourraient s'expliquer par des transformations entre les types particuliers de fibres. Les fibres de type FOG évoluent le plus souvent vers le type de fibres (SO ou FG) qui domine dans les muscles des animaux adultes.*

muscle squelettique / différenciation / porc

INTRODUCTION

Histochemical techniques allow the differentiation of several fibre types in skeletal muscles. The first differences between muscle fibres are usually found towards the end of fetal development. The fetal myofibres (or myotubes) can be classified into light-stained (type I, slow-twitch) and dark-stained (type II, fast-twitch) categories by the myofibrillar ATPase (mATPase) reaction. Gradual changes of fibre type frequencies have been demonstrated in various mammalian species (rat, rabbit, hamster and sheep) during skeletal muscle development. They are explained by transformations between individual fibre types and were observed chiefly during the first few weeks of postnatal life. The type II to type I transformation is conspicuous in postural muscles (*eg,* soleus) where it refers to the altered muscle function after birth. In contrast, an opposite fibre type conversion, *ie* the type I to type II transformation has been ascertained in muscles which are composed predominantly of type II fibres in adulthood (*eg,* biceps brachii muscle) (Gutmann *et al,* 1974; Kugelberg, 1976, 1980; Goldspink and Ward, 1979; Suzuki and Cassens, 1983). A direct correlation was proved between the histochemical mATPase staining and the myosin heavy chain (MHC) composition of muscle fibres (Gauthier and Lowey, 1979; Staron and Pette, 1986; Staron, 1991). A sequential appearance of various MHC isoforms was demonstrated from fetal to adult animal stages (Whalen *et al,* 1981; Gauthier, 1987; Harris *et al,* 1989). This MHC conversion is a molecular basis of fast- to slow-twitch (and *vice versa*) fibre type transformations.

From the viewpoint of mitochondrial enzyme, substantial differences among muscle fibres are detected only after the birth. The change of energy metabolism from oxidative to glycolytic pathway is explained by the decrease of intact mitochondria number (Van Den Hende *et al,* 1972). Since the slow-twitch fibres maintain a high activity of oxidative enzymes (*ie* slow-twitch oxidative type, SO) during postnatal development this developmental transformation does not change the mutual ratio of slow- and fast-twitch fibres. It takes place between the subpopulations of fast-twitch fibres. The fast-twitch oxidative-glycolytic (FOG) fibres convert to the fast-twitch glycolytic ones (FG). An increase of the FG type proportion was observed in developing ovine, bovine (Ashmore *et al,* 1972; Rehfeldt *et al,* 1987), rat (Maltin *et al,* 1985; Tamaki, 1985), mouse (Rehfeldt *et al,* 1987) and chick muscles (Ashmore and Doerr, 1971).

Determination of the fibre type frequencies during skeletal muscle differentiation in pig has given markedly various results. On the basis of the mATPase reaction, an increase of the SO type proportion was observed during the second half of the gestation and until the age of 16 weeks after birth (Swatland, 1975; Beermann *et al,* 1978; Szentuki and Cassens, 1979; Suzuki and Cassens, 1980). Using the oxidative enzyme activities as a criterion (*eg,* succinate dehydrogenase, SDH), an increase of the proportion of fibres with low oxidative capacity (*ie* FG type) was ascertained during postnatal growth (Cooper *et al,* 1970; Ashmore *et al,* 1972; Van Den Hende *et al,* 1972; Rehfeldt *et al,* 1987). On the basis of both the mATPase and the SDH activities demonstrated on serial muscle sections, it was proved that the proportion of fibres with low mATPase activity (*ie* SO type) increased and the proportion of fibres with low SDH activity (*ie* FG type) did not change in the course of postnatal development (Davies, 1972). However, Swatland (1977) found opposite developmental changes of fibre type frequencies.

Discrepancy in the above-mentioned results can be attributed to different materials studied (breed, age, muscle) and to various enzymes used for the fibre type deter-

mination. To ascertain whether there are any general trends during porcine muscle fibre differentiation, we carried out a histochemical analysis of 12 muscles with different fibre type composition in miniature pigs from 80 d of fetal development to 1 year of age.

MATERIALS AND METHODS

Four male miniature pigs (bred in the Institute of Animal Physiology and Genetics, Department of Genetics, Liběchov) were used at each of the following ages: 1, 6, 12, 21, 60 and 120 d and 1 year. Fibre type analysis was performed in 12 muscles: masseter (pars superficialis); pectoralis superficialis (2 parts - clavicularis and sternocostalis); biceps brachii; triceps brachii (caput laterale); trapezius thoracis; longissimus dorsi; sartorius; gracilis; gastrocnemius lateralis; soleus; and psoas major and diaphragm (pars costalis). Moreover, the level of differentiation was ascertained in the fetal muscles at 80 and 100 d of gestation (3 fetuses at each age). Tissue samples were taken from the central part of the muscles and were immediately frozen to –80°C by their immersion into petroleum ether cooled with dry ice-saturated acetone.

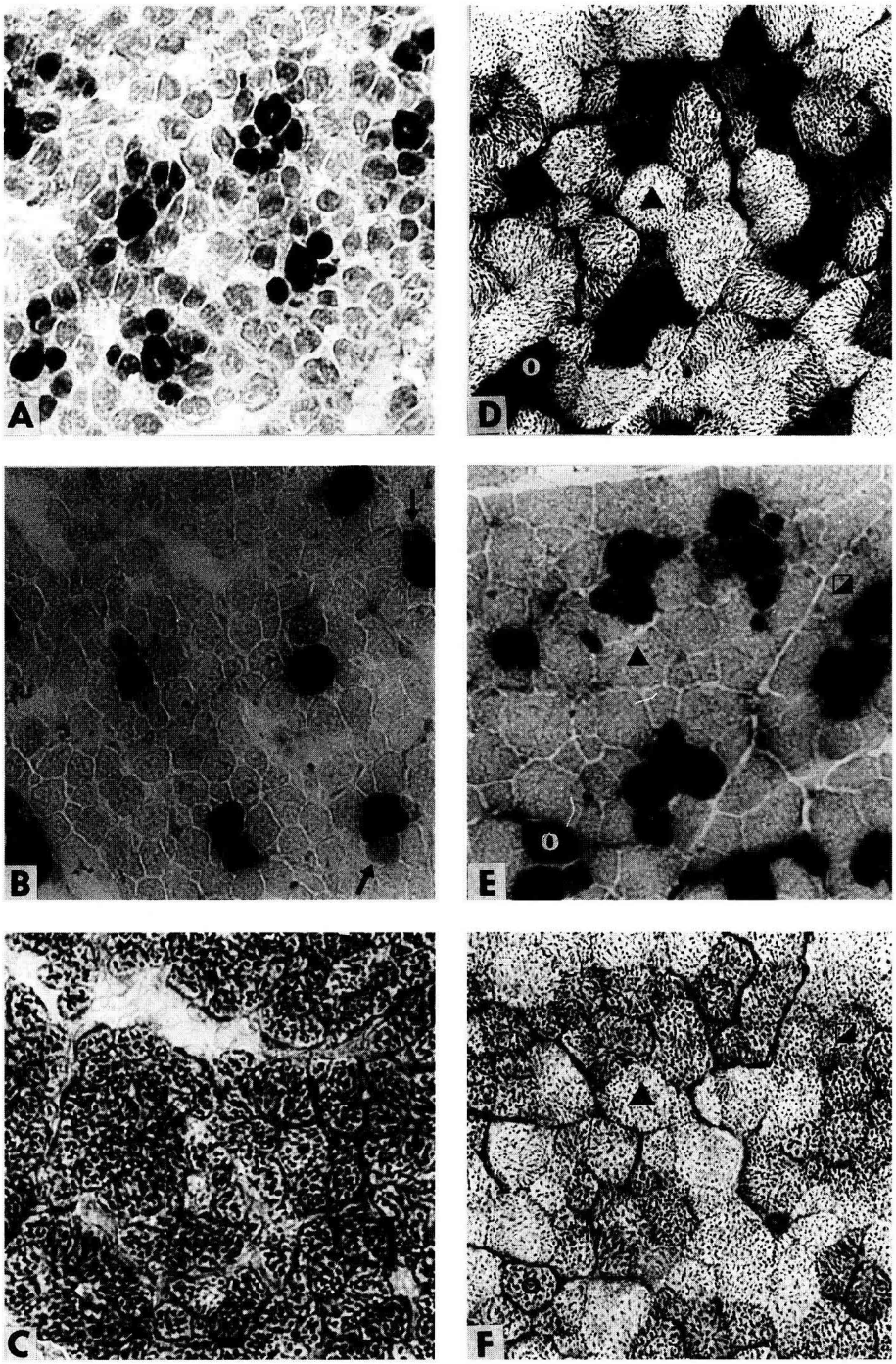
Histochemical demonstration of the mATPase activity alone can prove only the change in the proportion of slow- and fast-twitch fibres (*ie* FOG to SO transformation). Similarly, the detection of oxidative enzyme activities alone can characterize the reduction of oxidative capacity in some fast-twitch fibre only (*ie* FOG to FG transformation). Thus, the parallel histochemical demonstration of the mATPase and oxidative enzyme activities is indispensable for determination of the both developmental transformations. For this reason, serial 10 µm cross-sections were cut at –20°C in the Cryo-Cut II Microtome, air-dried at room temperature for 15 min and stained for the mATPase activity after acid preincubation at pH 4.2 (Guth and Samaha, 1970) and for oxidative enzyme activities (SDH, succinate dehydrogenase; NADH-tetrazolium reductase, NADH-TR; Lojda, 1965). The successive histochemical staining for SDH (or NADH-TR) and mATPase (Horák, 1983) was used starting from the 21st day of age when sufficient fibre diameters and histochemical differences between 3 fibre types were visible. This technique is preferable to the demonstration of individual enzyme activities on serial sections because it allows us to distinguish all 3 fibre types in one tissue section and thus speeds up an evaluation of samples.

Using the nomenclature of Peter *et al* (1972), the skeletal muscle fibres were classified into the SO, FOG and FG types by comparison of microphotographs taken from the same region of the serial sections stained for the mATPase with acid preincubation and oxidative enzyme activities or on microphotographs from a single section treated by the successive technique (fig 1D–F). The acid preincubation reverses a regular staining pattern of the mATPase. Thus, the SO type showed a dark staining and both the FOG and FG types were light. For comparison with the nomenclature of Padykula and Herman (1955), which is based only on the mATPase activity and which is often used in literature (see *Introduction),* the SO type corresponds to the type I and both the FOG and FG types are included in the type II. About 600–1 000 fibres per sample were evaluated for calculation of the individual type percentage. The statistical significance of differences in this parameter between 2 successive ages of postnatal development was evaluated by the *t*-test.

RESULTS

Prenatal muscle development

The mean length of gestation in miniature pigs corresponds to that of commercial pigs (120 d). It was not possible to distinguish with certainty a myotube developmental stadium from a muscle fibre stadium during late embryogenesis on the basis of the histochemical techniques used. For this reason, the muscular elements observed at this time are termed the fetal myofibres. Fetal myofibres showed no mATPase activity after acid preincubation and a weak homogeneous staining for SDH and NADH-TR activities at 80 d of gestation. They were mutually separated by considerable intercellular spaces. Some myofibres located centrally within the developing fasciculi demonstrated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almost twice the diameter of the ambient ones.

At 100 d of gestation, dark (slow-twitch, SO type) and light (fast-twitch, FOG and FG types) fetal myofibres were distinguished on the basis of mATPase after acid preincubation at pH 4.2 (fig 1A). Slight differences among myofibres were also observed in the oxidative enzyme activities, which were generally increased in comparison with the preceding fetal phase. The type SO myofibres usually presented a larger diameter. They were either individually dispersed in muscle fasciculi (*eg*, the gracilis muscle) or they formed small clusters composed of 2–4 fibres in the muscles in which type SO fibres prevailed in maturity (*eg*, the trapezius muscle). Thus, a distinct fibre type composition of studied muscles was already demonstrated in prenatal period.

Postnatal differentiation of fibre types

The characteristic cluster arrangement of fibre types in porcine muscle originated in the course of the first few weeks of postnatal development as a result of the increase of type SO fibre number in clusters and of the gradual changes in the oxidative capacity of the fibres. The fibres with intermediate mATPase activity were observed close to the SO fibre clusters (fig 1B) in all studied muscles at 1 d after birth. Their number continuously decreased up to approximately 21 d of age while the SO fibre number in clusters simultaneously increased. In older animals, the intermediate fibres were not found or were ascertained very rarely. They probably represent a transition stage in the course of fast- to slow-twitch fibre transformation.

The SDH and NADH-TR activities in the muscles of 1-d-old piglets (fig 1C) showed a higher level in comparison with the fetal period. Some fast-twitch fibres, usually situated on the periphery of muscle fasciculi, demonstrated a gradual decrease of their oxidative capacity from birth to 12 d of age. Type SO fibres retained high oxidative capacity in the course of the whole postnatal period. The SDH and NADH-TR staining patterns corresponded to each other at all studied ages.

The fibre type composition of the masseter muscle was quite distinct from the other muscles studied. This muscle consisted of type SO and FOG fibres only showing a mosaic distribution. Fibres of this muscle and the diaphragm generally demonstrated a higher level of oxidative enzyme activities than all the other muscles.

Changes of fibre type frequencies during postnatal development

In 1 d piglet muscles, determination of the frequencies of slow-twitch (SO type) and fast-twitch fibres was possible only on the basis of mATPase staining after acid preincubation. The fibres with intermediate mATPase activity were included in the type SO fibres when fibre type proportions were calculated. The fast-twitch fibres were considered as type FOG fibres on the basis of

Fig 1. Fibre type differentiation in the triceps brachii muscle. First histochemical differences were observed at 100 d of gestation using mATPase staining with acid preincubation (**A**). At 1 d after birth, the SO (dark) and FOG (light) types were distinguished together with intermediate fibres (arrows) on the basis of mATPase activity with acid preincubation (**B**); a serial section was stained for the SDH activity (**C**). The successive staining for NADH-TR and mATPase activities (**D**) distinguishes the SO (O), FOG (◢) and FG (▲) types on one tissue section (21 d of age). Serial sections to **D** stained for the mATPase activity with acid preincubation (**E**) and NADH-TR activity (**F**) are shown for comparison. The same magnification is used in all photographs (470 x).

their high oxidative capacity at this age (fig 1B,C).

From the 6th day of age, the differences in oxidative enzyme activities among fast-twitch fibres were sufficient for their classification into FOG type (medium to high activities) and FG type (low activity). Considerable variability of fibre type percentages was observed among the individuals of the same age. This fact, together with the low number of animals, caused the majority of differences in type frequencies between 2 successive developmental stages to be statistically insignificant. Thus, it is possible to speak only about general developmental trends which take place dur-

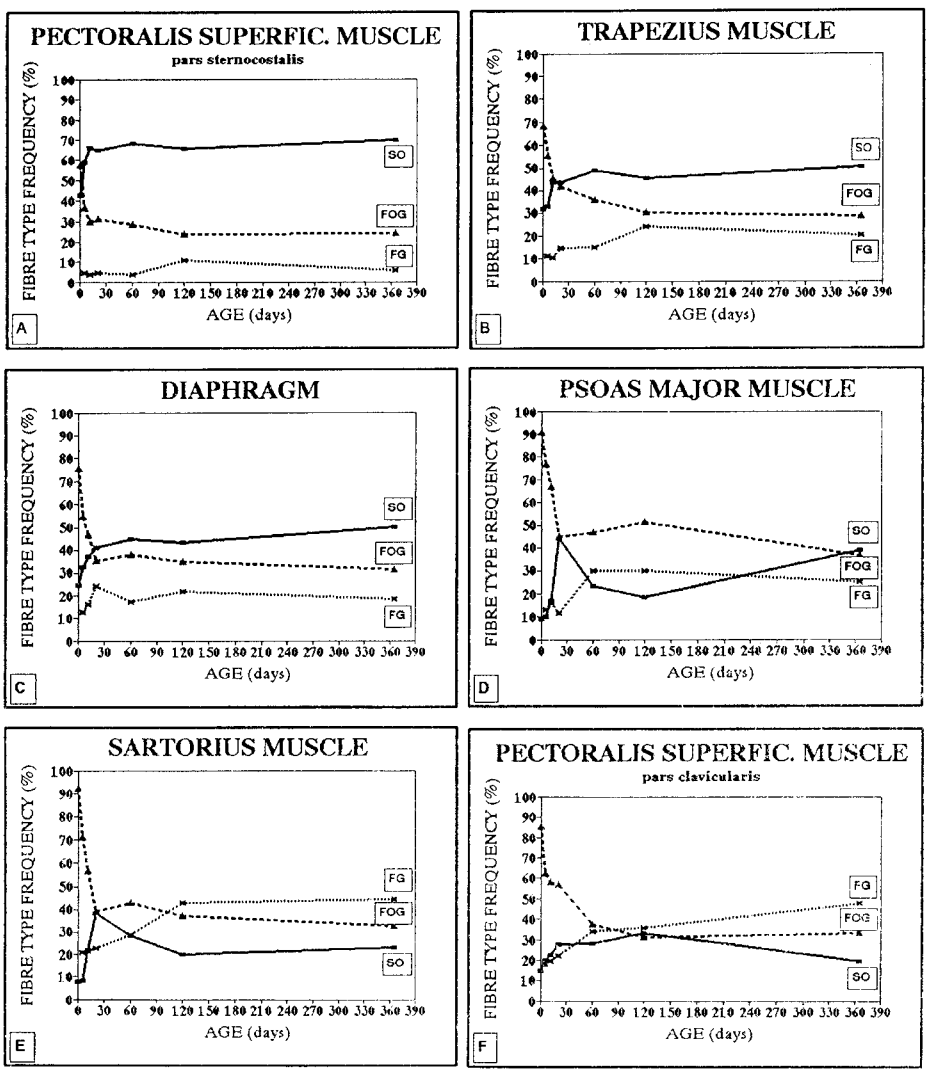

Fig 2. Changes of fibre type frequencies during postnatal muscle development.

ing muscle postembryogenesis. The FOG fibre percentage gradually decreased in all muscles studied. The SO fibre frequency increased quickly in the first 2–3 postnatal weeks while the increase of the FG fibre frequency was slower and it covered a longer period (to approximately 4 months of age) (fig 2 and 3).

A relationship was observed between the fibre type composition of adult mini-pig muscles (1 year of age) and the extent of changes of fibre type frequencies during postnatal period. The muscles composed mainly of type SO fibres in maturity (*eg*, the trapezius muscle, fig 2B) showed a higher proportion of SO fibres (about 25–40% *ver-*

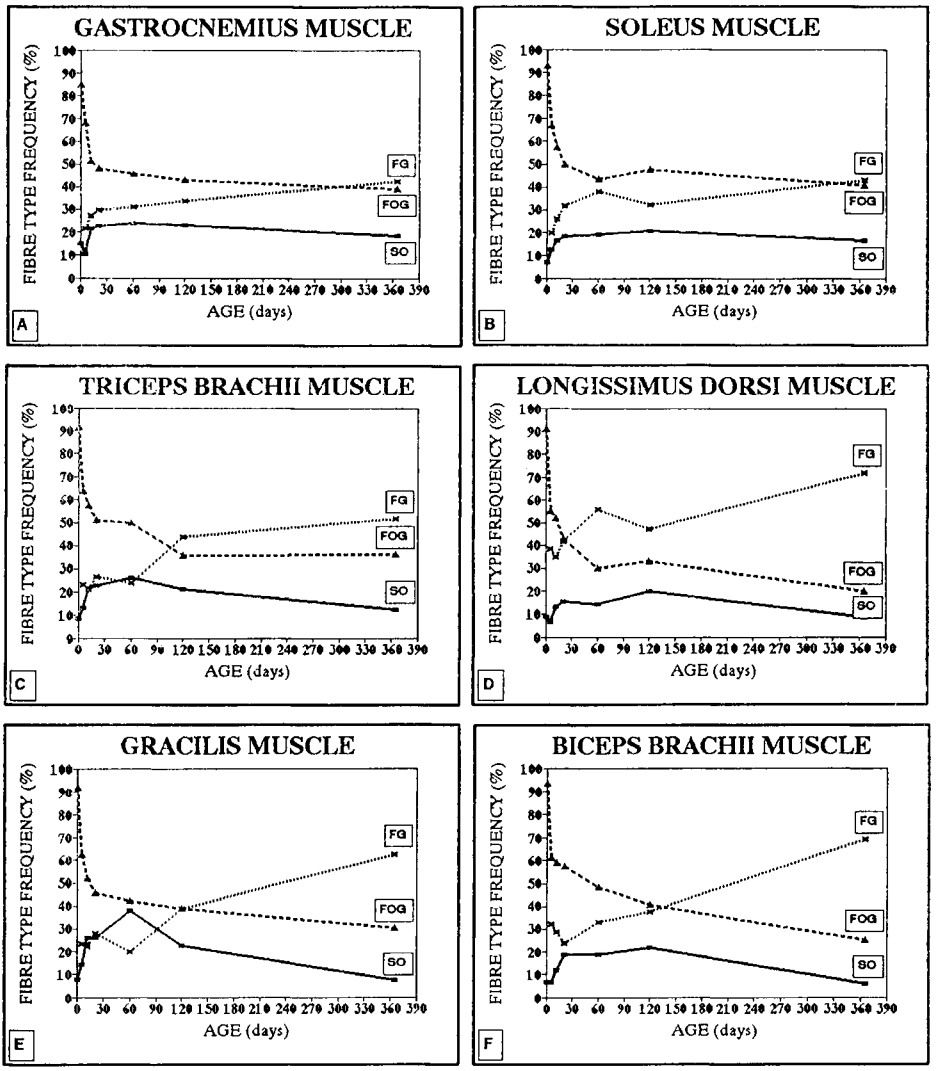

Fig 3. Changes of fibre type frequencies during postnatal muscle development.

Table I. Fibre type percentages (mean and SD) in skeletal muscles of miniature pigs. The skeletal muscles are arranged according to the decreasing type SO frequency at 365 d of age.

Skeletal muscle	*Fibre type*	*1 d*		*6 d*		*12 d*		*21 d*		*60 d*		*120 d*		*365 d*	
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Pectoralis superficialis p sternocostal	SO	42.5	4.7	58.9	3.7	66.3	1.5	64.8	2.4	68.2	2.9	65.8	7.5	70.1	6.7
	FOG	57.5	4.7	36.7	1.2	29.8	1.7	30.7	4.6	28.3	3.8	23.5	3.3	24.2	8.6
	FG	–		4.4	0.7	3.8	0.9	4.5	2.2	3.5	0.8	10.7	4.1	5.7	3.4
Trapezius thoracis	SO	32.0	1.6	33.1	0.9	44.2	1.6	44.8	5.1	49.6	3.8	45.5	1.3	50.3	6.4
	FOG	68.0	1.6	55.3	0.7	45.7	2.8	41.0	8.0	35.4	7.1	30.3	5.7	30.0	8.9
	FG	–		11.6	0.2	10.1	1.4	14.2	3.4	15.0	3.9	24.2	5.5	19.7	6.7
Diaphragm	SO	24.3	4.1	32.6	3.9	37.2	6.3	40.8	2.4	44.7	3.5	43.3	4.5	50.1	7.8
	FOG	75.7	4.2	54.5	7.2	46.6	5.8	35.1	7.4	37.8	3.3	34.7	8.8	31.5	6.8
	FG	–		12.9	3.3	16.2	0.6	24.1	5.0	17.5	6.8	22.0	5.4	18.4	5.7
Psoas major	SO	9.1	1.4	10.1	0.6	16.9	2.8	43.8	5.1	23.5	4.2	18.5	5.1	38.8	8.4
	FOG	90.9	1.1	76.7	1.3	66.9	2.2	44.6	1.4	46.6	1.9	51.4	8.0	36.3	6.6
	FG	–		13.2	1.5	16.2	5.1	11.6	4.7	29.9	3.7	30.1	5.2	24.9	6.9
Masseter	SO	11.4	1.6	17.4	0.3	18.9	4.4	23.3	1.7	25.1	1.3	25.7	9.5	32.1	9.7
	FOG	88.6	1.3	82.6	0.4	81.1	4.4	76.7	1.7	74.9	1.2	74.3	9.5	67.9	9.7
	FG	–		–		–		–		–		–		–	
Sartorius	SO	8.0	0.8	8.1	0.3	21.9	5.7	38.3	0.8	28.2	2.2	20.0	6.7	23.2	3.5
	FOG	92.2	0.8	71.1	3.5	56.6	2.6	38.9	0.3	43.1	5.3	37.2	3.6	32.4	2.2
	FG	–		20.8	3.8	21.5	6.3	22.8	1.3	28.7	3.2	42.8	7.6	44.4	3.7

Table I. Cont.

Skeletal muscle	*Fibre type*	*1 d* $\bar{x}$	*SD*	*6 d* $\bar{x}$	*SD*	*12 d* $\bar{x}$	*SD*	*21 d* $\bar{x}$	*SD*	*60 d* $\bar{x}$	*SD*	*120 d* $\bar{x}$	*SD*	*365 d* $\bar{x}$	*SD*
Pectoralis	SO	14.9	0.7	19.8	3.4	22.4	4.5	27.4	3.9	28.1	8.1	33.4	7.6	19.4	8.7
superficialis	FOG	85.1	0.7	62.4	9.4	58.1	1.1	56.6	9.4	37.6	1.3	30.7	8.1	33.0	7.4
p clavicularis	FG	–		17.8	6.0	19.5	3.4	22.0	7.6	34.3	9.3	35.9	3.9	47.6	9.7
Gastrocnemius	SO	14.9	3.3	10.6	0.2	21.3	4.6	22.8	5.3	23.7	2.5	23.2	1.5	18.3	5.0
	FOG	85.1	3.3	68.1	0.8	51.4	1.1	47.6	8.8	45.5	4.9	43.1	3.9	39.2	8.2
	FG	–		21.3	0.6	27.3	3.4	29.6	2.9	30.8	2.4	33.7	2.2	42.5	3.4
Soleus	SO	6.9	0.6	12.8	0.8	16.6	7.2	18.6	6.1	19.1	3.2	20.8	8.4	16.7	4.8
	FOG	93.1	0.6	67.0	0.2	57.3	0.1	49.6	0.3	43.2	4.2	47.2	6.4	40.4	3.7
	FG	–		20.2	0.6	26.1	7.1	31.8	6.4	37.7	1.2	32.0	1.9	42.9	3.1
Triceps	SO	9.4	0.6	12.3	0.8	21.0	6.0	22.1	1.9	27.9	4.3	20.7	0.3	11.1	0.9
brachii	FOG	90.6	0.6	64.0	4.9	58.3	2.1	50.8	8.3	50.0	6.8	35.8	8.5	36.2	9.4
	FG	–		23.7	4.1	20.7	3.9	28.1	6.3	22.1	2.9	43.5	8.2	52.7	8.2
Longissimus	SO	8.8	1.0	6.7	3.9	13.2	1.7	15.5	2.9	14.3	4.1	19.9	3.9	8.4	6.0
dorsi	FOG	91.2	1.0	55.1	6.4	52.0	4.1	42.8	0.3	30.0	1.7	33.0	1.7	19.9	2.3
	FG	–		38.2	9.9	34.8	3.0	41.7	3.2	55.7	3.3	47.1	5.8	71.7	8.3
Gracilis	SO	7.9	0.9	14.5	0.6	26.0	4.0	26.3	6.1	37.7	5.4	22.6	5.6	7.4	1.0
	FOG	92.1	0.9	62.2	1.9	51.9	3.3	45.6	3.7	42.3	2.8	38.7	3.4	30.2	3.7
	FG	–		23.3	1.3	22.1	0.5	28.1	2.6	20.0	4.0	38.7	2.5	62.4	4.4
Biceps	SO	6.5	0.6	6.8	1.3	12.1	3.2	19.0	9.2	18.7	3.1	21.9	5.1	5.8	1.3
brachii	FOG	93.5	5.6	61.2	8.8	59.1	8.4	57.1	9.2	48.3	3.2	40.5	9.2	24.9	8.9
	FG	–		32.0	6.2	28.8	9.1	23.9	8.2	33.0	9.2	37.6	3.8	69.3	9.2

sus 6–15% in the other muscles) already at the 1st day after birth. In these muscles, a marked increase of type SO frequency and only a mild increase of type FG frequency were observed. The type SO fibres filled gradually almost whole muscle fasciculi in these muscles. On the other hand, the muscles with the prevalence of type FG fibres in maturity (over 50%, *eg,* the biceps brachii muscle, fig 3C–F) showed a higher percentage of FG fibres (25–38% *versus* 5–20% in the other muscles) as early as the 6th day of age. The increase of the type SO frequency in these muscles was rather gradual and it terminated at about 12 d of age. However, the long-term increase of the proportion of type FG fibres was observed which took place mainly between 4 months and 1 year of age. It was usually accompanied by the decrease in the type SO frequency which reached the values observed at the 1st day of age (table I).

The masseter muscle showed also generally observed developmental trends. The type FOG frequency decreased and the type SO frequency increased mainly during the first 3 weeks of age (fig 4).

DISCUSSION

Histochemical analysis of skeletal muscles revealed that the domestication of pig together with selection for high meat production increased the proportion of type FG fibres in commercial pigs (Ashmore *et al,* 1973; Ashmore, 1974; Essén-Gustavsson and Lindholm, 1984). Comparison of our results with these studies shows that fibre type composition of the longissimus dorsi muscle in adult (1 year old) mini-pigs resembles rather that of the wild pig *(Sus scofa scrofa)* than of commercial pigs. Skeletal muscles of commercial pigs contained a higher fibre number than did miniature pigs (Stickland and Goldspink, 1978; Stickland and Handel, 1986). Regardless of these differences in adult animals we believe that our results ascertained from the study of 12 mini-pig muscles could contribute to the unification of the above-mentioned literature data about fibre type differentiation in pigs.

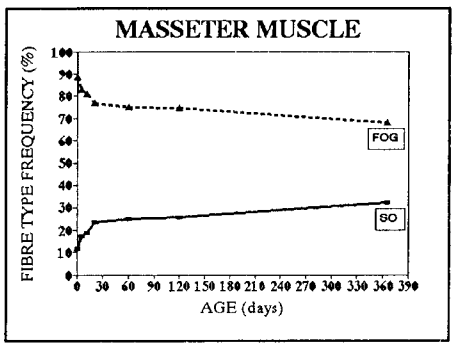

Fig 4. Changes of fibre type frequencies during postnatal development of the masseter muscle.

A direct relationship was demonstrated between histochemical and contractile properties of individual fibre types (Barnard *et al,* 1971). Thus, the studied miniature pig muscles could be classified as slow- or fast-twitch on the basis of their fibre type composition at 1 year of age. The general features of the postnatal muscle development in miniature pig were found in this study. These consisted of the reduction in the FOG fibre proportion and the increase in the SO and FG fibre frequencies. These changes of fibre type frequencies could probably be explained by developmental fibre type transformations. In slow-twitch muscles (with a higher prevalence of type SO fibres at 1 year of age, *eg,* the trapezius muscle), the transformation of type FOG fibres takes place primarily to the type SO fibres in the first few weeks after birth, while their conversion to the type FG fibres is considerably suppressed. On the other hand, fast-twitch muscles (with a predominance of type FG fibres at 1 year of age, *eg,* the biceps brachii muscle), demonstrate the transformation of type FOG fibres preferably to the type FG fibres, while their transformation to the type SO fibres is reduced. In addition, clear differ-

ences between slow- and fast-twitch muscles in fibre type composition were already observable in newborn piglets. Slow-twitch muscles showed a higher percentage of the type SO fibres at birth and a lower frequency of the type FG fibres at the 6th day of age in comparison with fast-twitch muscles. Thus, developmental transformations between fibre types gradually increase differences among skeletal muscles already present at birth reaching an adult fibre type composition. This suggestion is in accordance with the ontogenetic changes of fibre type frequencies which were observed in rat (Kugelberg, 1976; Tamaki, 1985; Maltin *et al,* 1989), hamster (Goldspink and Ward, 1979), pig (Swatland, 1975; Suzuki and Cassens, 1980) and sheep muscles (Suzuki and Cassens, 1983).

The reduction of the fibre SO frequency during the late postnatal period in some mini-pig muscles (this work), the hamster biceps brachii muscle (Goldspink and Ward, 1979) and the rat soleus muscle (Syrovy and Gutmann, 1977) suggests that there is probably yet another direction of transformation. Since the experimental elimination of muscle activity also reduces the frequency of type SO fibres (Gardiner, 1981; Spector, 1985), the above-mentioned change of fibre SO frequency might be explained by the natural reduction of motion activity of adult animals.

A change of the total fibre number within muscles during their normal postnatal growth (or a selective loss of specific fibre type) could be an alternative explanation of the changes in fibre type frequencies. A formation of new muscle fibres (hyperplasia) is completed at the third month of gestation (Swatland, 1973; Wigmore and Stickland, 1983) and the fibre number reached remains unchanged during the postnatal development in muscles of commercial and miniature pigs (Staun, 1963; Stickland and Goldspink, 1978; Stickland and Handel, 1986; Rehfeldt *et al,* 1987). Thus, the transformation between fibre types could offer a good explanation for the changes of fibre type frequencies presented in this paper for developing miniature pig muscles. The changes observed in the masseter muscle, which consists of the SO and FOG types only, give good support to this suggestion.

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REFERENCES

Ashmore CR (1974) Phenotypic expression of muscle fiber types and some implications to meat quality. *J Anim Sci* 38, 1158-1164

Ashmore CR, Doerr L (1971) Postnatal development of fiber types in normal and dystrophic skeletal muscle of the chick. *Exp Neurol* 30, 431-446

Ashmore CR, Thompkins G, Doerr L (1972) Postnatal development of muscle fiber types in domestic animals. *J Anim Sci* 34, 37-41

Ashmore CR, Addis PB, Doerr L (1973) Development of muscle fibers in the fetal pig. *J Anim Sci* 36, 1088-1093

Barnard RJ, Edgerton VR, Furukava T, Peter JB (1971) Histochemical, biochemical, and contractile properties of red, white, and intermediate fibers. *Am J Physiol* 220, 410-414

Beermann DH, Cassens RG, Hausmann GJ (1978) A second look at fiber type differentiation in porcine skeletal muscle. *J Anim Sci* 46, 125-132

Cooper CC, Cassens RG, Kastenschmidt LL, Briskey EJ (1970) Histochemical characterization of muscle differentiation. *Develop Biol* 23, 169-184

Davies AS (1972) Postnatal changes in the histochemical fibre types of porcine skeletal muscle. *J Anat* 113, 213-240

Essén-Gustavsson B, Lindholm A (1984) Fiber types and metabolic characteristics in muscles of wild boars, normal and halothane sensitive Swedish Landrace pigs. *Comp Biochem Physiol* 78A, 67-71

Gardiner PF (1981) Influence of reduced neuromuscular activity on the development of neonatal rat hindlimb muscle. *Dev Neurosci* 4, 382-388

Gauthier GF (1987) Vertebrate muscle fiber types and neuronal regulation of mysoin expression. *Am Zool* 27, 1033-1042

Gauthier GH, Lowey S (1979) Distribution of myosin isozymes among skeletal muscle fiber types. *J Cell Biol* 81, 10-25

Goldspink G, Ward PS (1979) Changes in rodent muscle fibre types during post-natal growth, undernutrition and exercise. *J Physiol* 296, 453-469

Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. *Exp Neurol* 28, 365-367

Gutmann E, Melichna J, Syrovy I (1974) Developmental changes in contraction time, myosin properties and fibre pattern of fast and slow skeletal muscles. *Physiol Bohemoslov* 23, 19-27

Harris AJ, Fitzsimons RB, McEwan JC (1989) Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. *Development* 107, 751-769

Horák V (1983) A successive histochemical staining for succinate dehydrogenase and 'reversed'-ATPase in a single section for the skeletal muscle fibre typing. *Histochemistry* 78, 545-553

Kugelberg E (1976) Adaptive transformation of rat soleus motor units during growth. *J Neurol Sci* 27, 269-289

Kugelberg E (1980) Adaptive fibre and motor unit transformation in rat soleus during growth. *In: Plasticity of Muscle* (D Pette, ed), Walter de Gruyter, Berlin, 111-117

Lojda Z (1965) Remarks on histochemical demonstration of dehydrogenases. II. Intracellular localization. *Folia Morph* 13, 84-96

Maltin CHA, Duncan L, Wilson AB (1985) Rat diaphragm: changes in muscle fibre type frequency with age. *Muscle Nerve* 8, 211-216

Maltin CHA, Delday MI, Baillie AGS, Brubb DA, Garlick PJ (1989) Fibre-type composition of nine rat muscles. I. Changes during the first year of life. *Am J Physiol* 257, E823-E827

Padykula HA, Herman E (1955) The specificity of the histochemical method for adenosine triphosphatase. *J Histochem Cytochem* 3, 170-195

Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE (1972) Metabolic profiles of 3 fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* 11, 2627-2633

Rehfeldt CH, Fiedler I, Wegner J (1987) Veranderung der Mikrostruktur des Muskelgewebes bei Labormausen, Rindern und Schweinen wahrend des Wachstums. *Z Mikr Anat Forch* 101, 669-680

Spector SA (1985) Effect of elimination of activity on contractile and histochemical properties of rat soleus muscle. *J Neurosci* 5, 2177-2188

Staron RS (1991) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in single human muscle fibers. *Histochemistry* 96, 21-24

Staron RS, Pette D (1986) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* 86, 19-23

Staun H (1963) Various factors affecting number and size of muscle fibers in the pig. *Acta Agric Scand* 13, 293-322

Stickland NC, Goldspink G (1978) Number of fibres in the skeletal muscle of miniature pigs. *J Agric Sci Camb* 91, 255-256

Stickland NC, Handel SE (1986) The numbers and types of muscle fibres in large and small breeds of pigs. *J Anat* 147, 181-189

Suzuki A, Cassens RG (1980) A histochemical study of myofiber types in muscle of the growing pig. *J Anim Sci* 51, 1449-1461

Suzuki A, Cassens RG (1983) A histochemical study of myofiber types in the serratus ventralis thoracis muscle of sheep during growth. *J Anim Sci* 56, 1447-1458

Swatland HJ (1973) Muscle growth in the fetal and neonatal pig. *J Anim Sci* 37, 536-545

Swatland HJ (1975) Histochemical development of myofibers in neonatal piglets. *Res Vet Sci* 18, 253-257

Swatland HJ (1977) Histochemical changes during muscle growth in pigs. *Zbl Vet Med A* 24, 248-251

Syrovy I, Gutmann E (1977) Differentiation of myosin in soleus and extensor digitorum longus muscle in different animal species during development. *Pflugers Arch* 369, 85-89

Szentuki L, Cassens RG (1979) Motor innervation of myofiber types in porcine skeletal muscle. *J Anim Sci* 49, 693-700

Tamaki N (1985) Effect of growth on muscle capillarity and fiber type composition in rat diaphragm. *Eur J Appl Physiol* 54, 24-29

Van Den Hende C, Muylle E, Oyaert W, De Roose P (1972) Changes in muscle characteristics in growing pigs. Histochemical and electron microscopic study. *Zbl Vet Med A* 19, 102-110

Wigmore PMC, Stickland NC (1983) Muscle development in large and small pig fetuses. *J Anat* 137, 235-245

Whalen RG, Seel SM, Butler-Browne G, Schwartz K, Bouveret P, Pinset-Harstrom I (1981) Three myosin heavy chain isozymes appear sequentially in rat muscle development. *Nature (Lond)* 292, 803-809