Anat. Histol. Embryol. **33,** 236–243 (2004)

ISSN 0340–2096

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Histochemical Skeletal Muscle Fibre Types in the Sheep

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With 3 figures and 7 tables

Received November 2003; accepted for publication December 2003

Summary

In this study, the differentiation of adult and postnatal muscle fibres in sheep longissimus thoracis muscle has been characterized. By using a variety of histochemical methods, we have investigated the m-ATPase and metabolic activities of skeletal muscle fibres in adult sheep and lambs aged between 1 day and 3 months. Types I, IIA, IIB and IIC fibres were identified. The results showed that the interpretation of the fibre type composition depends on the methods used. The findings also revealed that the fibre types IIA and IIB can be separated histochemically in sheep by using the correct m-ATPase technique, even at early stages of postnatal development, and that the origin of the four different fibres of the adult can be traced back to early postnatal stages.

Introduction

Ovine skeletal muscle fibres have been classified over the years according to different nomenclatures (Table 1), which have been indiscriminately used, sometimes even by the same author (Suzuki, 1971a,b, 1995; Suzuki and Cassens, 1983; Suzuki and Tamate, 1988).

In sheep all the studies that classify the skeletal muscle fibres agree in the difficulty of differentiating between the fibre types IIA and IIB, therefore the use of metabolic techniques seemed necessary. Thus, authors who identified the IIA and IIB fibres in the pig by means of the m-ATPase technique, in sheep used the NADH-TR metabolic technique to be able to identify the fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibres which correspond to the IIA and IIB, respectively (Suzuki and Cassens, 1983).

Furthermore the physiological differentiation of muscle fibres is a dynamic equilibrium which can vary during growth or as a response to the muscle work rate. Guth and Yellin (1971) concluded that muscle fibres are continuously changing during animal life as a functional demand adaptation and that the fibre type only reflects the fibre constitution at a certain moment. This theory is particularly interesting for the stockbreeder because it suggests a certain grade of induced variation in the fibre types during development. Several authors have shown variations in the proportion of different fibre types after birth in the femoral quadriceps, longissimus thoracis and serratus ventralis thoracis muscles of the sheep (White et al., 1978; Moody et al., 1980; Suzuki and Cassens, 1983, respectively). However, Henkel (1991) rejects the variations in the proportion of different muscle fibres and suggests muscle histochemistry as a tool for quantifying the effect of different treatments (handling and feeding) on the size of muscle fibres.

In this study we have defined the skeletal muscle fibre populations of the adult sheep by using different methods of m-ATPase and metabolic techniques, and we have analysed the postnatal development of these fibre populations between 1 day and 3 months of age.

Materials and Methods

Animals

We used eight adult sheep and 36 lambs of the Segureña breed from the same stockbreeder, and as a control, four Wistar rats. All of the ovines had passed the obligatory health tests and had the characteristic pattern of the species. Lambs were identified and weighed at birth, and the weight was checked weekly until the end of the study. Lambs lived on the farm where they were born until they were sacrificed. The feeding (weaning at 45 days of age and fattening based on pellets) and sanitary treatments were as usual for this breed in the Murcia region. Adult animals were sacrificed with a live weight of 35–40 kg. The time periods for the sacrifice of the lambs during the postnatal development to obtain the muscular samples were: 1, 15, 30, 45, 60 and 90 days after birth. Six animals were sacrificed at each time period. All the animals were killed following the guidelines of the Royal Decree 147/1993 (B.O.E. 12/03/1993).

Muscular samples

We obtained the muscular samples surgically; the longissimus thoracis muscle was dissected from the right side of the half carcass, and 1 cm samples were taken from the area adjacent to the last rib and at a constant depth of 1 cm. As control the tibialis cranialis muscle of the rat's right leg was used and was processed together with the sheep longissimus thoracis muscle. To avoid possible morphological and morphometric alterations of the fibres, the samples were frozen during the first hour after the animal's sacrifice in 2-methylbutane cooled in liquid nitrogen (Dubowitz and Brooke, 1973). Transverse serial sections (10μm) were cut in a Reichert Jung Cryocut cryostat at −20°C. The transverse orientation of the sections and the correct freezing of the samples were checked by a quick stain with haematoxylin–eosin. We obtained 60–80 serial sections of each sample that were kept at −40°C until the next day, for histochemical processing. For studying fibre differentiation

Table 1. Nomenclatures used by several authors to classify the different ovine muscle fibre types

Moody and Cassens (1968)	Red	Intermediate	White	
Ashmore and Doerr (1971)	βR	αR	αW	
Suzuki (1971a,b)	C, D	A	B	
White et al. (1978)	I	IIA	IIB	
Solomon et al. (1981)	βR	αR	αW	
Suzuki and Cassens (1983)	IC, ID	IIA	IIB	
Suzuki and Tamate (1988)	IC, ID	IIA	IIB	IIC
Carpenter et al. (1996)	SO	FOG	FG	
Whipple and Koohmaraie (1992)	βR	αR	αW	
Suzuki (1995)	SO1, SO2	FOG	FG	
Menzel (1999a,b)	STO	FTO	FTG	

during the postnatal development, adult longissimus muscle samples were used as control.

Histochemical techniques

The myosin-ATPase techniques used for adult animals are shown in Table 2. Method 1 corresponds to the alkaline pre-incubation of the system A described by Snow et al. (1982). Method 2 corresponds to acid pre-incubation (sodium acetate 0.2 M) of the same system. Methods 3 and 4 are acid pre-incubations (sodium acetate 0.1 and 0.2 M) and correspond to a modified form (Latorre et al., 1993) of the system C described by Snow et al. (1982). Method 5 in the Table 2 corresponds to the acid pre-incubation of Dubowitz and Brooke (1973) as modified by Gil (1986). To demonstrate the metabolic activity of the fibres, we used the nicotinic adenine-dinucleotide (reduced) tetrazolium reductase (NADH-TR) and menadione-linked α-glycerophosphate dehydrogenase (α-MGPDH) methods (Dubowitz and Brooke, 1973). These methods allow the separation of three fast fibre types (IIA, IIB and IIX) in rat skeletal muscle (Gorza, 1990; Latorre et al., 1993).

Morphometric and statistical analysis

Morphometric analyses were carried out using an interactive image analysis system (Quandimed 500; Leica, Barcelona, Spain). The parameter minimum diameter was selected as measurement of the fibre diameter to avoid possible errors because of tilted sections (Dubowitz and Brooke, 1973). In each muscle section at least 150 fibres of each type were analysed without differentiating between male and female animals (Blomstrand and Ekblom, 1982; Shorey and Cleland, 1983). The means, standard errors, standard deviations and statistical analyses of the smallest diameters were obtained through the Systat program (9; SPSS Inc., Chicago, IL, USA). The percentages of the fibre types were calculated by counting 500–1000 fibres from random superficial and deep area.

Table 2. Methods used to classify muscle fibres according to m-ATPase activity (Brooke and Kaiser, 1970)*

Alkali pre-incubation Method 1 Solution: 0.1 M $CaCl_2$, 0.07 M Na acetate, 0.075 M Na barbital; pH 10.2–10.6. Time 15 min. Temperature 20–22°C			
Method 2	Method 3	Method 4	Method 5
Acid pre-incubation 0.2 M Na acetate, adjusted with acetic acid, pH 4.6, 4.55 and 4.4; time 1–10 min, with interval at 1 min, temperature 20–22°C	0.1 M Na acetate, adjusted with 0.1 M acetic acid, pH 4.6, 4.35 and 4.2; time 5, 10 and 15 min, temperature 20–22°C	0.2 M Na acetate, adjusted with acetic acid, pH 4.6, 4.35 and 4.2, and diluted in H_2O 1 : 1; time 5, 10 and 15 min; 20–22°C	Solution A: Na acetate (Pm136.08), 1.94 g, Na barbital (Pm 206.18), 2.94 g, H_2O 100 ml. Solution B: 0.1 N HCl 50 ml from A, 101.5 ml from B, 20 ml NaCl (8.5%), (H_2O) to 250 ml and adjusted to pH 4.3, 4.55, 4.6 with 1 M NaOH; time 1–10 min, with interval 1 min, temperature 20–22°C
Wash solution H_2O, time 5 min, temperature 20–22°C	H_2O, time 5 min, temperature 20–22°C	H_2O, time 5 min, temperature 20–22°C	0.1 M Na barbital 2 ml, 0.18 M $CaCl_2$, 1 ml, H_2O 7 ml, adjusted to pH 9.4 with 0.1 N HCl; time 30 s
Incubation solution 0.1 M $CaCl_2$, 0.07 M Na acetate, 0.075 M Na barbital, 1.5 mg/ml ATP, adjusted to pH 9.45; time 60 min after acid and 30 min after alkaline pre-incubation	0.1 M $CaCl_2$, 0.07 M Na acetate 0.075 M Na barbital, 1.5 mg/ml ATP, adjusted to pH 9.45; time 60 min	0.1 M $CaCl_2$, 0.07 M Na acetate 0.075 M Na barbital, 1.5 mg/ml ATP, adjusted to pH 9.45; time 60 min	Wash solution with 1.5 mg/ml ATP, Adjusted to pH 9.4 with 1 M NaOH; Time 45 min
$CaCl_2$ 0.2 M, time 2 × 5 min	0.2 M, time 2 × 5 min	0.2 M, time 2 × 5 min	1%, time 2 × 5 min
$CoCl_2$ 2%, time 5 min	2%, time 5 min	2%, time 5 min	2%, time 5 min
Na barbital			0.01 M, time 5-10 min
$(NH_4)_2S$ 1%, time 30–60 s	1%, time 30–60 s	1%, time 30–60 s	1%, time 30–60 s

*Methods 1 and 2 are alkali and acid pre-incubation of system A described by Snow et al. (1982). Methods 3 and 4 are acid pre-incubations of system C described by Snow et al. (1982), but modified by Latorre et al. (1993). Method 5 is acid pre-incubation described by Dubowitz and Brooke (1973) as modified (Gil, 1986).

Results

Adult animals

Fibre types

The histochemical staining results of muscle fibre types in the sheep were compared with those of the rat. Fibres types I, IIA, IIB, IIX and IIC were identified in the rat muscle by m-ATPase staining (Table 3). Type I and IIC fibres in the sheep showed the same characteristic as in the rat. Furthermore, to identify the IIC fibres, it was necessary in both species to use very acid pre-incubation (method 2: pH 4.4; method 3: pH 4.35; method 4: pH 4.35; method 5: pH 4.3), which separates these fibres from the rest of the acid-labile population. The problem lies in analysing the degree of correspondence between the rest of the type II fibres in the sheep and types IIA, IIB and IIX in the rat. We therefore focus here on the results obtained with m-ATPase reactions.

Method 1. With alkaline pre-incubation we could clearly identify in the rat the type IIB fibres (weak staining) and types IIA, and IIX (intense staining) (Table 3). This method gave two type II populations of fibres in the sheep: one intensely stained and one moderately stained. The type differentiation was obtained over the pH range 10.2–10.6, although it was most frequently detected between 10.5 and 10.6 (Table 4; Fig. 1a,b).

Methods 2, 3, 4 and 5. In the rat, the results with these m-ATPase techniques (acid pre-incubations) were similar in the four methods: type IIA (negative), type IIB and type IIX (moderate) (Table 3). In the sheep we could not distinguish between type II fibres, they were all moderate or negative stained (Table 4). To find the type differentiation in sheep, we tried several pre-incubation times, but it did not affect the stain-based differentiation of the type II fibres. Short times (1–5 min) of acid pre-incubation for the four methods used, reflected moderate stain intensities for all the type II fibre population. The same fibres with a longer pre-incubation time (10 min) were negative as can be seen in Table 4.

The muscle fibres type identified in the sheep on the basis of m-ATPase activity could be classified in four types (Table 4):

- Type I: Alkaline-negative and acid-intense fibres.
- Type IIA: Alkaline-intense and acid-moderate/negative fibres.
- Type IIB: Alkaline-moderate and acid-moderate/negative fibres.
- Type IIC: Alkaline-intense and acid-intense/moderate. There were very few fibres of this type.

Metabolic techniques

The oxidative capacity (NADH-TR technique) in the rat was high in type I, IIA, IIC and IIX fibres and weakly-negative in type IIB fibres (Table 3).

The glycolytic activity (m-GPDH technique) in the rat was high in type IIB and IIX fibres, weak in IIA and IIC fibres and negative in type I (Table 3).

Table 3. Histochemical staining properties of muscle fibres types in the rat*

	I	IIA	IIB	IIX	IIC
Method 1					
10.2–10.4	–	+ + +	+	+ + +	+ + +
10.4–10.6	–	+ + +	+	+ + +	+ + +
Method 2					
4.6–4.55	+ + +	–	+	+	+ +
4.4	+ + +	–	+	+	+ +
Method 3					
4.6	+ + +	–	+ +	+ +	+ +
4.35	+ + +	–	+	+	+ +
4.2	+ + +	–	–	–	+ +
Method 4					
4.6	+ + +	–	+	+	+ +
4.35	+ + +	–	–	–	+
4.2	+ + +	–	–	–	+
Method 5					
4.6–4.55	+ + +	–	+	+	+ +
4.3	+ + +	–	–	–	+ +
NADH-TR	+ +	+ +	+	+ +	+ +
α-MGPDH	–	+	+ +	+ +	+

*Relative staining intensity of different fibre types: + + +, intense; + +, moderate; +, weak; –, negative.

Table 4. Histochemical staining properties of muscle fibres in the adult sheep*

	I	IIA	IIB	IIC
Method 1				
10.2–10.4	–	+ + +	+ + +	+ + +
10.5–10.6	–	+ + +	+ +	+ + +
Method 2				
4.6–4.55	+ + +	– (10′)/+ + (5′)	– (10′)/+ + (5′)	– (10′)/+ + (5′)
4.4	+ + +	–	–	+ +
Method 3				
4.6	+ + +	– (10′)/+ + (5′)	– (10′)/+ + (5′)	– (10′)/+ + (5′)
4.35	+ + +	–	–	+ +
4.2	+ + +	–	–	+ +
Method 4				
4.6	+ + +	– (10′)/+ + (5′)	– (10′)/+ + (5′)	– (10′)/+ + (5′)
4.35	+ + +	–	–	+ +
4.2	+ + +	–	–	+ +
Method 5				
4.6–4.55	+ + +	+ (10′)/+ + (5′)	+ (10′)/+ + (5′)	+ + +
4.3	+ + +	–	–	+ +
NADH-TR	+ + +	+ + +	–/+ + +	+ + +
α-MGPDH	–	+/+ + +	+ + +	–

*Relative staining intensity of different fibre types: + + +, intense; + +, moderate; +, weak; –, negative. Where very small differences in staining intensity occurred these are not shown in the table but are described in the text.

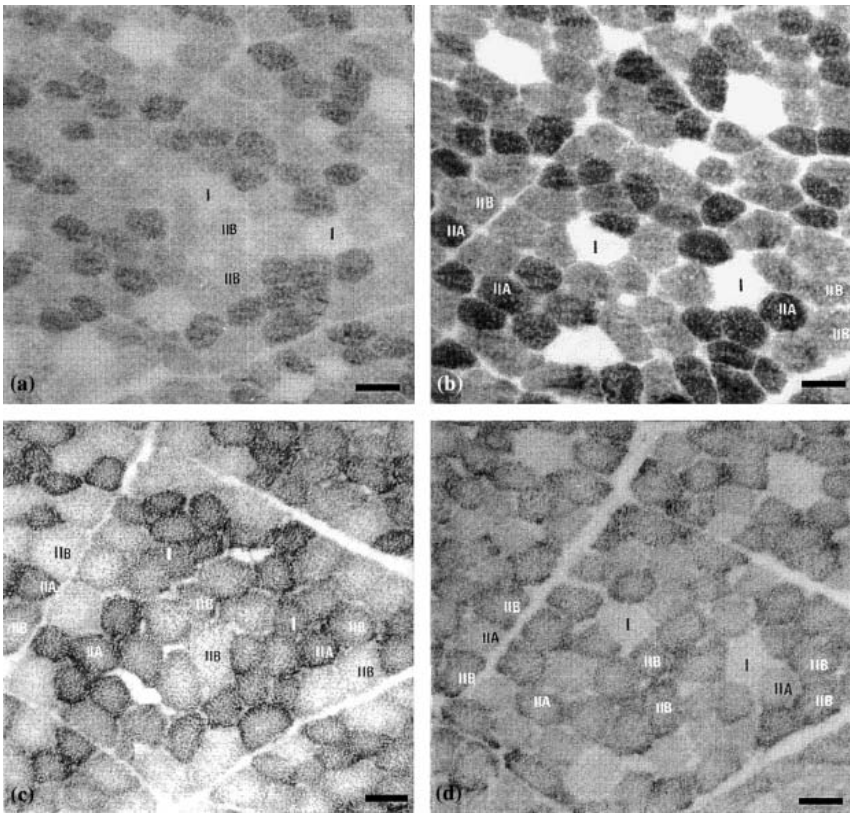

Fig. 1. Longissimus thoracis muscle from the adult ovine. (a) m-ATPase activity after alkaline pre-incubation at pH 10.2. Fibres types are: negative I, moderate IIB and intense IIA. (b) Serial section stained for m-ATPase activity after alkaline pre-incubation at pH 10.4. Fibres types are: negative I, moderate IIB and intense IIA. (c) Serial section stained for NADH-TR activity. Fibres types are: intense I and IIA, intense and negative IIB. (d) Serial section stained for m-GPDH activity. Fibres types are: negative I, weak IIA, intense IIB and IIA. Bar, 40 μm.

In the sheep, the alkaline-intense fibres (IIA) were mainly oxidative-intense and glycolytic-intense, thus corresponding to type FOG fibres. However, some of the fibres had a weak glycolytic activity (Table 4; Fig. 1c,d).

In the sheep, the alkaline-moderate fibres (IIB) were mainly oxidative-negative and glycolytic-intense, which would correspond to type FG fibres. However, some of the fibres had an intense oxidative activity (Fig. 1c,d).

Percentages of the fibre types

Type I and IIC fibres showed the lowest percentages. The percentage of alkaline intense fibres (IIA) was 36%, and 29.4% had a weakly glycolytic activity. The percentage of alkaline moderate fibres (IIB) was 53.7 and 33.3% had intense oxidative activity (Table 5).

Size of the fibres

The mean minimum diameters obtained for each fibre type are show in Table 6. Type I fibres were the largest ($P < 0.05$) and the alkaline-intense fibres (IIA) the smallest. The types I and alkaline-moderate fibres (IIB) had values larger ($P < 0.001$) than those of type IIA (Table 6).

Postnatal development

Fibre types

Fibre types I, IIC, alkaline intense (IIA) and alkaline moderate (IIB) were identified in longissimus thoracic muscle of the adult sheep (control muscle) on the basis of m-ATPase

Table 5. Mean percentages of the fibres types composition of the longissimus thoracis muscle in the adult and during the postnatal development (0–90 days)

	Fibre			
Age	I	IIA	IIB	IIC
Adult	10	36 (29.4*)	53.7 (33.3**)	0.3
1 day after birth	9.97	48.53	41.49	
15 days	9.92	34.36	55.71	
30 days	8.87	30.23	60.84	
45 days	10.61	37.79	51.63	
60 days	10.25	34.37	55.37	
90 days	8.57	31.81	59.6	

*Percentages of IIA fibres with weak glycolytic activity. **percentages of IIB fibres with intense oxidative activity.

Table 6. Mean minimum diameters of the longissimus thoracis muscle fibres during the postnatal development (0–90 days) and in the adult (μm ± SE)

	Fibre		
Age	I	IIA	IIB
Adult	26.33 ± 0.89	20.54 ± 0.40	24.27 ± 0.42
1 day after birth	10.45 ± 0.63	5.62 ± 0.30	6.59 ± 0.27
15 days	14.12 ± 0.84	11.25 ± 0.49	13.82 ± 0.34
30 days	14.35 ± 0.82	7.51 ± 0.41	9.75 ± 0.46
45 days	14.13 ± 0.50	11.54 ± 0.29	16.42 ± 0.21
60 days	13.97 ± 0.71	13.3 ± 0.32	17.85 ± 0.31
90 days	25.2 ± 0.76	13.05 ± 0.39	21.65 ± 0.28

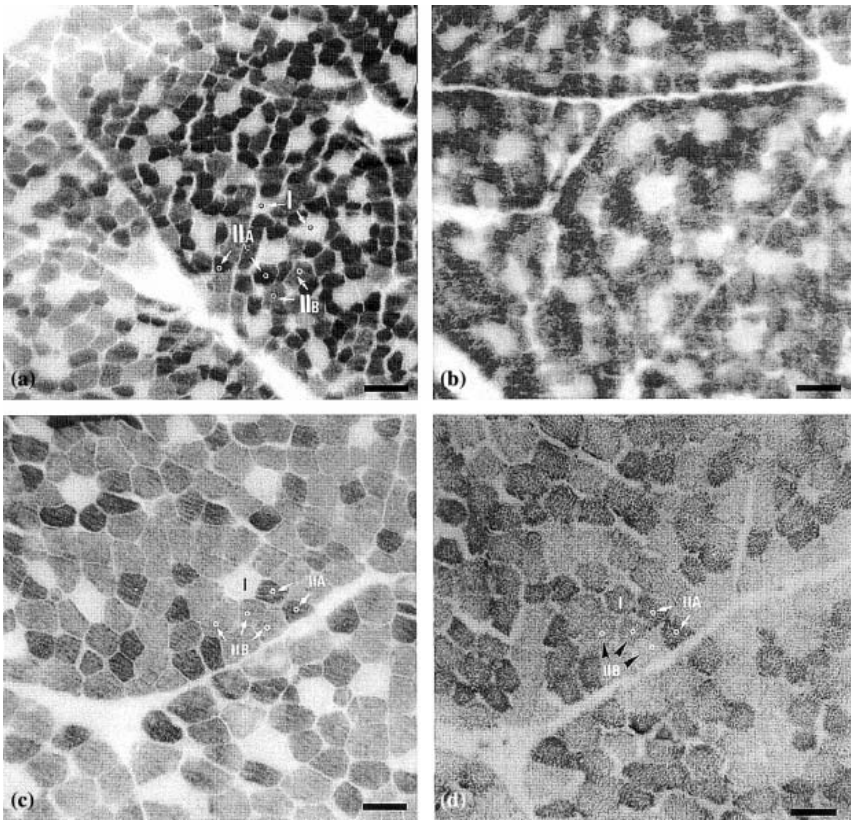

Fig. 2. Longissimus thoracis muscle from sheep. (a) Age: 1 day. m-ATPase activity after alkaline pre-incubation at pH 10.3, in the new-born animal. Fibres types are: negative I, moderate IIB and intense IIA. (b) Serial section stained for m-GPDH activity. Fibres types are: negative I, weak IIA and intense IIB. (c) Age: 45 days. m-ATPase activity after alkaline pre-incubation at pH 10.4. Fibres types are: negative I, moderate IIB and intense IIA. (d) Serial section stained for NADH-TR activity. Fibres types are: intense I and IIA, intense and negative IIB. Bar, 40 μm.

Table 7. Histochemical staining properties of muscle fibres during the postnatal development in the sheep*

	Age (days after birth)	I	IIA	IIB
Method 1 (10.2–10.6)	1–90	–	+ + +	+ +
Method 5 (4.6/4.3)	1–30	+ +	+/–	+/–
	45–90	+ + +	+/–	+/–
NADH-TR	1–90	+ + +	+ + +	+ +
α-MGPDH	1–90	–	+ + /+ + +	+ + /+ + +

*Relative staining intensity of different fibre types: + + +, intense; + +, moderate; +, weak; –, negative. Where very small differences in staining intensity occurred these are not shown in the table, but are described in the text.

activity. During the postnatal development the m-ATPase techniques that we used were method 1 and method 5.

Three fibre populations were present in the lambs at birth (Fig. 2a,b; Table 7):

- Alkaline-negative fibres: Histochemically, they were considered to represent type I fibres. Most of these fibres were located in the central area of the fascicles, they were rounded and remarkably large. However, in some fascicles small size fibres with identical histochemical characteristics could be distinguished. They clearly did not react with the M-GPDH glycolytic technique at this early age (Fig. 2b).
- Alkaline-intense fibres: These fibres were widely distributed across the fascicle though they tended to concentrate around the type I fibres (Fig. 2a). They have of a polygonal shape and their m-ATPase activity was as that of the IIA fibres described for the adult. With the acid pre-incubation m-ATPase technique, they could not be differentiated from the remaining type II fibres. The oxidative activity in these fibres was intense, they were moderate to intensely stained with the M-GPDH technique.
- Alkaline-moderate fibres: On the basis of myosin ATPase activity, these fibres were analogous to IIB adult fibres. They were mainly located in the periphery of the fascicle (Fig. 2a). All of them had a moderate oxidative activity in contrast to the fibres IIB described in the adult, their glycolytic activity was also moderate to intense.

Postnatal development (1–90 days) of the fibre types described at birth (Table 7; Figs 2c,d and 3a–d):

- Alkaline-negative fibres (type I): their intense stain for the m-ATPase technique with acid pre-incubation was not complete as was the case for the adult animals until 15–30 days after birth. They could be considered as adult type I fibres, at least from a histochemical point of view (Figs 2a,c and 3a,c).
- The alkaline-intense fibres (IIA) and the alkaline-moderate fibres (IIB) had an m-ATPase activity identical to types IIA and IIB, respectively, from the first days of postnatal development (Figs 2c and 3a,c).
- As in the adult animal, we detected during the postnatal development IIA fibres with weak glycolytic activity and IIB fibres with intense oxidative activity (Figs 2d and 3b,d).

Percentages of the fibre types

The percentage of alkaline-negative fibres (type I) was constant until the end of the postnatal development, while the percentage

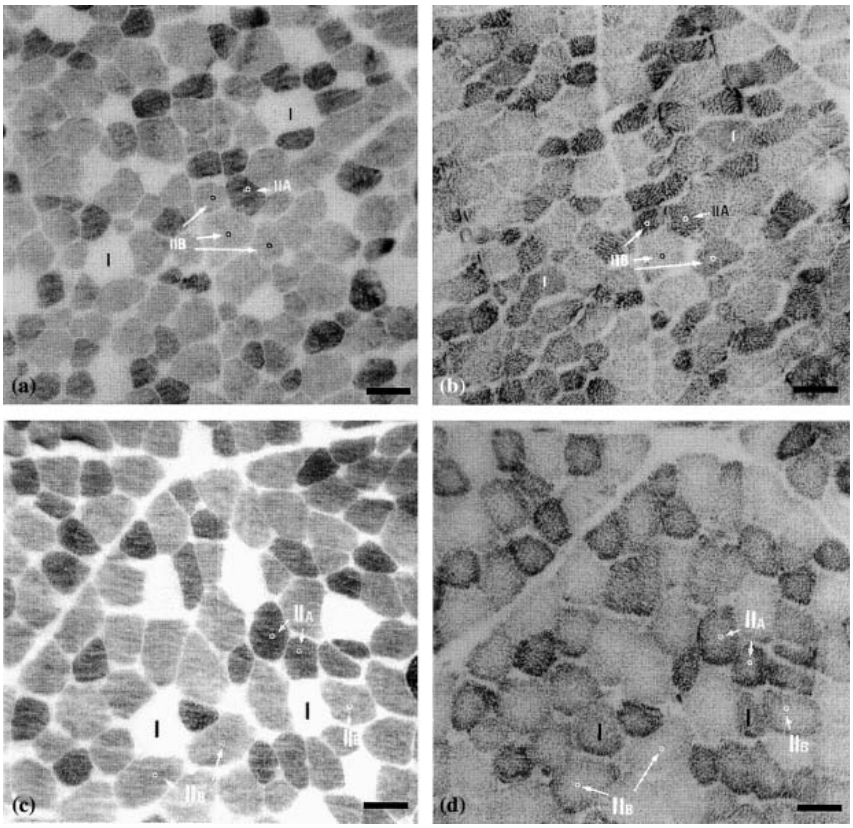

Fig. 3. Longissimus thoracis muscle from sheep. (a) Age: 60 days. m-ATPase activity after alkaline pre-incubation at pH 10.5. Fibres types are: negative I, intense IIA, moderate IIB. (b) Serial section stained for NADH-TR activity. Fibres types are: intense I and IIA, intense and negative IIB. (c) Age: 90 days. m-ATPase activity after alkaline pre-incubation at pH 10.4. Fibres types are: negative I, moderate IIB and intense IIA. (d) Serial section stained for NADH-TR activity. Fibres types are: intense I and IIA, intense and negative IIB. Bar, 40 μm.

of alkaline-intense fibres (IIA) decreased statistically only between birth and 15 days old. This decrease matched an increase in the percentage of alkaline-moderate fibres (IIB), which was present until the end of development (Table 5).

Size of the fibres

The mean minimum diameters values are show in Table 6.

Alkaline-negative fibres (type I) and alkaline-moderate fibres (IIB) always had larger values than type IIA fibres. During the postnatal development, the mean size values were 10.45 μm in type I and 15.37μm in type IIB. The alkaline-intense fibres (IIA) were the smallest, and during postnatal development, the minimum diameter reached a mean value of 10.38 μm (Table 6).

Discussion

Fibre types in the sheep

The use of the myosin ATPase technique in the sheep species only allowed the identification of two types of muscular fibres, the types I and II (Ashmore and Doerr, 1971; Suzuki, 1971a,b, 1995; White et al., 1978; Suzuki and Cassens, 1983; Suzuki and Tamate, 1988). However, some authors (Suzuki and Cassens, 1983; Suzuki, 1995; Carpenter et al., 1996; Menzel, 1999a,b), were able to identify more types of fibres in the sheep (IIA, IIB, IIC) using metabolic techniques. Nonetheless, our results showed that when applying the correct m-ATPase technique it is possible to distinguish at least three types of muscular fibres in the sheep as in others species (type I, type IIA and type IIB).

White et al. (1978); Suzuki and Cassens (1983) and Suzuki and Tamate (1988) classified the alkaline-resistant and acid-weak fibres (fibres type II) as IIA and IIB in order of their oxidative activity after the NADH-TR technique. Our results, to the contrary, revealed that a classification of the type II fibres based only on their oxidative activity is inaccurate. In the skeletal muscle of the ovine not all the alkaline-resistant fibres with high oxidative activity constitute the IIA fibre population. Moreover, it is not true that only those alkaline resistant fibres with oxidative-negative activity constitute the IIB fibre population. According to our results, the FOG and FG fibres cannot be compared with the type IIA and type IIB fibres, respectively. To accurately define the FOG and FG fibre populations, it is necessary to compare and contrast the two metabolic techniques (oxidative and glycolytic). In this sense, some of the alkaline-moderate fibres (IIB) identified in our study, are characterized by their atypical high oxidative activity. This group of fibres with the myosin-ATPase characteristics of the IIB fibres and the metabolic characteristics of the FOG fibres, have also been described in other species such as the pig (Gil et al., 2001), and could correspond to a particular type of fibres named IIX (Gorza, 1990). To be able to discern if the type IIB fibres with oxidative activity have some specific type of myosin different from the classic type IIB fibres (without oxidative activity), studies employing techniques such as inmunohistochemistry should be carried out. In this regard the results of Argüello et al. (1998) from the semitendinosus muscle of the goat confirmed four types of muscular fibres as identified based on their myosin content: I, IIA, IIA + IIX and IIX.

Fibre types percentages in the sheep

In the longissimus muscle Suzuki (1971a) found around 87% of fast fibres (grouping red and white fibres: A, 52% and B, 35%) and 12% of slow fibres (C + D). We agree with these results for the same muscle as regards type I fibres and as regards type II fibres as a whole. However, within the population of type II fibres our results differ from those of Suzuki.

Some studies (Ashmore et al., 1972; Suzuki and Tamate, 1988) suggest that in sheep the percentages and distribution of the different types of fibres, classified by means of the m-ATPase technique, are genetically determined in the semitendinosus, triceps brachii and abdominal cutaneous muscles. However, the percentage of the m-ATPase fibre types changed after birth in the quadriceps muscle (White et al., 1978) and in the longissimus muscle (Moody et al., 1980). Our results show that the percentages of the fibres I, IIA, IIB and IIC in the longissimus did not vary between day 30 and 90 of the development.

Suzuki and Cassens (1983) analysed in sheep the development of the fibre types percentages in the growing serratus ventralis thoracis muscle. According to these authors, the number of type II fibres (IIA plus IIB) decreases from birth (80%) until 4 weeks after birth (65%) and remains constant until the end of development. However, type I fibres increase their number from birth (10%) until 4-weeks old (35%) but then the percentage is also constant. Our results in the longissimus dorsi muscle agree with these authors in relation to the IIA and IIB fibres: we found that the percentage of IIA fibres only decreased between birth and 30 days after birth, whereas the percentage of type IIB fibres increased between birth and 15 days after birth. However, in our study type I fibres had the same percentage during development. The difference between our work and that of Suzuki and Cassens (1983) might be because of the fact that we studied different muscles.

Some publications (Ashmore et al., 1972; Hawkins et al., 1985) suggest a transformation of the αR fibres (of small size) into αW fibres (of a bigger size) as an explanation for the increase of muscular size during the development. We think that the developmental course of oxidative and/or glycolytic fibres should be specifically tested in future works. Some studies (Solomon et al., 1981) described different increases of percentages for certain aerobic fibres in the ovine longissimus muscle, and at 8 weeks after birth, Whipple and Koohmaraie (1992) found 48.6% of oxidative fibres and 41.6% of glycolytic fibres in this muscle.

Fibre types sizes in the sheep

It has been suggested (Hawkins et al., 1985) that the increase in the carcass fat content is related to an increase in red fibre size. This study also relates the size of the muscular fibres to the slaughtered weight, race and sex of the animals. (Suzuki and Tamate, 1988) proposed that in sheep the size of the type I fibres is similar to the size of the type IIB fibres in the hip and thigh muscles, as well as in other muscles (Suzuki, 1971a,b; Suzuki and Cassens, 1983). They also indicated that in sheep the glycolytic fibres (white) are not always bigger than the oxidative fibres (red). Our results confirm the large size of the fibres I and IIB and the smaller size of the IIA fibres. It is also noticeable that all three types of fibres identified with the m-ATPase technique significantly increased their size only between 60 and 90 days after birth.

In summary, with the use of an appropriate m-ATPase technique, the two fast fibre types IIA and IIB can be separated histochemically in skeletal muscle of sheep, even at early stages of postnatal development.

Acknowledgements

The Centro de Investigación y Desarrollo Agroalimentario (C.I.D.A.) de La Alberca (Murcia) supported this work. We thank Dr Paloma Peinado Ramón and Mr Michel Tomlinson for help preparing the English translation of the text.

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