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Influence of age on fibre type characteristics in the middle gluteal muscle of Andalusian foals

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Summary. 34 Andalusian foals of both sexes were divided into three age-groups (A = mean age 1 month, B = 7 months, C = 14 months). Samples of the right m. gluteus medius were stained for m-ATPase and NADH-TR in order to determine fibre type composition and size as well as the relative area occupied by each type. Results revealed no statistically significant variation in the proportion of type I fibre among the three age-groups. Significant differences were recorded, however, for type II fibres; an increase in the proportion of IIA fibres was accompanied by a decrease in IIB ones, the difference being most marked between groups A and B. IIB high-oxidative fibres also decreased, while IIB low-oxidative ones showed no significant variation. All fibre types increased significantly in size; types I and IIA recorded a threefold increase, whereas type IIB showed least growth. The relative area occupied by each type increased significantly between groups A and B, but only IIA fibres recorded a significant increase in relative area between groups B and C.

Key words: Andalusian foal, Muscle fibre, Age

Introduction

The characteristics of skeletal muscle fibre depend on a variety of factors, including the nature of the muscle concerned or of certain portions of it, (Kai, 1984; Bruce and Turek, 1985; Van den Hoven et al., 1985) breed, (Snow and Guy, 1980) training, (Essén-Gustavsson et al., 1989; López-Rivero et al., 1990c) and the age of the individual animal (Essén et al., 1980). The influence of age has not been analysed in any depth, particularly with a view to drawing conclusions regarding a foal's future locomotory capacity once it reaches adulthood, as some authors have proposed (Barlow et al., 1984).

With regard to equine muscle fibre composition, most authors report that the percentage of slow-twitch (type I, ST) fibres remains constant throughout postnatal development and growth (Lindhom and Piehl, 1974; Essén-Gustavsson et al., 1983; Henckel, 1983; Bechtel and Kline, 1987).

The percentages of subtypes IIA and IIB have been reported to vary with age, with an increase in IIA fibres and a decrease in IIB ones. (Essén et al., 1980). Fibre oxidative capacity increases with age, and all fibre types increase in size as the animal grows (Essén-Gustavsson et al., 1983).

The aim of the present study, as well as to confirm these reports, was to analyse the variations taking place in middle gluteal muscle fibre in Andalusian foals, a breed of great genetic purity.

Materials and methods

Horses

34 clinically-healthy Andalusian thoroughbred foals, ranging in age from 14 to 520 days, were used for this experiment. Animals were divided into 3 age-groups (Group A = mean age 1 month; Group B = mean age 7 months and Group C = mean age 14 months; Table 1).

Muscle Biopsy

Samples of m. gluteus medius were obtained following the Lindholm and Piehl technique (1974), using consistent anatomical landmarks to ensure that biopsies were always obtained from the same relative area of muscle and at a constant depth of 2.5 cm.

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Histochemistry

Samples were frozen in Isopentane previously cooled in liquid nitrogen (Dubowitz and Brooke, 1973) and stored at -80° C until use. Serial sections, 10 µm thick, were cut in a cryostat at -20° C, and stained for myofibrillar myosin adenosine triphosphatase (m-ATPase) after alkaline (pH 10.3) and acid (pH 4.2 and 4.4) preincubation. Fibres were identified as type I, IIA, IIB (black, white and grey respectively, after preincubation at pH 4.4; Fig. 1a and IIC (grey after preincubation at pH 4.2) (Brooke and Kaiser, 1970). Since the relative proportion of type IIC fibres was consistent only in the youngest age-group, and never exceeded 2-3% of the total, there were treated as IIB fibres (Essén-Gustavsson and Lindholm, 1985). Serial sections were stained for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) (Dubowitz and Brooke, 1973) (Fig. 1b). Type II fibre metabolism may be oxidative or non-oxidative (glycolytic) (Essén et al., 1980); here, fibres which stained intensely or moderately for NADH-TR (dark blue stain) were classified as IIB «oxidative» (IIBox), while those which presented an even light-blue stain were designated IIB «non-oxidative» (IIBno). For quantitative analysis, a minimum of 500 fibres from randomly-selected fields were counted in each sample.

Morphometry

The area and lesser diameter of type I, IIA and IIB fibres identified by m-ATPase after preincubation at pH 4.4 were determined for a minimum of 25 fibres of each type in a number of randomly-selected fields using an Ibas-2 Kontron computerized image analyzer. Lesser diameter was defined as the shortest line joining two points of an ellipse determined by transverse section and which passes through its geometric centre. This parameter would appear to be more reliable than fibre areas an expression of fibre size, since it is not affected by the possible oblique nature of the cut.

The relative cross-sectional area that a fibre type occupied in the biopsies (Pf, where f = type I, IIA or IIB) was calculated by dividing the product of the fibre type percent (%) and the mean area of the fibre type (mfaf) by the sum of these products for all fibre types (Sullivang and Armstrong, 1978):

$$Pf = \frac{(\%f * mfaf)}{\Sigma\ (\%f * mfaf) * 100}$$

Statistical methods

The values for each variable, based on kurtosis and skewness data provided by the computerized image analyzer program STAT-1, approached normal distribution for each biopsy. Routine statistical methods were used to calculate the mean and standard deviation (SD). The homogeneity of the data obtained for each group was tested using a one-way ANOVA and a two-way interactive ANOVA, levels of significance being expressed as p values. Differences between groups were determined by means of Tukey's Studentised Range Test using the General Linear Models procedure developed by Statistical Analysis Systems (SAS Inc, 1982).

Results

Fibre-type composition

Data obtained are shown in Table 2. Significant differences in IIBno properties between sexes ($p < 0.05$) were found only in group C, where percentages were greater in males than in females. A two-day ANOVA showed no interaction between groups and sexes ($p > 0.05$).

Differences between sexes were also recorded for IIBox fibres, the proportion being greater in females than in males ($p < 0.05$) (Table 2). Here, a group*sex interaction was recorded, although its negligible statistical significance ($p = 0.0483$) did not justify the separation of the groups by sex.

Tukey'–s T-test showed that the increase in the percentage of slow twitch (type I) fibres recorded from group A to group B was no significant ($p > 0.05$). The proportion of fast-twitch fibre subgroups (IIA and IIB) varied from group to group, with a significant difference ($p < 0.05$) betwen group A and group B. The proportion of IIBno fibres did not vary significantly, although a statistically significant difference was recorded in the percentage of IIBox ones between groups A and B.

Area and lesser diameter

Data are shown in Table 3. One-way ANOVA and group sex interaction tests, performed as for fibre composition, showed that both area and lesser diameter of IIA and IIB fibres were greater in females than in males in group C ($p < 0.05$). A significant group*sex interaction was recorded only for fibres area in type IIA fibres from group B ($p = 0.0342$). Since no significant interaction was recorded for lesser diameter in this group ($p = 0.2504$), the groups were not separated by sex, as lesser diameter is considered a more reliable measurement (Brooke and Kaiser, 1970; Banker and Engel, 1986). All fibre types were found to increase significantly in size from one group to the next: IIB fibres double in size, while I and IIA fibres recorded a threefold increase over the course of the experiment.

Relative area

The relative area occupied by each fibre type increased significantly between groups A and B for all types; between groups B and C, however, the only

Table 1. Grouping of foals, showing mean age in days(*), range, number of males/females and total number for each group.

	MALE	FEMALE	TOTAL
Group A			
N	7	5	12
MEAN	36	43	39
RANGE	14-57	18-54	14-74
Group B			
N	3	6	9
MEAN	209	210	210
RANGE	199-215	206-218	199-218
Group C			
N	7	6	13
MEAN	478	420	433
RANGE	372-520	354-451	354-520

* No significant differences were recorded between the mean age of males and females within each group.

Table 2. Middle gluteal muscle fibre type composition in Andalusian foals of different ages. Percentages of each fibre type are shown by sex and as a total. Data obtained by Tukey's T test among the three groups (A, B and C) and one-way ANOVA within each group and between groups are also shown.

	I	IIA	IIB	IIBno	IIBox
Group A (1 month)					
MALE n = 7	15.1 ± 2.7	25.7 ± 2.8	59.2 ± 4.6	29.2 ± 4.0	30.0 ± 1.9
FEMALE n = 5	16.1 ± 2.3	27.2 ± 2.7	56.7 ± 2.4	26.2 ± 2.0	30.5 ± 1.3
TOTAL n = 12	15.5 ± 2.5	26.3 ± 2.7[bc]	58.2 ± 3.9[bc]	27.9 ± 3.6	30.3 ± 1.6[bc]
Group B (7 months)					
MALE = 3	14.8 ± 4.1	32.9 ± 2.6	52.3 ± 2.6	31.1 ± 1.2	21.2 ± 2.3
FEMALE n = 6	20.1 ± 4.9	31.3 ± 1.3	48.5 ± 4.9	25.6 ± 5.8	23.0 ± 5.1
TOTAL n = 9	18.3 ± 5.1	31.9 ± 1.7[a]	49.7 ± 4.5[a]	27.5 ± 5.4	22,3 ± 4.3[a]
Group C (14 months)					
MALE n = 7	18.9 ± 1.6	35.4 ± 4.6	45.6 ± 5.3	27.9 ± 6.2	17.7 ± 7.0
FEMALE n = 6	18.3 ± 3.1	34.0 ± 3.0	47.6 ± 3.0	20.7 ± 3.7	27.0 ± 6.1
TOTAL n = 13	18.5 ± 2.3	34.6 ± 3.8[a]	46.9 ± 4.4[a]	24.6 ± 6.2*	22.3 ± 4.3[a]

* Differences between sexes ($p < 0.05$)
a. Different from group A ($p < 0.05$)
b. Different from group B ($p < 0.05$)
c. Different from group C ($p < 0.05$)

significant difference recorded ($p < 0.05$) was for IIA fibres (Table 4).

Discussion

The absence of any statistical difference in the proportion of slow-twitch fibres in the middle gluteal muscle of Andalusian foals of different ages coincides with findings reported for other breeds (Lindholm and Piehl, 1974; Bechtel and Kline, 1987). However, the slight increase (around 3%) recorded in the younger age groups (A and B) was similar to that reported by Bechtel and Kline (1987) in a linear study on Standardbred and Quarterhouse foals. It is widely accepted that the I/II ratio is determined genetically (Barlow et al., 1983) since each breed presents a characteristic ratio depending on its functional requirements, lower in sprinters and highter in stayers (Snow and Guy, 1980; López-Rivero et al., 1989). The variations detected with age were too slight to be taken into consideration, at least during the first year of life and when assessing biopsies taken at a uniform depth.

The proportion of fast-twitch subtypes IIA and IIB tends to vary substantially between age-groups (Essén et al., 1980). In this study of Andalusian foals, the percentage of IIA fibres rose significantly from group A to group B ($p < 0.05$; Table 2), while the increase - though still present - was no longer significant from group B to C ($p > 0.05$). The percentage of IIB fibres decreased at a similar rate (Table 2). Ronéus and Essén-Gustavsson (1986), studying the effect of muscular dystrophy on foals, reported that from 12 to 70 days old the percentage of IIA fibres was always smaller than that of IIB fibres in healthy Standardbred foals; a similar finding has been reported by Essén et al. (1980) and Essén-Gustavsson et al. (1983). We have obtained similar results in the Andalusian horse, but a preliminary study of Arabian horses revealed that the percentage of IIA and IIB fibres was the same at one year of age (Martínez-Galisteo, 1990). All this points to age being responsible for a transformation of

Table 3. Middle gluteal muscle fibre type size in Andalusian foals of different ages, showing the area (μm²) and lesser diameter (μm) of each fibre type by sex and as a total. Data obtained from Tukey's T test between totals, and a one-way ANOVA within and between groups are also shown.

	AREA (x10²)			Lesser diameter		
FIBRE TYPE	I	IIA	IIB	I	IIA	IIB
Group A						
1 months						
MALE = 7	5.8	10.0	22.7	23.5	30.8	45.3
	±0.8	±1.7	±2.5	±1.9	±3.1	±2.5
FEMALE n = 5	7.2	10.9	23.5	25.4	31.0	45.2
	±1.7	±1.3	±3.6	±3.1	±2.0	±4.3
TOTAL n = 12	6.4[bc]	10.3[bc]	23.0[bc]	24.3[bc]	30.9[bc]	45.3[bc]
	±1.7	±1.3	±3.6	±3.1	±2.0	±4.3
Group B						
7 months						
MALE n = 3	15.6	20.0	36.8	38.1	42.9	57.6
	±2.6	±3.6	±4.4	±3.2	±4.9	±3.4
FEMALE n = 6	14.6	19.4	30.0	36.9	43.5	56.1
	±2.5	±3.2	±5.0	±3.7	±3.2	±6.9
TOTAL n = 9	14.9[ac]	19.6[ac]	37.6[ac]	37.5[ac]	43.3[ac]	56.6[ac]
	±2.5	±3.1	±4.6	±3.3	±3.5	±5.8
Group C						
14 months						
MALE n = 7	17.9	28.8	46.5	40.7	51.2	64.9
	±4.6	±3.0	±4.7	±5.6	±2.8	±3.2
FEMALE n = 6	20.6	34.3	54.1	44.1	54.4	69.7
	±3.6	±2.9	±6.7	±4.2	±2.7	±4.3
TOTAL n = 13	19.1[ab]	31.3[ab]	50.0[ab]	42.2[ab]	53.1[ab]	67.1[ab]
	±4.2	±4.0*	±6.4*	±5.1	±3.4*	±4.4*

Differences between sexes ($p < 0.05$)
a. Different from group A ($p < 0.05$)
b. Different from group B ($p < 0.05$)
c. Different from grouu C ($p < 0.05$)

Table 4. Relative area occupied by each fibre type in biopsies taken from the middle gluteal muscle of Andalusian foals of different ages, expressed as % (sd) of each fibre type in each group. Data from Tukey's T test between groups are also shown.

	RELATIVE FIBER AREA (%) (*)		
	I	IIA	IIB
Group A (n = 12)			
1 month			
mean±sd	5.9±1.4[bc]	16.4±3.0[bc]	77.7±3.4[bc]
Group B (n = 9)			
7 months			
mean±sd	9.9±3.3	22.6±2.2[c]	67.4±4.7
Group C (n = 13)			
14 months			
mean±sd	9.6±2.6	28.9±4.4	61.4±5.8

*. No differences between sexes withing groups ($p < 0.05$)
b. Dofferemces with respect to group B ($p < 0.05$)
c. Differences with respect to group C ($p < 0.05$)

IIB fibres into IIA, a phenomenon previously reported by several authors for other breeds of horse, either as a function of age, (Essén et al., 1980; Essén-Gustavsson et al., 1983; Henckel, 1983) or as the result of training (Essén-Gustavsson and Lindholm, 1985; Essén-Gustavsson et al., 1989; López-Rivero, 1990c).

Such changes in the contractile properties of fibres can only come about as a result of prior modifications of metabolic profile, and more particularly of oxidative metabolism. This would naturally account for the decrease in IIB fibres, which were thus divided into two groups: those with a clearly oxidative metabolism (which stained intensely or moderately to NADH-TR), and those of low oxidative capacity (light-staining). This subdivision is feasible because both type I and type IIA fibres - at least in the middle gluteal muscle - always have oxidative metabolism (Essén et al., 1980). The percentage of IIBno fibres did not vary from one age-group to another, whereas that of IIBox fibres was significantly greater in group A than in group B (Table 2), suggesting that these are transformed into IIA fibres, while the IIBno fibre population either remains constant throughout postnatal development (at least during the first year of life) or displays a most a negligible decrease. These findings concur with the data reported by Bechtel and Kline (1987) for Standardbred and Quarterhorse foals

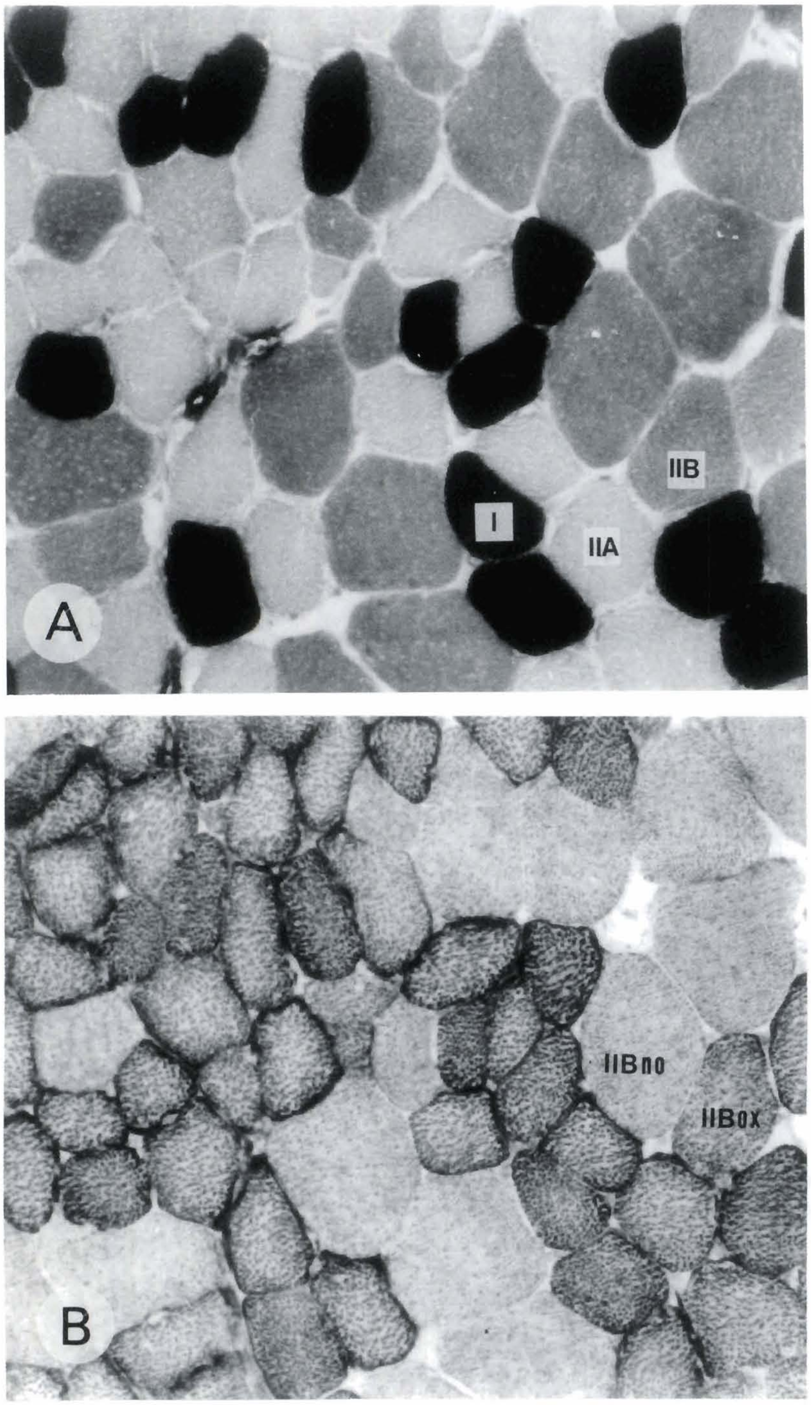

Fig. 1. Serial sections of m. gluteus medius from one year old Andalusian foal. **a.** m-ATPase after acid preincubation (pH 4.4). I, Type I fibres; IIA fibres and IIB, types IIB fibres. **b.** NADH-TR. IIBox, oxidative type IIB fibres and IIBno, non-oxidative type IIB fibres, (× 160)

with a similar age, which indicate that the percentage of FG fibres was the same at one year old as at birth. This transformation theory is borne out by the presence of fibres with an intermediate stain between IIB and IIA, which Snow (1983) has designated type IIAB and which are identifiable by m-ATPase after acid preincubation at pH 4.4; these intermediate fibres always have a clearly oxidative metabolism. All this gives rise to a clear differentiation between the effects of growth and the effects of training; an intensive training schedule leads to an increase in the oxidative capacity of all fibre types, including FT fibres; the percentage of FT fibres in this case decreases considerably (López-Rivero et al., 1990c).

Area and lesser diameter of the type IIB fibres were the largest in all age-groups, followed by types IIA and I. All fibres grew during the first year, but not at the same rate: the relative area occupied by types I and IIA almost tripled from group A to group C, whereas IIB fibres showed only a twofold increase, the greater rise being recorded between groups A and B.

IIA and IB fibres were found to be larger in females than in males; this finding coincides with data obtained by López-Rivero et al. (1990a,b), who report that Andalusian mares have greater type IIB fibres than stallions of the same breed. This may indicate that fast-twitch fibres develop earlier in mares. López-Rivero et al. (1990a) attribute the difference in size to the greater degree of physical activity in stallions, although this factor can be ruled out in the present study, since both sexes were undergoing exactly the same amount of physical activity.

Comparison of fibre size in one-year-olds and adults (López-Rivero et al., 1990a,b) reveals, as expected, that I and IIA fibres increase in size with the growth of the animal. Surprisingly, however, there is little difference in IIB fibre size between one-year-olds and adults, suggesting that these are the first fibres to stop growing during postnatal developement.

The relative area occupied by IIB fibres was always greater than that of I or IIA fibres in all age-groups. The significant increase in type IA area recorded between groups A and B was caused by a considerable growth in size over this period, since the number of type I fibres did not increase significantly. This may be a response to the postural requirements of the animal, given the substantial weight increase over the same period and the fact that relative area is a highly reliable indicator of muscle function (Andrews and Spurgeon, 1986). From seven months onwards, few variations were recorded in relative area, a finding also reported by Lindholm and Piehl (1974), although here an increase in the relative area occupied by IIA fibres from 7 to 14 months (group B and C) was accompanied by a decrease in that of IIB fibres.

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