

# SKELETAL MUSCLE HISTOCHEMISTRY OF ANDALUSIAN HORSES: A COMPARATIVE STUDY WITH OTHER BREEDS

## CARACTERISTICAS HISTOQUIMICAS DEL MUSCULO ESQUELETICO EN CABALLOS ANDALUCES: ESTUDIO COMPARADO CON OTRAS RAZAS

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### Additional Keywords

Muscle fibre. Thoroughbred. Arabian horse.

### Palabras clave adicionales

Fibra muscular. Pura sangre inglés. Caballo árabe.

### SUMMARY

The purpose of this study was to characterize the histochemical and morphometric profiles of the skeletal muscle in adult Andalusian horses, and compare it with those of Thoroughbred and Arabian horses. Muscle biopsies were taken from the *Gluteus medius* of 32 Andalusian horses (15 mares, 17 stallions), 24 Thoroughbred (17 mares, 7 stallions), and 15 Arabians (7 mares, 8 stallions), ranging from 5 to 24 years. Consistent sex differences in fibre type composition and fibre area were observed. A highly significant difference among breeds was also obtained, probably being related to the type of work for which the breeds have been selected. Results also imply that histochemical and morphometric properties of the skeletal muscle in Andalusians provide only a moderate endurance potential. However, since genetic factors are of importance, the endurance potential of a breed could be significantly increased by means of a correct genetic program.

### RESUMEN

El presente estudio trata de caracterizar los perfiles histoquímico y morfométrico del mús-

culo esquelético en caballos Andaluces adultos, y compararlos con los de caballos Purasangre y Arabes. Se obtuvieron biopsias del músculo *Gluteus medius* de 32 caballos Andaluces (15 yeguas, 17 sementales), 24 Purasangre (17 yeguas, 7 sementales), y 15 Arabes (7 yeguas, 8 sementales), de 5 a 24 años de edad. Se observaron importantes diferencias en el porcentaje y tamaño de las fibras entre sexos y también entre razas, según el tipo de trabajo para el que cada raza ha sido específicamente seleccionada. Las propiedades histoquímicas y morfométricas del músculo esquelético en el caballo Andaluz sólo le proporcionan una capacidad de resistencia moderada. No obstante, dada la importancia de los factores genéticos, el potencial de resistencia de los caballos Andaluces podría modificarse significativamente mediante un adecuado programa genético.

### INTRODUCTION

Mammalian skeletal muscle is composed of various fibre types with different metabolic and contractile properties (Brooke and Kaiser, 1970;

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Lindholm and Piehl, 1974). Based on differences in the alkaline lability of myosin adenosine triphosphatase myofibrillar (mATPase) activity, muscle fibres can be classified into two main types: type I (low activity) and type II (high activity). The type II fibres can be further subdivided into IIA and IIB fibres on the basis of differences in their mATPase acidic lability. Because there is a positive correlation between mATPase activity and contraction velocity, it is appropriate to refer to type I fibres as slow-twitch fibres and type II fibres as fast-twitch fibres.

The proportion and size of fibre types in adult skeletal muscle are the result of the interaction of several factors. It has been extensively documented that while muscle fibres have great plasticity to adapt both their metabolic function and muscle fibre composition to environmental influences, hereditary factors are also of great importance in this respect. Thus, there appears to be a strong genetic component determining the presence of primary- and secondary-generation cells which are, in general, the forerunners of slow- and fast-twitch fibres, respectively (Kelly and Rubinstein 1980). Several studies of horses over the last 10 years have shown that muscle fibre composition and size vary significantly between breeds, and in accordance with the type of work for which the breed has been selectively bred (Gunn 1978; Snow and Guy 1980 and 1981; López-Rivero *et al.* 1989 and 1990a). Spanish (Andalusian) horses were used in the past for agricultural and breeding purposes, but now they tend

to be used more for riding and recreational purposes requiring moderate endurance potential. Few studies have attempted to evaluate muscle characteristics in Andalusian horses. The study reported here was carried out in order to characterize the histochemical and morphometric profiles of the skeletal muscle in adult Andalusian horses, and compare these characteristics with those of Thoroughbreds and Arabian horses in order to obtain information regarding their functional implications.

## MATERIALS AND METHODS

A total of 71 clinically healthy horses, 32 Andalusian, 24 Thoroughbred, and 15 Arabian, were used for this study. The horses, which ranged in age from 5 to 24 years, were allotted according to breed and sex (**table I**). None of them had been subjected to any particular training programme. Stallions, however, had been given some exercise (light riding, harnessing), while mares had not been exercised at all.

Muscle samples from the right *Gluteus medius* were obtained by the percutaneous needle biopsy technique described by Lindholm and Piehl (1974). Care was taken to standardize the location and depth of the sample, as described by López-Rivero *et al.* (1992a). All biopsies were taken by the same person, at a depth of 5 cm, and always within the same relative area. Samples were quick-frozen in isopentane kept in liquid nitrogen and stored at -80 °C until analysed.

Serial transverse sections, approxi-

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**Table I.** Number of horses and mean age ( $\pm$ SD) of each group. (Número de caballos y edad media (y desviación típica) de cada grupo).

Breed	Sex	Number	Age (years)	
			mean	SD
Andalusian	Mares	15	13.13	4.33
	Stallions	17	10.53	4.62
Throughbred	Mares	17	9.71	2.11
	Stallions	7	12.86	3.44
Arabian	Mares	7	11.57	3.11
	Stallions	8	13.00	6.18

mately 10  $\mu$ m thick, were incubated for  $\text{Ca}^{++}$ -activated myofibrillar adenosine triphosphatase (mATPase) at pH 9.4 after preincubation at pH 10.3, 4.5, and 4.2. Muscle fibres were classified into type I, IIA, and IIB (Brooke and Kaiser 1970) according to the mATPase staining characteristics at the different levels of preincubation acidity (**figure 1 a,b,c**). A semiquantitative evaluation of the oxidative capacity of fibre types was obtained from serial sections stained with reduced nicotinamide dinucleotide dehydrogenase tetrazolium reductase (NADH-TR) (**figure 1 d**).

The relative frequency of these fibre types was determined by counting at least 400 fibres within a specimen. The mean cross-sectional area of I, IIA, and IIB fibres was measured for all biopsy specimens by means of a computerised video-display image analysis system (Ibas-2 Kontron). Between 25 and 50 random measurements were made for each fibre type and the results for each individual were averaged according to fibre type.

Mean values (and SD) for muscle fibre type composition and fibre areas

were obtained separately for stallions and mares by routine statistical procedures. Unpaired tests (proportions and means) were used to demonstrate the hypothesis that no difference is present in fibre type proportion and fibre type sizes within each breed for different ages.

## RESULTS

No significant differences attributable to age in fibre type proportions and sizes were obtained here ( $p > 0.05$ ). Consequently, the values presented for each breed and sex represent pooled mean values for all ages.

As it can be seen in **table II** in general, mares had a higher percentage of type I fibres ( $p < 0.001$ ), a lower percentage of IIA fibres ( $p < 0.001$ ), and smaller type IIB fibres ( $p < 0.05$ ) than stallions.

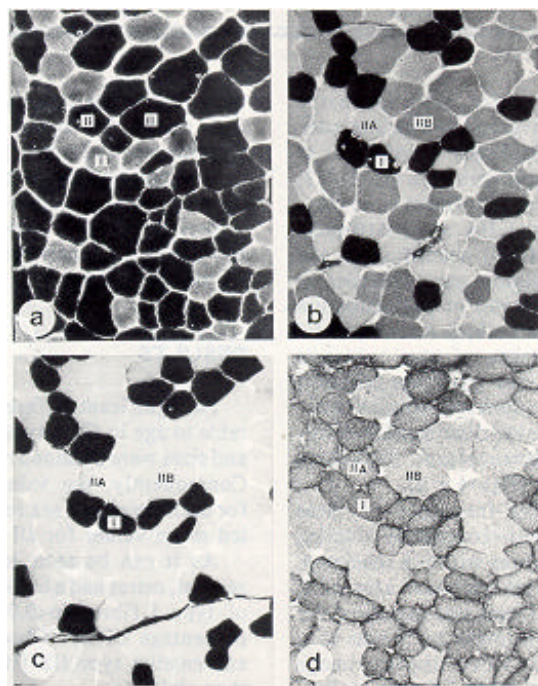
A highly significant difference between breeds was observed with regard to the percentage of type I and type IIA fibres ( $p < 0.001$ ), as well as in the cross-sectional area of type II fibres (IIA  $p < 0.05$ ; IIB  $p < 0.001$ ).

Thoroughbreds had the lowest and Arabians the highest percentage of type I fibres, whereas Andalusians are situated midway between the other two breeds regarding this variable. Thoroughbreds showed a significantly higher percentage of type IIA fibres than the other two breeds. Finally, the cross-sectional area of

type IIA and type IIB fibres were significantly larger in Andalusian horses than in Thoroughbreds and Arabians

## DISCUSSION

Results from this study imply consistent differences between sexes.



**Figure 1.** Myosin ATPase staining after preincubation at pH 10.3 (a), 4.5 (b) and 4.2 (c), and NADH-TR activity (d) of one biopsy specimen removed from the *Gluteus medius* muscle of an Andalusian horse. Types I, IIA and IIB fibres are marked. (Tinción de miosín ATPasa tras incubación a pH, 10.3 (a), 4.5 (b) y 4.2 (c), y de NADH-TR (d) de una biopsia del *Gluteus medius* de un caballo Andaluz. Las fibras I, IIA y IIB están identificadas).

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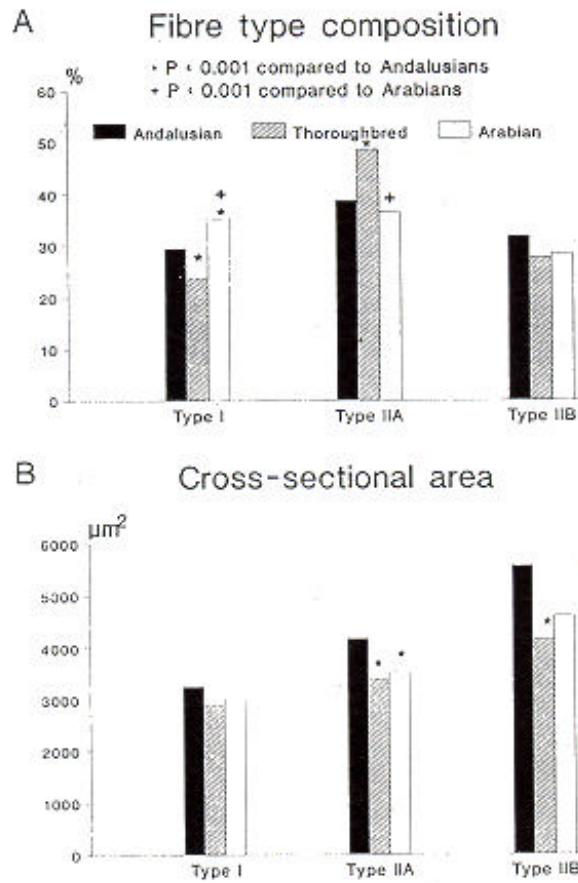
In a given breed, the percentage of type I fibres was greater in mares than in stallions. A similar sex variation has been found in Thoroughbred (Snow and Guy, 1981). It is possible that the sex difference observed here may reflect the greater selection pressure that could be applied to stallions for breeding purposes. The significantly increased IIA-to-IIB fibre ratio and the smaller IIB fibres found in stallions show that the mean relative cross-sectional area occupied by IIA fibres in biopsy specimens is significantly greater for males than for females, at the expense of type I and type IIB fibres. This characteristic should be beneficial for work of moderate intensity and longer duration, because type IIA fibres frequently have a higher oxidative capacity than type IIB fibres, a character that makes them more fatigue-resistant than IIB type (Essén-Gustavsson and Lindholm, 1985).

In this study, significant differences in percentages and cross-sectional areas of various fibre types were detected in the skeletal muscle between Andalusian and Thoroughbreds or Arabians. The highly significant difference in the proportion of slow- to fast-contracting muscle fibres among breeds is consistent with other studies on horses (Gunn, 1978; Snow and Guy, 1980 and 1981; López-Rivero *et al.*, 1989). These differences might be related to the type of performance for which the animal is best suited. The type I-to-II fibre ratio of equine muscle does not change as a result of endurance training (López-Rivero *et al.*, 1991), and research on equine athletes has shown that the type of athletic ability to which an individual is best suited is directly related to muscle fibre type composition in certain muscles (Snow and Guy, 1981; Wood *et al.*, 1988). Moreover, fibre composition and size of equine muscle

**Table II.** Mean values ( $\pm$ SD) of fibre types and cross-sectional areas and results of the homogeneity tests of proportions and means. (Valores medios ( $\pm$  desviación típica) de los porcentajes y áreas de sección transversal fibrilares en cada raza y sexo, y resultados del test de homogeneidad para proporciones y medias).

Breed	Sex	No.	Fibre composition (%)			Cross-sectional area ( $\mu\text{m}^2$ )		
			I	IIA	IIB	I	IIA	IIB
Andalusian	Mares	15	32(7)a	33(5)a	35(6)	3217(864)	4198(1257)a	6379(1335)a
	Stallions	17	27(6)a	42(5)a	31(5)	3226(880)	4168(1032)a	5017(1424)a
Thoroughbred	Mares	17	25(5)b	48(8)b	28(11)	2999(543)	3434(584)b	4399(838)b
	Stallions	7	21(6)b	50(6)b	29(6)	2777(836)	3487(905)b	3419(634)b
Arabian	Mares	7	42(10)c	30(6)a	28(6)	3000(290)	3488(301)ab	4145(1136)ab
	Stallions	8	30(5)c	42(4)a	28(3)	3195(813)	3819(927)ab	4873(907)ab

Significance for the three breeds or the two sexes: \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ . Breeds with different letters within a column are statistically different as analyzed by Scheffe's test.



**Figure 2.** Mean fiber type composition (% A) and cross-sectional area ( $\mu\text{m}^2$  B) in Andalusian, Thoroughbred and Arabian horses. (Tipos medios de fibras musculares (% A) y área de sección transversal ( $\mu\text{m}^2$  B) en caballos Andaluces, Purasangre y Arabes).

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are unaffected by the paternal factor within a given breed, but a tendency to influence the ratio of slow- to fast-contracting fibres was observed (Rivero *et al.*, 1992). All these conclusions support the role of inheritance.

Although a single biopsy from the *Gluteus medius* muscle is a poor estimator of the whole muscle due to the substantial variability of fibre composition and size as a function of sampling depth (López-Rivero *et al.*, 1992a), differences in type I-to-II ratio between breeds are very noticeable and they indicate therefore a functional differentiation of the skeletal muscle in each breed. Because slow-twitch muscle fibres are highly oxidative, they are designed for endurance activities and utilize oxygen in their energy processes. The Arabian horse may be the best suited for endurance work, whereas the Thoroughbred may be the most suitable for racing (**figure 2 a**), because the fastest sprinters have the highest proportion of fast-twitch fibres (Snow and Guy, 1980).

With regard to fibre size, the comparative analysis revealed that Andalusians tended to have larger myofibres than Thoroughbred and Arabians (**figure 2 b**). It is not yet known with any certainty whether the size of muscle fibres is a hereditary characteristic or the result of external factors. Size

differences in type II (particularly IIB) fibres among mature untrained horses of several breeds observed in the current study, do suggest that genetic factors are also involved in the predetermination of fibre size in horses. Formerly, we found, in general, minimal effects of endurance training on the myofibre size of mature Andalusians and Arabians, but a tendency towards the increase in certain fibre type sizes (especially type I and IIB) was found in Andalusians (López-Rivero *et al.*, 1992b).

The largest type II fibres found in Andalusians represent another disadvantage for aerobic metabolism, because there is a correlation between cross-sectional fiber area and oxygen diffusion time (Hill, 1965); moreover, the supply of oxygen and nutrients is faster and more efficient in small fibres than in larger fibres.

In conclusion, this study suggests that histochemical properties of the skeletal muscle on Andalusian horses provide only a moderate endurance potential. Notwithstanding, based in the influence of hereditary factors on muscle fibre composition in horses, we suggest that the histochemical and morphometric profiles of the skeletal muscle of an entire equine breed could be significantly modified by means of a correct genetic programme.

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