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Variability of muscle fibre type composition in a number of genealogical bloodlines in Arabian and Andalusian horses

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Summary

Muscle biopsies were obtained from three different depths of the gluteus medius muscle in 118 horses (33 Arabians and 85 Andalusians), ranging from 42 to 56 months old. All the horses were kept under identical environmental conditions from birth. For each breed, the horses were allotted according to a number of different paternal and maternal bloodlines. Muscle biopsy specimens were analyzed for fibre type distribution (types I, IIA and IIB). Significant differences in muscle fibre type composition were observed among horses belonging to different bloodlines. A very significant influence of the maternal bloodline on the percentages of types I and IIB fibres ($P<0.001$) was observed in Andalusian horses. In addition, the paternal bloodline had a significant effect on the percentage of type IIA fibres in Arabian and Andalusian ($P<0.01$ in both) horses. Consistent variations in fibre types were also confirmed between breeds and between sexes. It is concluded that the proportions of equine muscle fibre types are significantly correlated with genetic factors.

Keywords: equine, genetics, inheritance, skeletal muscle, muscle fibres, bloodlines

Variationen der Muskelfaserzusammensetzung bei einigen geneologischen Blutlinien von Pferden der Rasse Araber und Andalusier

Bei 118 untrainierten Pferden, 33 Araber und 85 Andalusier im Alter von 42 bis 56 Monaten, wurden Muskelbiopsien aus 3 verschiedenen Tiefen des M. glutaeus medius entnommen. Alle Pferde wurden von Geburt an unter identischen Management- und Haltungsbedingungen aufgezogen. Innerhalb jeder Rasse wurden die Pferde anhand der verschiedenen väterlichen und mütterlichen Blutlinien eingeteilt. Die Muskelbioptate wurden histochemisch hinsichtlich der Verteilung der unterschiedlichen Muskelfasern (Typ I, IIA und IIB) untersucht. Pferde, die verschiedenen Blutlinien angehörten, wiesen signifikante Unterschiede in der Muskelfaserzusammensetzung auf. Bei den Andalusischen Pferden hatte die mütterliche Blutlinie einen hochsignifikanten Einfluß ($p<0,001$) auf den Anteil der Typ I- und IIB-Fasern. Desweiteren hatte die väterliche Blutlinie bei Arabern und Andalusiern einen signifikanten Einfluß auf den Anteil der Typ IIA-Fasern ($p<0,01$ für beide Rassen). Es konnten übereinstimmende Variationen der Anteile der Muskelfasertypen zwischen den Rassen und zwischen Geschlechtern festgestellt werden. Aus den Ergebnissen geht hervor, daß beim Pferde die Anteile der verschiedenen Muskelfasertypen signifikant mit genetischen Faktoren korreliert sind.

Schlüsselwörter: Pferd, Genetik, Vererbung, Skelettmuskel, Muskelfasern, Blutlinien

Introduction

Over the past two decades, great interest has been shown in factors affecting histochemical properties of equine skeletal muscle. Now, there is sufficient evidence that certain muscle characteristics vary in a number of ways: breed, age, sex, training, anatomical and pathological aspects, etc. (*Snow* and *Valberg* 1994). Moreover, several studies have reported a significant correlation between muscle characteristics and performance capacity in horses (*Snow* and *Guy* 1981; *Rivero* et al. 1993a). It can be assumed that some variations in muscle properties may be related to inherent traits. Significant differences in these attributes are present in newborn foals of the same breed, in yearlings before and after training and among several breeds of relatively untrained horses (*Snow* and *Valberg* 1994). All these findings support the role of inheritance in skeletal muscle histochemistry. In a categorical study of monozygous and dizygous human twins (*Komi* et al. 1977), it was concluded that there is a predominant genetic influence on muscle fibre type composition. Similar conclusions have been reported in other mammals (*Nimmo* et al. 1985).

However, no specific studies have been designed to investigate genetic influence on muscle fibre type composition within breeds of horses. The existence of several bloodlines within a given horse farm provides a good model for the examination of the degree of association between muscle characteristics and genetics in horses. Generally, each bloodline (either paternal or maternal) is designated by the most influential animal belonging to that bloodline. The purpose of the present pilot study was to compare the muscle fibre type composition among Arabian and Andalusian horses belonging to different genealogical bloodlines, in order to determine whether inbreeding effects from a common ancestor are associated with significant variations of these muscle characteristics.

Materials and methods

Horses

The population consisted of 118 clinically healthy, untrained horses: 33 Arabians (AR, 17 mares and 16 stallions) and 85 An-

dalusians (AN, 43 mares and 42 stallions), which ranged from 42 to 56 months old. The stallions have not been gelded. All the horses belonged to the same farm under the directorship of the Spanish Cria Caballare. Therefore, all horses were raised under identical management practices and environmental conditions from birth.

Classification of horses

For each breed, the horses were allotted according to their genealogical bloodline (Tab. 1). In this table there are two different paternal (PBL) and eight maternal (MBL) bloodlines in AR horses, and four PBLs and nine MBLs in AN horses. For each individual horse, the influence of each founder horse was quantified. For that purpose, the percentage of genes of each founder animal (stallion or mare) in the genealogy of a given horse (Pi) was calculated according to the formula (*Falconer* 1989):

$$Pi=\Sigma\ (1/2)^n$$

where 'n' is the number of generations between that founder animal and the horse under study. This summation is the number of paths to the common ancestor. The individual horse was included in that genealogical bloodline with the highest Pi value. However, in order to be these categories distinct enough, a given horse was allotted to a given bloodline only if Pi was above 15%.

Muscle biopsies

For each horse, three biopsy specimens were obtained at depths of 20 (superficial), 40 (middle) and 60 mm (deep sampling depth) from the right gluteus medius muscle as previously detailed (*Rivero* et al. 1995) and according to *Lindholm* and *Piehl* (1974). The sampling area is topographied on the ventral compartment of the gluteus medius muscle (*Bruce* et al. 1993), and all sampling sites therefore belonged to the same compartment of the muscle. Upon collection, the muscle samples were frozen by immersion in isopentane (30 sec) kept at freezing point in liquid nitrogen. All samples were stored at –80°C until analyzed.

Histochemistry

Transverse serial-sections of 10 μm were cut on a cryostat microtome at –20°C and incubated for myosin adenosine triphosphatase (mATPase, Enzyme Comission number 3.6.1.3) activity at pH 9.4 after three pre-incubations at pH 10.3, 4.5 and 4.2. Staining methods used were those of *Dubowitz* (1985). The myofibres were classified into type I, IIA and IIB according to the mATPase staining pattern. The relative frequencies of types I, IIA and IIB fibres were determined by typing at least 500 fibres per biopsy specimen.

Statistical analysis

Two-factor analysis of covariance (ANCOVA) with PBL and MBL as the factors and sampling site as covariate, was used, within each breed, to test the hypothesis that no difference is present in fibre type composition among the different bloodlines. Additionally, breed and sex effects were also examined by two-factor ANCOVA with muscle sampling depth as the factor of covariation. When a particular F ratio was significant ($P<0.05$), a post-hoc Tukey's studentized range test was performed to compare multiple means.

Results

The F values of the two-factor ANCOVA carried out to test differences among PBLs and among MBLs are summarized in Tab. 2. Significant differences in the percentage of type IIA fibres were recorded between at least two of the PBLs in both AR and AN horses ($P<0.01$ in both). However, PBL-associated variations in percentages of types I and IIB fibres were not significant ($P>0.05$). There were very significant differences in the proportions of these two later fibre types among MBLs in ANs ($P<0.001$). While the percentage of type I fibres varied significantly between only two MBLs of AN horses, the proportion of type IIB fibres differed significantly among a great number of MBLs within this breed. The percentage of type IIA fibres was homogeneous in all MBLs in AN horses ($P>0.05$). Significant va-

Tab. 1: Number of stallions and mares and mean age of horses included in each paternal and maternal bloodline.

	Paternal bloodlines				Maternal bloodlines									Total	Mean age (months±SD)
Gender	1	2	3	4	1	2	3	4	5	6	7	8	9		
					Arabian horses										
Stallions	9	7			0	5	4	1	1	2	0	3		16	43.87±1.27
Mares	11	6			2	6	5	2	0	0	2	0		17	48.06±2.84***
Total	20	13			2	11	9	3	1	2	2	3		33	45.97±3.04
					Andalusian horses										
Stallions	18	5	6	14	5	9	0	3	3	16	2	3	2	43	44.47±1.40
Mares	19	9	5	9	6	7	4	3	2	17	2	0	1	42	46.46±2.67***
Total	37	14	11	23	11	17	4	6	5	33	4	3	3	85	45.56±2.45

***, $P<0.001$ compared to stallions

Tab. 2: F values of a two-factor analysis of covariance made to test inbreeding effects from a common paternal and maternal ancestor on muscle fibre type composition. The effect of sampling depth was used as the factor of covariation.

Fibre types	Paternal effect	Maternal effect	Depth effect
Arabian horses (n=33)			
I	0.40	1.48	84.94***
IIA	5.44**	0.90	3.43
IIB	0.34	1.11	92.10***
Andalusian horses (n=85)			
I	1.08	4.09***	279***
IIA	4.15**	1.63	3.14
IIB	1.23	4.11***	320.96***

n, number of horses. ***, **, * The effect is significant at P<0.001, P<0.01, P<0.05, respectively

riations in fibre type composition were not recorded among MBLs in ARs (P>0.05).

There were systematic and significant differences in muscle fibre type composition among the three sampling sites (Tab. 3). The percentage of type I fibres increased significantly (P<0.001) going from the most superficial to the deepest sampling site of the muscle. Conversely, the proportion of type IIB fibres decreased (P<0.001). A reduced, but significant (P<0.05), increase in the percentage of type IIA fibres with increasing depth was also observed. The effect of breed and sex was highly significant with respect to the relative frequency of the various fibre types (Tab. 3). At a given depth of the muscle AR horses had higher percentages of types I and IIA fibres (P<0.01 in both) and a lower proportion of type IIB fibres (P<0.001) compared with ANs. Stallions had a higher percentage of type IIA fibres and a lower proportion of type IIB fibres (P<0.001 in both fibre types) than mares. However, no significant differences in the percentage of type I fibres were observed between sexes (P>0.05).

Discussion

The gluteus medius muscle is the most frequently sampled when studying the effects of training, growth, and pathology in the horse because it is a major propulsive muscle active in locomotion and it is easily accesible (*Lindholm* and *Piehl* 1974). The considerable variability of fibre types within the equine gluteus medius muscle, particularly as a function of sampling depth, indicates that a single muscle biopsy is a poor estimator of the whole muscle (*Rivero* et al. 1992). *Lexell* et al. (1985) concluded that it is possible to minimize the variance involved in the procedure of sampling living muscles, by taking for each individual multiple biopsies from different depths of the muscle. In the present study, we examined three biopsies representative of superficial, middle and deep regions of the muscle. Thus, it is possible to establish the depth-related rate of change of muscle fibre types and subsequently to obtain more accurate information regarding the histochemical

Tab. 3: Mean values (±sd) are shown for muscle fibre type composition (%) at 3 sampling depths of the M gluteus medius of male (M) and female (F) Arabian (AR) and Andalusian (AN) horses

Sampling site	Breed	Sex		Fibre types I	IIA	IIB
Superficial	AR	F	(n=17)	19(4)	36(4)	45(6)
		M	(n=16)	21(5)	37(5)	42(6)
	AN	F	(n=43)	17(4)	34(5)	49(5)
		M	(n=42)	21(6)	36(7)	43(6)
Middle	AR	F		28(11)	38(6)	34(10)
		M		32(11)	38(4)	30(11)
	AN	F		26(7)	36(4)	38(7)
		M		27(7)	37(6)	36(7)
Deep	AR	F		46(14)	37(6)	17(16)
		M		40(9)	40(6)	20(10)
	AN	F		39(9)	35(6)	26(9)
		M		38(10)	37(6)	25(10)
			Breed	8.55**	6.82**	21.47***
			Sex	1.35	7.45**	8.96**
			Depth	326.51***	5.87*	275.86***

The F values of a two-factor analysis of covariance where the factors were breed and sex, and the sampling depth was the factor of covariation are also shown . ***, **, * Means that a ratio is significant at P<0.001, P<0.01 and P<0.05 levels, respectively. n, number of horses

profile of the muscle. The significant variations of fibre types among sampling depths substantiate former studies and their functional significance of these variations has been extensively interpreted (*Rivero* et al. 1992).

The most important observations of the present study were the significant variations in muscle fibre type composition seen among horses from different PBLs and MBLs. Of particular importance was the strong influence of the MBL on the percentage of type I fibres recorded in AN horses. An important aspect of this study was the rigid control exerted in selecting the horses. Only horses under identical environmental conditions, varying in a very narrow age range and balanced for gender were included in the study. It seems, therefore, that variations observed in muscle fibre type composition are certainly under genetic influence.

The significant effect of the MBL on the percentage of slow-contracting fibres observed in AN horses is consistent with the results reported by *Wood* et al. (1988) in Quarter horses. They found significant variations in the type I-to-type II fibre ratio among horses allotted according to their bloodline. Our findings are also coherent with the high coefficient of inheritance (96%) estimated for that fibre ratio by *Komi* et al. (1977) in humans. This also has been supported by studies in other species, including an investigation using two inbred strains of mice (*Nimmo* et al. 1985). The latter concluded that the heredability of fibre type percentage, total fiber number and relative size of type I and type II fibres were highly significant. Furthemore, the results indicated a polygenic mode of inheritance. Thus, the significance of the genetic component in determining the individual variations observed in muscle fibre types may well be a direct consequence of important genetic influences determining the presence of primary and secondary generation muscle cells, which are, in general, the forerunners of slow- and fast-twitch muscle fibre types, respectively.

Our results also suggest significant PBL- and MBL-related modifications in both subtypes of fast-twitch fibres (types IIA and IIB). A significantly higher percentage of fast-twitch high oxidative fibres was also found in a bloodline of Quarter horses bred to run in comparison to other bloodline of horses not bred to run (*Wood* et al. 1988). Many studies in horses have reported an increase in the percentage of type IIA fibres accompanied by a decrease in that type IIB fibres associated with both training and growth (*Snow* and *Valberg,* 1994). However, it is not precisely known to what extent proportions of these fibre types are related with inherent traits or how far they may be exclusively attributable to environmental factors.

The significant differences in muscle fibre type composition observed in the present study between AR and AN horses are not new findings (*Snow* and *Guy* 1981; *Rivero* et al. 1989). The higher percentages of type I and IIA fibres observed in AR horses compared with AN horses, constitute an excellent muscular adaptation for aerobic requirements during long-distance exercises of submaximal intensity. The higher percentage of type IIB fibres, frequently with low oxidative capacity, recorded in AN horses in comparison with AR horses, may represent a disadvantage for aerobic metabolism. This may be an important factor limiting endurance potential in this breed.

The higher percentage of type IIA fibres and the lower proportion of type IIB fibres observed in stallions compared with mares also substantiate the results of *Rivero* et al. (1993b) for AN and AR horses and *Roneus* et al. (1991) for Thoroughbreds. Interestingly, mares used in the present study were, on average, slightly older than stallions (Tab. 1). As the percentage of type IIA fibres increases significantly over the first 4–6 years in horses whereas the proportion of type IIB decreases (*Roneus* et al. 1991; *Rivero* et al. 1993b), differences between sexes seen in the present study cannot be attributed to an age-linked effect. These variations can be neither associated with sampling effects, because despite a biopsy taken from the same absolute depth of the gluteus medius muscle might be relatively more superficial in stallions than in mares, the proportion of type IIA fibres increased significantly as a function of sampling depth (Tab. 3). Thus, the finding of a higher percentage of type IIA fibres in stallions in comparison with mares, is difficult to reconcile with a presumibly more superficial location of sampling sites within the muscle. Moreover, as all the horses were kept under identical conditions, present results clearly confirm that significant sex-linked differences in the proportions of fibre II subtypes exist in the horse.

Conclusion

This study shows that muscle fibre type composition is not homogeneous within a breed of horses, but significant variations exist among horses with different genealogies. From these observations, it can be now confirmed that proportions of muscle fibre types in the horse are significantly associated to genetic factors. The inbreeding effect from a common maternal ancestor seems to have stronger influences on the proportion of the two main fibre types compared with the inbreeding effect from a common paternal ancestor. Nevertheless, there seems to be a significant influence of the paternal bloodline in determining the content of fast-twitch type IIA muscle fibres. Our results also confirmed significant variations in muscle fibre type composition between breeds and gender. The results have implications for the use of muscle biopsies as an aid for the selection of horses for breeding purposes. Further studies testing muscle characteristics in horses from several generations are required to estimate the coefficient of inheritance of fibre types in equine skeletal muscle.

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