

A Histochemical Assessment of the Capillary Blood Supply of the Middle Gluteal Muscle of Thoroughbred Horses

A preliminary report

P. HENCKEL

August Krogh Institute, Copenhagen University,
Universitetsparken 13, DK-2100 Copenhagen 0, Denmark

Summary

Eight Thoroughbred horses of varying ages were investigated by needle biopsy sampling of the middle gluteal muscle. Fibre types, mean fibre areas, capillary/fibre ratios and the number of capillaries per mm² are given for all horses. The foals had a lower proportion of IIA fibres and a higher proportion of IIB fibres than the rest of the horses. The capillary density was also lower in the foals. As these horses had smaller mean fibre areas, this was predominantly caused by lower capillary/fibre ratios. The effect of maturation was investigated by resampling the foals seven months later. On both occasions these biopsies were further analyzed for properties of the individual fibre types. During that period the fibre type distribution changed to that of the rest of the horses. The mean fibre areas increased as a result of the increase in the mean area of all fibre types. Although proliferation of capillaries was observed around fibres of all types, this did not affect the capillary densities which remained unaltered.

Introduction

Several studies on human muscles have shown a close correlation between maximal oxygen uptake and capillary density. In equine muscles, a correlation of fibre type distribution with gross performance capacity has so far been established (Snow and Guy, 1980). By adding information of capillary density to fibre type distribution, a combination of peripheral and central properties is probably obtained, and one might improve the predictive value of histochemical muscle characteristics to performance potentials. Furthermore, knowledge of fibre type distribution and capillary density may provide useful information in preparing optimal training programmes for individual horses. The present investigation was undertaken to demonstrate the capillary supply of the middle gluteal muscle of Thoroughbred horses at various ages.

Material and Methods

Eight Thoroughbred horses (both stallions and mares) comprising 3 six-month-old foals (group I), 2 two-year-old and 1 three-year-old (group II) and 2 above the age of ten (group III) were investigated. Samples were collected from the middle gluteal muscle by the percutaneous needle biopsy technique (Lindholm and Piehl, 1974). The needle had an outer diameter of 6 mm. Samples were processed and sectioned using conventional techniques. The foals were resampled seven months later to study the effects of maturation. Fibre typing was performed on ATP-ase stains following acid pre-incubation at pH 4.4 and pH 4.6 and alkali pre-incubation at pH 10.3. Capillaries were visualized by the α -amylase-PAS stain (Andersen 1975), and counts were carried out on areas of not less than 0.5 mm² which were considered to be representative with respect to distribution. All horses were analyzed for fibre types, number of capillaries per mm², capillary to fibre ratio and mean fibre area. The three foals were on both occasions further analyzed for mean fibre type area, mean number of capillaries around the fibre types and the percentage area of a cross-section of muscle occupied by the different fibre types. Area measurements were performed by computer-assisted planimetry (Andersen and Henckel, in preparation). Because of the low number of samples, results are given in mean values and ranges of the groups. Differences between groups have not been tested for significance for the same reason. Consequently, these preliminary results can only indicate possible differences between the groups.

Results

The relative fibre type distributions are listed in Table 1. On average, the mean fibre area was 1957 μm^2 (1705–2209) for group I, 3116 μm^2 (2322–3845) for group II and 3053 μm^2 (2396–3709) for group III. The capillary/fibre ratio was \bar{m} 0.97 (0.79–1.19) in group I, \bar{m} 1.86 (1.72–1.98) in group II and \bar{m} 2.05 (1.85–2.25) in group III. The capillary densities were \bar{m} 497 cap./mm² (417–607), \bar{m} 627 cap./mm² (488–852) and \bar{m} 692 cap./mm² (607–777) for groups I, II and III respectively. After seven months the fibre type distribution in the foals was \bar{m} 7.9% (7.4–8.3) for type I fibres, \bar{m} 34.3% (25.6–42.1) for type IIA and \bar{m} 57.8% (49.6–67.0) for type IIB fibres. The percentage fibre type area distribution of the foals changed from \bar{m} 2.7% (0.0–4.7) to \bar{m} 3.6% (3.3–3.9) for type I fibres, from \bar{m} 9.0% (3.9–12.0) to \bar{m} 21.4% (16.4–29.9) for type IIA fibres, and from \bar{m} 88.5% (84.2–93.4) to \bar{m} 75.0% (66.2–80.3) for type IIB fibres. The mean fibre area in the foals changed to 2560 μm^2 (2400–2716). The capillary/fibre ratio increased from \bar{m} 0.97 to \bar{m} 1.21 (1.02–1.36). The capillary density remained virtually the same with \bar{m} 470 cap./mm² (427–501), compared to \bar{m} 497 cap./mm² (417–607) at the first sampling. The mean fibre type areas in the foals changed from \bar{m} 784 μm^2 (682 and 885) to \bar{m} 1176 μm^2 (1106–1247) for type I fibres, from \bar{m} 930 μm^2 (865–1042) to \bar{m} 1601 μm^2 (1225–1799) for type IIA fibres and from \bar{m} 2280 μm^2 (1883–2496) to \bar{m} 3353 μm^2 (3336–3380) for type IIB fibres. The mean number of capillaries around the fibre types changed from \bar{m} 2.53 (2.00 and 3.06) to \bar{m} 3.03 (2.75–3.18) for type I fibres, from \bar{m} 2.76 (2.06–3.52) to \bar{m} 3.88 (3.24–4.37) for type IIA fibres and from \bar{m} 2.99 (2.29–3.60) to \bar{m} 3.46 (3.09–3.78) for type IIB fibres.

TABLE 1. Fibre type distributions in all horses.

Group	Horse no.	Type I	Group mean	IIA	Group mean	IIB	Group mean
I	1	10.7%		21.5%		67.8%	
	2	6.9%	5.9%	7.8%	19.0%	85.3%	75.2%
	3	0.0%		27.6%		72.4%	
II	4	9.5%		30.6%		59.9%	
	5	15.1%	10.3%	40.9%	34.5%	44.0%	55.1%
	6	6.4%		32.1%		61.5%	
III	7	11.5%	9.4%	41.7%	35.4%	46.8%	55.3%
	8	7.2%		29.1%		63.7%	
Total Mean		8.3%		28.9%		62.7%	

Discussion

The capillary density is influenced by the mean fibre areas, the capillary/fibre ratio and to some extent by the relative fibre type distribution, due to the various diffusional capacities of the fibre types. Consequently any change of these parameters would automatically affect the capillary density. Because of the large variations among the horses with regard to mean fibre areas, the capillary/fibre ratio itself provides little information on diffusional capacities of the muscle as a whole. However, this ratio may be of use with repeated sampling to ascertain whether proliferation or reduction of capillaries has taken place. Among the horses, the foals displayed the lowest capillary densities, and it could be shown that this was a result of both smaller mean fibre areas and lower capillary/fibre ratios. The results further indicated that fibre areas as well as capillary/fibre ratios increased with maturation. Of the individual fibre types, the highest diffusional capacities (small fibres surrounded by many capillaries) were displayed by type I fibres and the lowest by type IIB fibres, which is in accordance with their respective capacities for aerobic metabolism. The maturation effect on fibre areas included all fibre types. Similarly, proliferation of capillaries occurred around fibres of all types, resulting in unchanged capillary densities with maturation during that period. The actual densities were within the range of those reported for Standardbred trotters (Henckel 1982), but the Thoroughbred horses appear to have smaller mean fibre areas and lower capillary/fibre ratios.

The effect of maturation on the relative fibre type distribution was interesting. During the seven months the type IIA fibres increased in distribution from 19.0% to 34.3%, while the type IIB fibres decreased from 75.2% to 57.8%. This was not a result of any specific training régimes, although a change in the activity level of the horses may have had an influence on this change. It points to a rather sensitive period when fibre type conversion

may be taking place, and it may open some possibilities of affecting the basic distribution of fibre types during that period. The physiological significance of the relative fibre type distribution is rather limited in equine muscles and may at worst be misleading due to the large differences in fibre type areas. A more appropriate way would be to calculate the percentage area occupied by the individual fibre types in cross-sections of the muscles. In terms of such calculations the effect of maturation was expressed as an increase in the area occupied by type IIA fibres from 9.0% to 21.4% and a decrease in the area of type IIB fibres from 88.5% to 75.0%, which clearly reveals the different results obtained by this method.

There appeared to be no clear differences between the horses in group II and III with regard to any of the measurements. Only group I differed in almost all measured variables. This might indicate a mature state of the muscle around the age of two, which is earlier than reported for Standardbred trotters (Henckel 1982).

There was a large variation in capillary densities within all age groups. This might indicate that differences dependent on hereditary factors may not be outlined by training, so that this parameter may become a useful tool for evaluating future performance potentials of very young horses.

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