

Muscle Histochemistry and Biochemistry of Thoroughbred Horses during Growth and Training

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Summary

Biopsies of the middle gluteal muscle were obtained from 14 Thoroughbred yearlings before the start of training. The horses were then trained and raced through their two-year-old season, and biopsies were again taken during the last week of the season.

The muscle samples were analyzed for fibre composition, fibre areas and enzyme activities. By the end of the racing season, the proportion of type IIA fibres had increased from 31% to 38%, while the proportion of type IIB fibres had decreased from 56% to 49%. There was no significant change in fibre areas.

Increased oxidative capacity of the muscle was demonstrated by about a two-fold increase in citrate synthase (28 to 60 μ mol/g/min) and 3-OH-acyl-CoA dehydrogenase (14 to 23 μ mol/g/min). The glycolytic enzyme lactate dehydrogenase was unchanged, while triose-phosphate dehydrogenase decreased in activity.

There was a large individual variation among horses in the proportion of fibre types and enzyme activities in muscle.

Introduction

Oxygen uptake, transport capacity of the cardiovascular system and total haemoglobin are all important physiological variables for a horse's performance. The muscle biopsy technique (Bergström 1962; Lindholm and Piehl, 1974) and analyses of skeletal muscle for biochemical and histochemical properties have shed new light on muscle function and structure in many animal species including the horse (Essén *et al.*, 1980; Lindholm and Piehl, 1974; Guy and Snow, 1977).

With exercise of different speed and duration the skeletal muscle undergoes changes in morphological and biochemical character (Lindholm and Saltin, 1974). It is also well known that racehorses differ individually in muscle fibre composition, muscle fibre area and enzymes of aerobic and anaerobic metabolism (Essén *et al.*, 1980; Snow and Guy, 1981; Essén-Gustavsson and Lindholm, unpublished data). Studies in man and animals have shown that, while muscle fibres have a great plasticity to adapt both their metabolic

function and muscle fibre distribution to environmental influences, hereditary factors are also of great importance in this respect (Howald 1982).

The Thoroughbred horse has been selectively bred for superior galloping speed for some centuries. It is not known which physiological characteristics make this breed faster than other breeds. It is known, however, that Thoroughbreds have a somewhat higher proportion of fast-contracting type II muscle fibres in the middle gluteal muscle than Standardbred trotters and other horses (Snow and Guy, 1980; Essén-Gustavsson and Lindholm, unpublished data).

The present investigation was carried out to determine:

1. the metabolic changes of skeletal muscles of Thoroughbreds with growth and training before and after their first year of racing and
2. the relationship between these muscle characteristics and the horse's track performance.

Material and Methods

Fourteen Thoroughbreds, six mares, three stallions and five geldings, from three different stables at Täby race track, Stockholm, Sweden, were studied. Two muscle biopsies from the middle gluteal muscle were obtained on each of two occasions from each horse: in September 1980, just before the start of training when the horses were 1.5 years of age, and in October 1981 when the horses were 2.5 years of age. The second biopsy was taken within one week of their last race at the end of the two-year-old racing season. All horses were regularly trained by professional trainers from October 1980. At the beginning of the training the horses trotted for about 15 minutes daily, and in December they were worked at the canter for the first time. At the end of March they were walked, trotted and cantered for about 45 minutes daily. According to individual fitness, sprint training started for some of the horses in April and for some in May. The date of their first start varied. Three horses started for the first time in May, two in June, two in July, four in August and three in September. All the horses remained sound throughout the two-year-old racing season, except for minor injuries in some of the horses which did not stop the training for any prolonged period of time.

The usual technique for biopsy sampling in horses was used (Lindholm and Piehl, 1974). One piece of muscle for histochemical analysis was mounted on a specimen holder with OCT embedding medium (Tissue Tek II, Miles Laboratory) and frozen in isopentane pre-cooled in liquid nitrogen, while a second piece was frozen immediately in liquid nitrogen for biochemical analysis. Both samples were stored at -80°C until analyzed. The performance was analyzed by allocating points per start (5 for the first, 4 for the second etc.) and dividing the total number of points by the number of starts.

Fibre area measurements

The sections stained for myofibrillar ATP-ase after acid pre-incubation (pH 4.6) were used for fibre area analysis. The sections were set under a 'Visopan' microscope with a grid to an electronic planimeter (MOP, Digiplan) over it. The fibres were identified on the board (I, IIA and IIB), and the mean area was measured from 25 randomly selected fibres of each kind.

Biochemical analysis

The muscle biopsies were freeze-dried, dissected free of connective tissue and blood under a dissection microscope and weighed. Analyses were made of triose-phosphate dehydrogenase (TPDH), citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD) (Essén *et al.*, 1980) and lactate dehydrogenase (LDH) (Essén *et al.*, 1983).

Statistical analysis

The results from the horses of each age were compared using the t-test of paired observations. The results are presented as means, standard deviation and range.

Results

During the first racing season, as two-year-olds, none of the horses competed over more than 1600 metres, and the usual distance was 1100–1200 metres. The horses contested an average of six races each (range 3–8) during this season.

Fibre composition and fibre area (Table 1)

There was no difference in the type I/type II fibre ratio between the 1.5-year-old and the 2.5-year-old horses, both of which displayed 12% type I fibres. Of the two fast-twitch fibre types, the percentage of type IIA fibres increased significantly ($p < 0.01$) from a mean of 31% (range 15–47%) as 1.5-year-olds to a mean of 39% (range 33–54%) at the end of the two-year-old season. A corresponding decrease occurred in the percentage of type IIB fibres from 56–49% ($p < 0.01$). All fibre types displayed large individual differences between horses in both age groups.

There was no significant change in fibre area between the first and second year, but there was a tendency towards an increase in type I and type IIA fibre areas and a decrease in type IIB fibre area.

Enzyme activity (Table 1)

The oxidative enzymes CS and HAD increased significantly ($p < 0.001$) over the 12-month period of training. CS showed a more than two-fold increase in activity from an average of 28 $\mu\text{mol/g/min}$ to 60 $\mu\text{mol/g/min}$, whereas HAD increased in activity from 14 to 23 $\mu\text{mol/g/min}$. There was a large individual variation of enzyme activities in each age group. The seven horses which displayed the highest CS activity as 2.5-year-olds also displayed the highest CS activity as 1.5-year-olds ($p < 0.01$). A high CS activity during the first year always correlated with a correspondingly high HAD activity. This correlation was not as clear in the second year when the horses were 2.5-year-olds. Of the two glycolytic enzymes, TPDH decreased in activity from 1802 to 1569 $\mu\text{mol/g/min}$ ($p < 0.05$), whereas LDH activity did not change in the two age groups.

Track performance

Track performance, measured as either mean place or amount of prize money, was not correlated to a high or low percentage of any of the three fibre types. Neither was track performance correlated to a high percentage of oxidative enzymes (CS and HAD) or glycolytic enzymes (TPDH and LDH).

TABLE 1. Fibre types and enzyme activities from 14 Thoroughbred horses and fibre area from 7 Thoroughbred horses at 1.5 and 2.5 years of age.

Year	Age (years)	Fibre type (%)				Fibre area ($\mu\text{m}^2 \cdot 10^{-2}$)				Enzyme activity ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)			
		I	IIA	IIB	I	IIA	IIB	CS	HAD	TPDH	LDH		
1980	1.5	\bar{x} 12	31	57	18.4	28.2	44.5	27.6	14.0	1802	1576		
		SD 4.5	8.1	11.6	2.7	3.8	6.5	5.0	1.9	278	235		
		Range 6-24	15-47	34-74	14.3-22.2	21.0-32.2	40.2-49.2	20-36	11-18	1483-2458	1293-1978		
1981	2.5	\bar{x} 12	39	49	21.4	30.3	41.6	60.1	22.6	1569	1536		
		SD 3.5	5.7	8.4	4.4	3.6	4.9	9.8	3.7	165	179		
		Range 7-20	33-54	26-59	17.5-21.7	25.0-35.5	36.4-48.8	48-85	16-31	1347-1799	1277-1800		
Level of significance		p < 0.01				p < 0.001				p < 0.05			

 \bar{x} = mean.

Discussion

The training programme carried out between 1.5 and 2.5 years of age was quite similar for all the horses before April. From April onwards, the fitness and ability of the individual horse determined the start of intensity and duration of the sprint training. The date for the first race also varied between the horses from May to September. It is a well known fact that early fitness for racing varies widely among horses even if the horses are similarly trained. This difference is most probably largely due to genetic factors, but it could also be due to the amount and type of activity performed earlier in life before training. In this study no difference in fibre composition or enzyme activity was observed between the horses starting early or late during their two-year-old racing season.

With 12% type I fibres in the middle gluteal muscle the fibre composition is similar to that found in both a group of 14 adult Thoroughbreds (Essén-Gustavsson and Lindholm, unpublished data), where seven stayers had 14% and seven sprinters 16% type I fibres, and in Thoroughbreds in England which had 12.5% type I fibres (Snow and Guy, 1980). In Standardbred trotters, usually more than 20% of fibres are type I (Lindholm and Piehl, 1974; Taylor and Brassard, 1980), while in other studies 15–20% type I fibres have been reported (Essén *et al.*, 1980; Essén-Gustavsson *et al.*, 1983). It is obvious that the type I/type II fibre ratio varies among horses, and it seems that Thoroughbred horses have a lower proportion of type I fibres in comparison to other horse breeds with the exception of the Quarterhorse (Snow and Guy, 1980).

The increase in the percentage of type IIA fibres and CS and HAD activities, as well as the decrease in the percentage of type IIB fibres and TPDH activity during the period of 1.5 to 2.5 years of age are most probably due to the training received. Similar changes have been observed after intense training in Thoroughbreds (Guy and Snow, 1977) and after intense training and racing in Standardbred trotters (Essén-Gustavsson and Lindholm, unpublished observations).

Since Thoroughbred horses have reached about 80% of their final adult weight at 1.5 years of age (Evans *et al.*, 1977), growth cannot be the major factor causing the metabolic changes found in the muscle between 1.5 and 2.5 years of age. The fact that the seven horses with the highest CS activity at 2.5 years of age also displayed the highest activity at 1.5 years of age suggests that the ability for achieving a high CS activity may be inherent. Standardbred yearlings also showed large individual differences in both the fibre composition and CS activity (Essén-Gustavsson *et al.*, 1983). Perhaps the individual differences in CS activity seen in 1.5-year-old Thoroughbreds are due to the physical activity performed by each horse before 1.5 years of age.

When compared to trained Thoroughbreds, three or more years of age, the CS and HAD activities are quite similar. The mean of the six horses with the highest CS activity in the 2.5-year-old group was similar to that of five Thoroughbred stayers (72 $\mu\text{mol/g/min}$) we had previously measured, and the mean of the remaining eight horses of the same age group was similar to that of six Thoroughbred sprinters (54 $\mu\text{mol/g/min}$) (Essén-Gustavsson and Lindholm, unpublished data). As the 2.5-year-old horses have not raced longer than 1600 metres it is too early to make any predictions about stayer or sprinter abilities.

While the fibre area did not change significantly during the year of training and racing, there was a tendency towards a decrease in type IIB fibre area. In Standardbred trotters

fibre area increases in all fibre types up to 1.5 years of age (Essén-Gustavsson *et al.*, 1983). However, in comparison to trained adult Thoroughbred horses, the type IIB fibres in the 2.5-year-old horses were larger and the type I and type IIA fibres were smaller. This indicates that training and racing after 1.5 years of age tend to increase the size of the two oxidative fibres and to decrease the size of the glycolytic type IIB fibres. Since the type IIA fibre percentage is higher in well trained and well performing adult trotters, it is obvious that a high oxidative capacity in the muscle is very important for a racehorse of whatever breed.

In this study neither enzymes nor fibre composition were correlated to track performance after the first racing season. In adult Standardbred trotters, on the other hand, a higher percentage of type IIA muscle fibres and high CS activity are positively correlated to track performance (Essén-Gustavsson and Lindholm, unpublished data). As far as Thoroughbreds are concerned, the lack of racing in their two-year-old season affords little information about their ultimate ability. It is possible that the more individual and harder training of the three-year-old season may have a greater influence on the type IIA fibre percentage in particular, but also on the CS activity in individual horses, bearing in mind that the training of Thoroughbreds has been shown to have a positive influence on these variables (Guy and Snow, 1977). The third biopsy planned for October 1982, after the 3-year-old season, may provide some answers.

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Skeletal Muscle Characteristics of Foals at Two to Four Weeks and Eight Months of Age

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Summary

Fibre type composition and enzyme activity data from the gluteus medius muscle of foals at two to four weeks and eight months of age demonstrated a significant reduction in fast twitch highly oxidative (FTH) fibres from 37.1% to 25.6% and an increase in fast twitch fibres of intermediate oxidative capacity (FTinter) from 20.6% to 27.6% of the population. Slow twitch (ST) fibres (14.7% and 16.0%) and fast twitch low oxidative (FT) fibres (29.4% and 30.8%) did not change over the period.

A significant increase in phosphofructokinase (PFK) and decrease in succinate dehydrogenase (SDH) activities over this same period suggested that the foals enhanced the anaerobic, rather than the aerobic, component of muscle metabolism during the first eight months of life.

Fibre cross-sectional areas at two to four weeks of age were substantially smaller than those in older foals and adult horses.

Introduction

The skeletal muscles of the horse, and in particular those concerned with locomotion, have been studied with increasing interest in relation to performance capabilities over the past ten years. Histochemical and biochemical studies on muscle samples obtained by needle biopsy techniques (Lindholm and Piehl, 1974; Snow and Guy, 1976; Essén *et al.*, 1980) have demonstrated fibre type and enzyme activity characteristics comparable to those found in man and other mammalian species. While the relationship of muscle characteristics and their adaptation to athletic ability in humans have been well documented (Costill *et al.*, 1976; Saltin *et al.*, 1977), there have been few similar studies on the development of these characteristics in the horse (Gunn 1978, 1979, 1980; Essén-Gustavsson *et al.*, 1983).

The present study was carried out to determine the basic fibre composition and enzyme activities of aerobic and anaerobic metabolism in the *gluteus medius* muscle of foals at an early age. This is to form the basis of further studies of the development of skeletal muscle in the horse.