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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.

Materials and Methods

Eighteen foals from Arab-cross and Thoroughbred-cross mares on the University of Queensland Veterinary School Farm were used in this study. The mares foaled at pasture and the foals ran with their dams until weaned at approximately six months of age. Muscle samples were obtained from the *gluteus medius* muscle, using the needle biopsy technique of Lindholm and Piehl (1974). On each occasion, at two to four weeks and eight months of age, two samples were taken from each foal at a point approximately 5 cm dorsocaudal to the tuber coxa on the left side. One sample for histochemical analysis was mounted in embedding medium (Tissue-Tek II, Miles Laboratories) on a specimen holder and frozen in isopentane cooled in liquid nitrogen, while that for enzyme analysis was frozen immediately in liquid nitrogen. Both samples were stored at -80°C until analyzed.

Histochemical analysis. From each muscle sample, serial cross-sections were cut in a cryostat at -20°C and mounted on coverslips for histochemical staining. Muscle fibres were first identified as slow twitch (ST) or type I, and as fast twitch (FT) or type II, by staining for myofibrillar adenosine triphosphatase (ATP-ase) after pre-incubation at pH 10.3 and 4.3 (Brooke and Kaiser, 1970). The FT fibres were further classified as having high (FTH), intermediate (FTI) or low (FTL) staining intensity for NADH dehydrogenase (Novikoff *et al.*, 1961). From photomicrographs of each section, 200 or more fibres were classified.

Fibre cross-sectional areas. From seven of the samples taken from foals at two to four weeks of age, fibre cross-sectional areas were determined by direct planimetry on photomicrographs of the ATP-ase sections pre-incubated at pH 10.3.

Biochemical analysis. Muscle samples were weighed and prepared for analysis of phosphofructokinase (PFK) and succinate dehydrogenase (SDH) activities, according to the methods of Baldwin *et al.* (1973) and Costill *et al.* (1976), respectively. The activities are expressed as $\mu\text{mol g}^{-1}\cdot\text{min}^{-1}$.

Statistical analysis. Results from the foals at each age were compared by Student's 't' tests and the results are presented as mean values with standard deviations unless otherwise indicated.

Results

Biopsy samples were collected from 14 foals at two to four weeks of age and from these same foals plus four additional foals at eight months of age.

Fibre composition. The relative composition of fibre types was determined from nine of the foals at two to four weeks of age and from nine foals at eight months of age. In the sections stained for ATP-ase (pH 10.3), ST and FT fibres were readily distinguished (Fig. 1a). The ST fibres were small, relatively uniform in size and represented approximately 15% of the fibre population (Table 1). These fibres were unstained or only very faintly stained for ATP-ase at pH 10.3. In addition, some small fibres which stained moderately at pH 10.3 (and were darkly stained at pH 4.3) were classified as ST. The FT fibres (85%) varied in size but showed a uniform intensely dark staining reaction for ATP-ase at pH 10.3. These fibres were unstained at pH 4.3.

TABLE 1. Mean fibre composition (\pm SD) of *gluteus medius* muscle in foals at two to four weeks (n = 9) and eight months (n = 9) of age.

Age	Percentage fibre types			
	ST	FTH	FTI	FTL
two to four weeks	15 \pm 4.9	35 \pm 3.6	21 \pm 5.9	29 \pm 5.3
eight months	16 \pm 5.2	25 \pm 5.8	28 \pm 7.0	31 \pm 7.2
t-value	0.540	4.076	2.305	0.461
df	16	16	16	16
level of significance	NS	p < 0.001	p < 0.05	NS

TABLE 2. Mean cross-sectional areas (\pm SD) of fibres in *gluteus medius* muscle of seven foals at two to four weeks of age.

Foal	Fibre area (μm^2)			
	ST	FTH	FTI	FTL
1	236 \pm 74	378 \pm 136	673 \pm 237	1551 \pm 439
2	335 \pm 119	473 \pm 146	912 \pm 309	1737 \pm 505
3	334 \pm 117	446 \pm 148	701 \pm 195	1587 \pm 467
4	462 \pm 207	782 \pm 256	1427 \pm 479	2231 \pm 760
5	301 \pm 107	408 \pm 165	710 \pm 195	1460 \pm 344
6	624 \pm 207	834 \pm 223	1426 \pm 428	2292 \pm 723
7	508 \pm 160	640 \pm 208	1029 \pm 267	1996 \pm 488
Mean of all foals	385 \pm 179	553 \pm 240	975 \pm 422	1814 \pm 606

In the sections stained for NADH dehydrogenase, the ST fibres showed a uniformly high staining intensity. Individual FT fibres, however, showed either a high (FTH), intermediate (FTI) or low (FTL) intensity staining reaction (Fig. 1b). In a few of the foals, the distinction between FTH and FTI fibres was not as clear, and there appeared a range of

staining intensity. The FTL fibres were distinguished by their low staining intensity and their large size.

Fibre cross-sectional areas determined in seven foals at two to four weeks of age are presented in Table 2. Mean values were ST 385 ± 179 , FTH 553 ± 240 , FTI 975 ± 422 and FTL $1814 \pm 606 \mu\text{m}^2$. The mean fibre area was $982 \mu\text{m}^2$.

Muscle enzymes. Biopsies were obtained from 11 of the foals at both two to four weeks and eight months of age and analyzed for enzyme activity. A 't' test of paired observations showed a significantly higher ($p < 0.05$) PFK activity in the older foals, while SDH activity was similar at both ages (Table 3).

TABLE 3. Mean SDH and PFK activity (\pm SD) in *gluteus medius* muscle of the same 11 foals at two to four weeks and eight months of age.

Age	Enzyme activity ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)	
	SDH	PFK
two to four weeks	9.7 ± 3.0	157.1 ± 26.1
eight months	7.5 ± 3.4	209.3 ± 42.9
t-value*	1.882	2.808
df	10	10
level of significance	NS	$p < 0.05$

*'t' test of paired observations

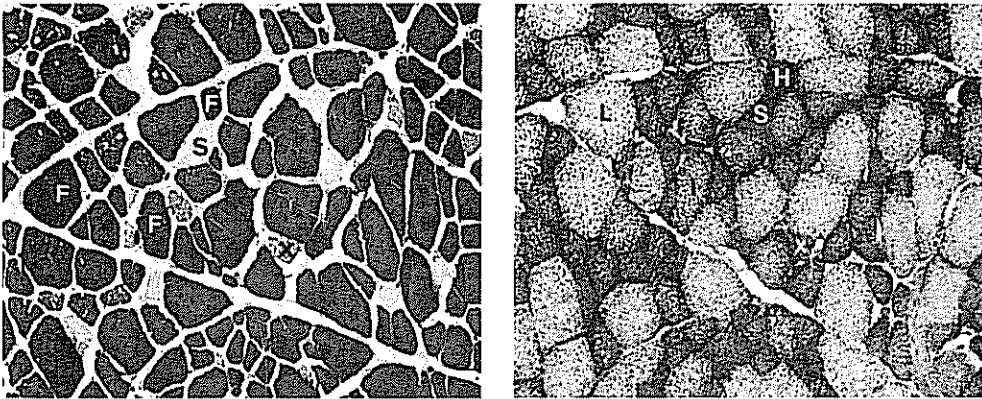


FIGURE 1. Serial cross-sections of *gluteus medius* muscle from a foal aged 18 days.
 (a) ATP-ase, pH 10.3. ST fibre – S, FT fibres – F, and medium stained fibre classified as ST – X.
 (b) NADH dehydrogenase. Same fibres as in (a). ST fibre – S, FT fibres – H (highly stained), I (intermediate stained) and L (low stained). Medium stained fibre (for ATP-ase in (a)) classified as ST – X.

Discussion

The horse is actively mobile at or very shortly after birth and would therefore be expected to have a well differentiated complement of fibres in its skeletal muscles of locomotion as has been shown to be the case in the guinea pig (Dubowitz 1965). The results of the present study suggest that this is the case, at least in foals of two weeks of age.

A number of differing classifications of the fibre types in mammalian muscle have been proposed. In the studies on skeletal muscle in the horse, the slow twitch (ST), fast twitch high oxidative (FTH) and fast twitch low oxidative (FT) system used by Lindholm and Piehl (1974) has been most widely adopted. We have chosen this system as the basis for the present study and modified it slightly to include three intensities of NADH staining in the fast twitch fibre population.

In the foals, as in older horses, the FT fibre was the predominant type in the *gluteus medius* muscle. The proportion of ST fibres (15%) was a little lower than in older Standardbred horses (Lindholm and Piehl, 1974, 24% ST; Taylor and Brassard, 1981, 27% ST) and similar to that (17%) in two-month-old Standardbred foals (Essén *et al.*, 1980) and adult Arabs (14.4%) and Thoroughbreds (12.5%) (Snow and Guy, 1980). The use of NADH staining for subgrouping the FT fibres revealed three recognizable categories showing either high, intermediate or low staining intensity to the reaction. Using this technique, Lindholm and Piehl (1974) only described two groups of fast twitch fibres, FT (light stained) and FTH, some of which stained as darkly as the ST fibres and some of which stained more lightly. Taylor and Brassard (1981) described only FT and FTH fibres in adult Standardbreds, while Essén *et al.* (1980) reported type II (i.e. FT) fibres showing dark, medium and light staining reactions for NADH in Standardbreds aged from two months to 28 years. It appears, therefore, that a range of staining intensity exists within the FT fibre population, suggesting a spectrum of aerobic capacity. If the FTH and FTI groups in the present study are combined, the values of 58% and 53%, at two to four weeks and eight months, respectively, for high to intermediate staining fast twitch fibres approximate the 49 to 50% FTH reported by Lindholm and Piehl (1974). These figures differ considerably from those of Taylor and Brassard (1981) who found 28 to 33% FTH and 41 to 45% FT fibres in the *gluteus* of Standardbreds in Canada. In comparison, the Thoroughbred *gluteus medius* muscle contained 51% FTH and 37% FT fibres, as shown by the SDH staining reaction (Snow and Guy, 1980).

The reasons for the variation in the proportions of FTH and FT fibres may lie with either the breeding of the horses or the level and type of training or both. Little is known of the influence of breeding on the composition of horse muscle, whereas the percentage of FTH fibres has been shown to increase with training in the horse at the expense of FT fibres (Lindholm and Piehl, 1974; Guy and Snow, 1977). The same is true in the rat and guinea pig (Barnard *et al.*, 1970; Baldwin *et al.*, 1973).

As an enzyme of the citric acid cycle, SDH reflects the capacity for aerobic metabolism and has been shown to be significantly correlated to age (and training) in Standardbreds from six months of age (Lindholm and Piehl, 1974). The lack of significant change in this enzyme by eight months of age is surprising, since an increase in SDH could well have been expected, given the foals' increasing activity over the period. The decline in staining intensity of NADH by eight months of age agrees with this lack of rise in SDH and a possible tendency for SDH to decline. The significant rise in PFK activity reflects an

increase in the anaerobic capacity of the foals and implies a substantial absolute increase in the enzyme when the muscle growth over the period is considered. The reasons for this difference in enzyme activities are not obvious but are presumably related to the growth and activity of the foals during this particular age span.

The cross-sectional fibre areas in the foals at two to four weeks of age are similar to those in other foals of equivalent age (Essén-Gustavsson, personal communication). When compared to Standardbreds of six months to two years of age the fibre areas are much smaller (ST 2539, FTH 2689, FT 4791 μm^2 ; Lindholm and Piehl, 1974), suggesting a growth factor of 3 for FT fibres and of 5 or 6 for FTH and ST fibres during the next six to twelve months. The mean fibre area in the foals was 982 μm^2 compared with 2500 μm^2 in foals at seven to eight months of age (Essén-Gustavsson *et al.*, 1983), representing a 2.5-fold increase in the mean fibre cross-sectional area. It has been predicted that Thoroughbreds increase the transverse sectional area of their semitendinosus muscle by a factor of 10 between bodyweights of 50 and 500 kg (Gunn 1980). A growth rate of this order could account for the differences in fibre areas between young foals and adult horses.

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