Muscle Fiber Type Changes in the Middle Gluteal of Quarter and Standardbred Horses from Birth Through One Year of Age

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Summary

Percentages of the three muscle fiber types were determined in the middle gluteal muscle of 11 Quarter horses (QH) and 6 Standardbreds (STB) at 1 day, 3 months, 6 months and 1 year of age. Muscle biopsies were taken 2 cm below the superficial fascia from the middle portion of the middle gluteal muscle. Biopsy samples were mounted on chucks, sectioned on a cryostat and stained for myosin ATPase (pH 9.4) and succinate dehydrogenase. Fibers were classified as fast twitch glycolytic (FG), fast twitch oxidative glycolytic (FOG) or slow twitch oxidative (SO). The percentages of all 3 fiber types were significantly different between breeds. Quarter Horses had a higher ($P < .001$) percentage of FG fibers and a lower ($P < .001$) percentage of FOG fibers, and a significantly lower ($P < .001$) percentage of SO fibers than STB. Small changes occurred in the percent FG, FOG and SO fibers as a function of age in both QH and STB. However, the only age-related significant change was an increase ($P < .05$) in percent FOG fibers from QH.

Index terms: Horse, growth.

Introduction

Studies (Snow and Guy, 1980; Snow and Guy, 1981; Stull and Albert, 1981) indicate that the percentage of different muscle fiber types varies substantially with the breed and type of horse. Studies of human muscle (Saltin *et al.*, 1977; Inbar *et al.*, 1981) have shown that the fiber type composition of the vastus lateralis, a muscle used actively in running, is correlated with athletic performance. Snow (1983) reported that significant differences were found between the percentage of type I muscle fibers in the middle gluteal muscle of Thoroughbred horses classed as stayers versus Thoroughbreds classed as sprinters or medium-distance runners. The gluteus medius is the major propulsion muscle in the horses' hind limbs. In order to interpret fiber type information from very young animals and make meaningful projections, the dynamics of muscle fiber type populations as a function of age must be known.

Suzuki and Cassens (1980) found that the percentage of type I (slow-twitch) muscle fibers increased from birth to 8 weeks of age in several muscles of the pig. In young hamsters (Goldspink and Ward, 1979), per cent slow-twitch type fibers decreased over time in the biceps brachii, while the percent slow twitch type fibers increased over time in the soleus. In the horse, Lindholm and Piehl (1974) reported that the percent fast twitch highly oxidative fibers in the middle gluteal was higher in adult horses than in 6-month-old foals. The percent fast-twitch low oxidative fibers was lower in adult horses than in six-month-old foals. Essen, *et al.*, (1980) found that a sub-class of fast twitch fibers, type II C, was more predominant in the middle gluteal of foals than in older horses. Others (Essen-Gustavsson *et al.*, 1983; Henckel, 1983) have reported increasing percentages of type II A fibers and decreasing percentages of type II B fibers as a function of increasing age in foals 6 months of age and older. Thornton and Taylor (1983) reported that very young foals may enhance their anaerobic, rather than aerobic components of muscle metabolism during the first few months of life.

The purpose of the present study was to systematically investigate changes in per cent muscle fiber types with increasing age. Beginning with young foals, middle gluteal biopsy samples were removed from the same individuals over a one year span of time.

Materials and Methods

Eleven Quarter Horses (QH) and 6 Standardbred horses (STB) were studied. Muscle biopsies were taken 2 cm below the fascia from the middle portion of the M. gluteus medius of each horse at 1 day, 3 months, 6 months and 1 year of age. The biopsy location was identified as being a site on the croup at an angle of 45° dorsocaudal from the tuber coxae. In 1-day-old foals, the biopsy site was 5–6 cm from the tuber coxae, while in yearlings the site was 8–10 cm from the tuber coxae. The exact location of the biopsies in all horses from 1 day through 1 year of age was influenced by individual muscle size. The biopsies were alternately taken from the left and right middle gluteal on each subsequent sampling occasion to minimize possible interference of scar tissue and muscle regeneration

All foals were kept on grass-alfalfa pasture with free access to creep feed of approximately 70% digestibility and 18% crude protein until weaning at 5 months of age. After weaning and until 1 year of age, foals were kept in large dry lots or on pasture. When not on pasture, foals received mixed grass-legume hay or haylage of about 16% crude protein on a dry basis at a rate of 2% of body weight daily and supplemental grain at an average rate of 1% of body weight daily. Growth rates and health of foals throughout the study were normal for age.

Before each biopsy, the hair at the biopsy site was shaved and the skin was thoroughly cleansed. Three ml of lidocaine without epinephrine were injected beneath the skin and fascia as a local anesthetic. A stab incision was made through the skin and fascia, and biopsies were retrieved from 2 cm below the fascia using a Love-Gruenwald Invertebral Disc Rongeur with 3 × 10 mm cups and a seven inch shaft.

Histochemical analysis. Muscle samples for histochemical analysis were mounted onto chucks, cut into 10 μm sections on a cryostat at −20°C and stained for myosin ATPase (pH 9.4) (Padykula and Herman, 1955) and succinate dehydrogenase (Nachlas *et al.*, 1957). The percentage of fibers staining negative for succinate dehydrogenase (SDH) was designated as the per cent FG fibers and the percentage of fibers staining

negative for myosin ATPase was designated as the per cent SO fibers. The percentage of FOG fibers was determined by the formula % FOG = 100 − (% SO + % FG) (Snow and Guy, 1980). At least 200 total fibers per section were characterized for each stain from 2 or 3 locations within a section.

Statistics. Linear regression coefficients of fiber types versus age for each horse were calculated. A regression coefficient for a given fiber type represents the rate of change in fiber type per cent through 1 year of age. These regression coefficients were used as the data on which further statistical analyses were based (Allen *et al.*, 1983). t-tests were used to test whether the regressions of fiber types on age were different than zero. Analysis of variance of regression coefficients was used to compare changes in fiber types between breeds. The animal within breed mean square was used as the error term for testing differences in fiber type changes between breeds because animals were nested within breed. Analysis of variance of mean per cent fiber types averaged over all ages was used to compare per cent fiber type populations between breeds.

Results

Table 1 lists the fiber type percentage for samples taken from QH and STB at 1 day, 3 months, 6 months and 1 year of age and also, mean fiber types averaged across all ages are listed for each breed. Quarter horses had a higher ($P < .001$) percentage FG fibers, lower ($P < .001$) percentage FOG fibers and lower ($P < .001$) percentage SO fibers than Standardbreds.

Table 2 shows mean linear regression coefficients for the regression of three fiber types on age, the standard error of the mean, the *t*-value and the probability of obtaining a larger *t*-value if the regression coefficient were actually equal to zero. This information is presented averaged over both breeds, and also separately according to breed.

When both Quarter Horses and Standardbreds were analyzed together, there was an

TABLE 1 Age differences in percentage of muscle fiber types of Standardbred (STB) and Quarter Horses (QH)*

	FG		FOG		SO	
Age	QH	STB	QH	STB	QH	STB
1 day	40.4 ± 2.9 n = 10	30.0 ± 5.0 n = 6	51.5 ± 2.9 n = 10	57.7 ± 4.0 n = 6	8.1 ± 1.9 n = 10	12.3 ± 1.6 n = 6
3 months	44.0 ± 3.7 n = 10	32.4 ± 3.0 n = 5	45.5 ± 4.8 n = 10	55.4 ± 4.8 n = 5	10.5 ± 2.8 n = 10	12.2 ± 3.4 n = 5
6 months	42.6 ± 3.5 n = 9	34.2 ± 4.4 n = 5	45.6 ± 3.0 n = 9	50.4 ± 4.7 n = 5	11.8 ± 1.7 n = 9	15.4 ± 1.4 n = 5
1 year	38.9 ± 3.4 n = 10	29.5 ± 2.6 n = 6	48.5 ± 3.6 n = 10	54.7 ± 3.6 n = 6	12.6 ± 3.8 n = 10	15.8 ± 1.6 n = 6
Combined age means	41.5 ± 3.4 n = 11	31.3 ± 3.9 n = 6	47.8 ± 3.7 n = 11	54.8 ± 4.2 n = 6	10.7 ± 2.7 n = 11	13.9 ± 2.1 n = 6

*Values are given as mean per cent ± S.D. The n values for QH less than 11 and STB less than 6 reflect missing samples. FG = fast twitch glycolytic; FOG = fast twitch oxidative glycolytic; SO = slow twitch oxidative

TABLE 2. t-Tests of mean regression coefficients for percentage of muscle fiber types in middle gluteal of Quarter and Standardbred Horses from birth through one year of age.

Fiber type	Coefficient	Std error of the mean	t	P
Combined Horses (n = 16)				
FG	7.13	2.78	2.56	0.022
FOG	−9.93	3.32	−2.99	0.009
SO	2.80	1.66	1.69	0.112
Quarter Horses (n = 11)				
FG	6.98	3.69	1.89	0.088
FOG	−10.56	4.21	−2.51	0.031
SO	3.58	1.70	1.70	0.120
Standardbreds (n = 5)*				
FG	7.45	4.21	1.77	0.151
FOG	−8.55	5.85	−1.46	0.218
SO	1.10	2.78	0.39	0.713

*Both the 3 and 6 month samples were missing from the same STB horse, therefore, only five animals were used for the analysis

FG = fast twitch glycolytic; FOG = fast twitch oxidate glycolytic; SO = slow twitch oxidative

increase ($P < .05$) in per cent FG fibers and a decrease ($P < .01$) in per cent FOG fibers as a function of increasing age. No significant change ($P > .05$) was detected in per cent SO fibers.

Discussion

In this study, small changes were observed in the percentages of the different fiber types as a function of age. An approximate 12% decline in the per cent FOG fibers was found from one day of age through 6 months of age for both Quarter Horse and Standardbred foals. However, at 1 year of age, the values were similar to the initial 1 day levels. The overall mean regression coefficient for FOG fibers (−9.93) reflects the decreasing per cent FOG fibers for the first 6 months after birth.

Regression analysis over all horses from 1 day through 1 year of age also detected a slight tendency for the percentage of FG fibers to increase due to the initial rise up to 3 to 6 months after foaling. The per cent FG fibers at one year also returned to approximately the same level as at day 1. Although per cent SO fibers numerically increased throughout the first year of age in both QH and STB foals, the increase was not statistically significant ($P > .05$).

The significant differences seen in the per cent fiber type populations of FG, FOG and SO fibers between Quarter Horses and Standardbreds are not surprising in light of their respective athletic specializations. The Quarter Horse breed, developed for strength, agility and short distance sprinting speed could be expected to have a higher percentage of FG fibers and a lower percentage of SO fibers than the Standardbred, which is a middle distance sprinter. The larger per cent FOG fibers in Standardbreds could indicate less reliance on the easily fatigued FG fibers.

Had this study not examined the per cent fiber types in very young foals, the conclusions reached concerning per cent fiber type changes as a function of age would have been identical to those of other researchers (Essen-Gustavsson *et al.*, 1983; Henckel, 1983) who sampled foals at 6 months of age and beyond. These investigations noted an increase in the percentage of type II A fibers and a decrease in the percentage of type II B in foals as they grew from 6 months of age to 1 year and beyond. A similar conclusion could be reached in this study by looking only at the 6-month and 1-year-old foals. However, the increase in per cent FOG fibers in foals from 6 months to 1 year of age was preceded by 6 months of decreasing per cent FOG fibers. Likewise, the decrease in per cent FG fibers in foals from 6 months to 1 year of age was preceded by 3 to 6 months of increasing per cent FG fibers.

A continuous but nonsignificant ($P > .05$) trend of increased per cent SO fibers from 1 day through 1 year of age was also noted in this study. The 2-cm sampling depth in newborn foals is relatively deeper within the middle gluteal muscle than at subsequent later sampling occasions. If the per cent SO fibers do not change as a function of age, a slightly higher proportion of SO fibers could be detected in muscle samples from the horses at younger ages. However, our results indicate fewer SO fibers at younger ages. The possibility remains that an increase in per cent SO fibers occurs in foals from birth through 1 year of age.

The trend in this study for per cent FG fibers to increase from 1 day through 6 months of age and for per cent FOG fibers to decrease is consistent with the conclusions of Thornton and Taylor (1983) that very young foals enhance their anaerobic components of muscle during the first several months of life. However, in this study the degree of change seen in the per cent fiber types over the first year was small. This observation is consistent with the advanced physiological muscle maturity of newborn foals. Differences in the per cent FG, FOG and SO fiber types (Raub *et al.*, 1986) between adult and fetal equine middle gluteal were greater than the differences between neonatal and older foals found in this study.

References

Allen, O. B., Burton, J. H. and Holt, J. O. (1983). Analysis of repeated measurements from animal experiments using polynomial regression. *J. Anim. Sci* **57**, 765–770.

Essen, B., Lindholm, A. and Thornton, J. (1980). Histochemical properties of muscle fibre types and enzyme activities in skeletal muscle of Standardbred trotters of different ages. *Equine Vet. J.* **12**: 175–180.

Essen-Gustavsson, B., Lindholm, A., McMiken, D., Persson, S. G. B. and Thornton, J. (1983). Skeletal muscle characteristics of young Standardbreds in relation to growth and early training. *In: Equine Exercise Physiology* Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge, pp. 200–210.

Goldspink, G. and Ward, P. S. (1979). Changes in rodent muscle fibre types during post-natal growth, undernutrition and exercise. *J. Physiol.* **296**, 453–469.

Henckel, P. (1983). A histochemical assessment of the capillary blood supply of the middle gluteal muscle of thoroughbred horses. *In: Equine Exercise Physiology* Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.), Granta Editions, Cambridge, pp. 225–228.

Inbar, O. Kaiser, P. and Tesch, P. (1981). Relationship between leg muscle fiber type distribution and leg exercise performance. *Int. J. Sports Med.* **2**, 154–159.

Lindholm, A. and Piehl, K. (1974). Fibre composition, enzyme activity and concentration of metabolites and electrolytes in muscles of Standardbred horses. *Acta Vet. Scand.* **15**, 287–309.

Nachlas, M. M., Tsou, K. C., DeSouza, E., Cheng, C. S. and Seligman, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* **5**, 420–436.

Padykula, H. A. and Herman, E. (1955). The specificity of the histochemical method of adenosine triphosphate. *J. Histochem. Cytochem.* **3**, 170–195.

Raub, R. H., Kline, K. H., Lawrence, L. M. and Bechtel, P. J. (1986). Distribution of muscle fiber type in fetal equine gluteus medius muscle. *J. Equine Vet. Sci.* **6**, 148–150.

Saltin, B., Henricksson, J., Nygaard, E., Andersen, P. and Jansson, E. (1977). Fiber types and metabolic potential of skeletal muscles in sedentary man and endurance runners. *Ann. N. Y. Acad. Sci.* **301**, 3–29.

Snow, D. H. (1983). Skeletal muscle adaptations: A review. *In: Equine Exercise Physiology*. Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge, pp. 160–183.

Snow, D. H. and Guy, P. S. (1980). Muscle fibre type composition of a number of limb muscles in different types of horses. *Res. Vet. Sci.* **28**, 137–144.

Snow, D. H. and Guy, P. S. (1981). Fibre types and enzyme activities of the gluteus medius in different breeds of horse. In: *Biochemistry of Exercise IV-B*. Poortmans, J. and Niset, G. (eds.) University Park Press, Baltimore, pp. 275–282.

Stull, C. L. and Albert, W. W. (1981). Comparison of muscle fiber types from 2-year-old fillies of the Belgian, Standardbred, Thoroughbred, Quarterhorse and Welsh Breeds. *J. Anim. Sci.* **51**, 340–343.

Suzuki, A. and Cassens, R. G. (1980). A histochemical study of myofiber types in muscle of the growing pig. *J. Anim. Sci.* **51**, 1449–1461.

Thornton, J. R. and Taylor, A. W. (1983). Skeletal muscle characteristics of foals at two to four weeks and eight months of age. *In: Equine Exercise Physiology*. Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge, pp. 218–224.