Changes in Muscle Fiber Type Variation within the Middle Gluteal of Young and Mature Horses as a Function of Sampling Depth

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Summary

A cross section of the middle gluteal muscle going from the superficial surface toward the os coxae was removed from ten adult horses and four foals (two to five days of age). Muscle samples were removed from the cross sections at depths of 1, 2, 4, 6 and 8 cm below the superficial fascia in adult horses, and at depths of 1, 2, 3 and 4 cm below the superficial fascia in foals. Sections were stained for myosin ATPase (pH 9.4) and succinate dehydrogenase (SDH). Fibers were classified as fast twitch glycolytic (FG), fast twitch oxidative-glycolytic (FOG) or slow twitch oxidative (SO) based on staining characteristics. The percentage of FG and SO fiber types varied as a function of sampling depth in both groups. Percent FG fibers decreased and per cent SO fibers increased with increasing sampling depth. Per cent FOG fibers did not change as a function of sampling depth for either age group. Both young and adult horses showed similar muscle fiber type patterns.

Index terms: Sampling site; growth, foals.

Introduction

Investigators have documented wide variations in muscle fiber type composition between different equine muscles (Lindholm and Piehl, 1974; Gunn, 1978; Snow and Guy 1976, 1980). Other species including dogs (Gunn, 1978), cats (West, *et al.*, 1986) and man (Henrickson-Larsen, *et al.*, 1983) have substantial fiber-type variation within certain muscles. A study of superficial and deep areas of six different equine limb muscles (Snow and Guy, 1980) did not find changes in fiber type with depth. Raub *et al.* (1985) found pronounced variation of fiber types within the middle gluteal of a mature pony. Van den Hoven *et al.* (1985) found differences in percent slow-twitch fibers as a function of intramuscular location in equine triceps brachii, longissimus dorsi, biceps femoris and middle gluteal. Bruce and Turek (1985) found a greater percentage of slow-twitch (ST) type fibers in deeper regions of the middle gluteal muscles of two Thoroughbreds when compared to more superficial regions of the same muscles.

They also found a trend for per cent ST fibers to increase longitudinally in the middle gluteal, when moving away from the area of the tuber coxae to the head of the femur.

Muscle samples for numerous studies have been obtained by biopsy techniques. For conclusions drawn from such samplings to be meaningful, one of two conditions must exist. Either muscle fiber types must be distributed uniformly throughout a muscle, or the pattern of fiber type distribution must be known. Additionally, distribution of muscle fiber type patterns between young and adult horses could be important in longitudinal age studies where muscle size varies. Several metabolic parameters of equine muscle from different aged horses have been examined (Lindholm and Piehl, 1974; Essen-Gustavsson *et al.*, 1983; Lindholm *et al.*, 1983; Thornton and Taylor, 1983; Henckel, 1983).

Variation in fiber type patterns between fetal and adult equine middle gluteal has been reported (Raub, *et al.*, 1986); however, no studies have compared neonatal and adult equine muscle fiber type patterns. The purpose of this study was to examine variation of fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG) and slow-twitch oxidative (SO) fibers at different depths in the middle gluteal of neonatal and adult horses, and to compare the fiber type variation between these two groups of horses.

Materials and Methods

Ten small, mature, stock-type horses (5 mares, 5 geldings) of unknown breeding (average live weight, 365 kg), ranging in age from 3–15 years, and four foals from two to five days of age (one Standardbred filly, one Quarter Horse colt, two Quarter Horse fillies) were used in this study. The mature horse muscle samples were obtained from a commercial packing plant and the foals were obtained from the University of Illinois Veterinary Clinic following death from causes unrelated to the muscular system.

Entire cross sections, 2–3 cm thick, were removed from the center portion of the left middle gluteal from each horse. Muscle samples 0.5 cm in diameter were removed 2 cm on either side of a line running from the superficial fascia to the os coxae. In adult horses, the origin of the line was 10 cm dorsocaudal to the tuber coxae at an angle of 45°. In foals, the origin of the line was 6 cm dorsocaudal to the tuber coxae (Fig. 1). These locations were selected to represent common muscle biopsy sites. Adult horses were sampled at depths of 1, 2, 4, 6 and 8 cm below the fascia, and foals were sampled at depths of 1, 2, 3 and 4 cm below the fascia. The 4-cm depth in foals, and the 8-cm depth in adults represented the deepest portion of the middle gluteal in animals of these two sizes.

Histochemical analysis. Muscle samples were mounted on labeled card squares with embedding medium (Tissue-Tek II) and frozen in liquid nitrogen. Samples were later mounted and 10 μm sections cut on a cryostat at −20°C. Sections were stained for myosin ATPase (pH 9.4) (Padykula and Herman, 1955) and succinate dehydrogenase (SDH) (Nachlas *et al.*, 1957).

The percentage of fibers failing to stain for SDH was designated as the per cent FG fibers. The percentage of fibers failing to stain 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). This method for determining three fiber types has been shown to agree closely with serial sectioning methods (Raub *et al.*, 1985). At least 200 fibers per section were characterized for each stain.

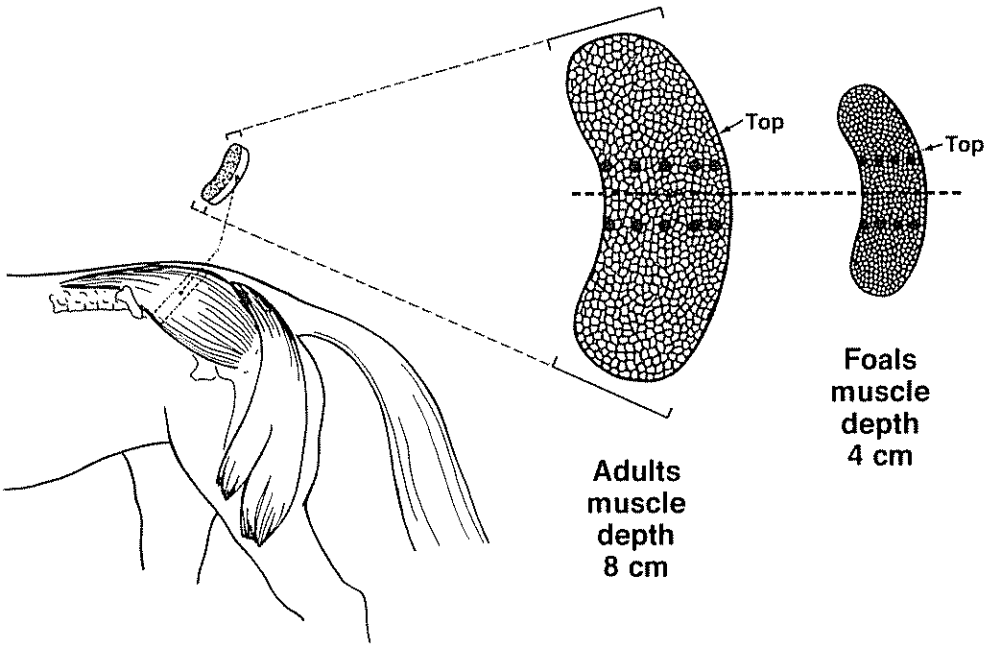

FIGURE 1. Gluteus medius sampling locations for adult horses and foals.

Statistics. Linear regression coefficients of fiber types versus sampling depth for each horse were calculated. A regression coefficient for a given fiber type represents the rate of change in fiber type per cent with sampling depth. These regression coefficients were used as the data on which further statistical analyses were based (Allen *et al.*, 1983). The t-test was used to determine whether the regressions of fiber types versus depth were different than zero. Analysis of variance of coefficients was used to compare ages and individuals. The animal within age mean square was used as the error term for testing age differences because animals were nested within age. Fiber type percentages from the 1 cm sampling depth in adults were omitted when developing the regression equations to account for differences in relative muscle size between foals and adults. Fiber type percentages from the 1 cm sampling depth in adults were reported in Table 1 to allow for constant absolute depth comparisons between adult horses and foals.

Results

Table 1 lists the three fiber type percentages at the various sampling depths for both adult horses and foals. Values are means of the fiber type percentage from the two samples obtained at the designated depth. Per cent fiber types for the two sample locations at a given depth were averaged after analysis of variance showed that sampling location within a given depth was not a statistically significant source of variation.

Both per cent FG and SO fiber types changed as a function of sampling depth. Adult horses had a 65% reduction of FG fibers going from the most superficial to the deepest sampling site, while FG fibers in foals declined 38%. In adult muscle, there was four-

TABLE 1 Per cent fiber type composition of the gluteus medius muscle from adult horses and foals at various sampling depths

Sampling depth (cm)	Percentage fiber type		
	FG	FOG	SO
Adults			
1	35.9 ± 6.3	53.3 ± 5.7	10.8 ± 2.7
2	33.9 ± 5.7	52.4 ± 6.2	13.7 ± 2.9
4	29.8 ± 5.3	52.1 ± 5.6	18.1 ± 5.5
6	18.4 ± 8.4	54.9 ± 8.3	26.7 ± 5.9
8	12.4 ± 9.5	43.5 ± 14.0	44.1 ± 20.3
Foals			
1	38.4 ± 5.1	53.2 ± 7.0	8.4 ± 2.1
2	33.5 ± 3.7	55.3 ± 5.5	11.2 ± 2.0
3	29.4 ± 7.0	54.2 ± 7.2	16.4 ± 1.8
4	23.7 ± 9.3	52.9 ± 16.9	23.4 ± 9.4

Values derived from means ± SD of the two sample locations at the same depth. For adult horses n = 10 and for foals n = 4.
FG = fast glycolytic; FOG = fast oxidative glycolytic; SO = slow oxidative

fold increase in per cent SO fibers between the most superficial and the deepest sampling site. In foal muscle, the increase was three-fold.

Table 2 shows the mean regression coefficient for each fiber type, the standard error of the mean, t-value for assessing whether a coefficient is different than zero, and the probability of obtaining a greater t value if a coefficient is actually equal to zero. Values are presented for all horses, and for adults and foals separately. A t-test of the coefficients for the three fiber types shows that both FG and SO coefficients (means for all horses) are different ($P < .0001$) than zero, while the FOG coefficient is not different ($P > .05$) than zero. Similar results are obtained when adults and foals are analyzed separately. Per cent FG fibers declined significantly ($P < .0001$) while per cent SO fibers increased significantly ($P < .0001$) with increasing sampling depth.

Fiber type profiles of foals versus adult horses were compared with analysis of variance of regression coefficients. The percent FG fibers in the gluteus medius decreased with sampling depth to a greater ($P < .05$) extent in adult horses than in foals and the distribution of per cent SO fiber types was not significantly different ($P > .05$) between ages. There was no significant difference ($P > .05$) in FOG fiber type distribution between foals and adult horses.

Differences in the fiber type profile slopes for all three fiber types were significant ($P < .05$) between individual horses within a given age.

Discussion

In this study, marked variation in the relative proportion of FG and SO fiber types was seen in the middle gluteal as a function of sampling depth. The percent FOG fibers showed little variation throughout cross sections of the middle gluteal. The results of this study agree with those obtained by Raub *et al.* (1985) who found marked differ-

TABLE 2 Regression coefficients for percent fiber types as a function of sampling depth in the middle gluteal of horses and foals

Fiber type	Coefficient	Standard error of mean	T value	P value
Adults plus foals				
FG	−6.74	0.46	−14.75	0.0001
FOG	−1.58	0.94	−1.67	0.1062
SO	8.42	1.15	7.31	0.0001
Adults				
FG	−7.52	0.50	−15.09	0.0001
FOG	−2.11	1.21	−1.75	0.0958
SO	9.76	1.47	6.64	0.0001
Foals				
FG	−4.79	0.61	−7.83	0.0001
FOG	−0.23	1.33	−0.17	0.8670
SO	5.06	1.00	5.04	0.0015

FG = fast glycolytic; FOG = fast oxidative glycolytic; SO = slow oxidative.

ences in the fiber type distribution throughout the middle gluteal of a pony, and with Bruce and Turek (1985) and Van den Hoven *et al.* (1985) who found cross sectional differences in middle gluteal in a Thoroughbred and Dutch Warmblood, respectively.

Because of the major involvement of the middle gluteal in hind leg propulsion and the accessability of the muscle, it has often been used as a biopsy site. However, this study indicates that the equine middle gluteal does not exhibit uniform fiber type distribution. This can present some problems in interpretation of data from single biopsy studies of the equine middle gluteal.

Henriksson-Larsen *et al.* (1983) found differences in the distribution of fiber type between individuals for the human tibialis anterior. Different fiber type distribution patterns between individuals may account for some of the reported differences in per cent fiber types between individuals. This source of variation could conceivably be magnified when dramatically different body or breed types are compared. In this study, the regression coefficients for both FG and SO fiber types were all of the same sign between horses. This indicates that all horses displayed similar patterns of fiber type change as a function of sampling depth; however, the magnitude of fiber type changes with sampling depth differed between individuals. The obvious problem is one of obtaining biopsy samples from the same relative location within the middle gluteal between horses, particularly when horses of differing muscle mass are compared.

Another area of difficulty is longitudinal studies where muscle biopsies are taken from horses at various stages of growth. Because the muscle mass is changing, the question arises as to whether biopsies should be taken from the same absolute depth in relation to the fascia, or whether samples should be taken at a relative constant depth. If the latter option is elected, the muscle size might be determined by a noninvasive technique (possibly ultrasound)

The current study showed similar middle gluteal muscle fiber type patterns to exist

in both foals and mature horses. The middle gluteal from adult horses displayed a greater degree of FG fiber type variation throughout the muscle than foal muscle. However, since breed differences between age groups were not controlled in this study, this finding is difficult to interpret. The information obtained from this study indicates that care should be exercised in sampling and interpreting data obtained from the biopsy of equine middle gluteal muscle.

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Relating Maximal Oxygen Consumption to Skeletal Muscle Mitochondria in Horses

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Summary

Generally, large animals have smaller capacities for weight-specific resting and maximal oxidative metabolism than small animals, though horses represent a remarkable exception to that rule. Despite a body mass of several hundred kilograms, their weight-specific maximal oxygen consumption ($\dot{V}O_2max/Mb$) is similar to that predicted for a 100 g animal. To address the question as to how horses achieve these extremely high rates of oxygen consumption, $\dot{V}O_2max$ was determined in 4 Standardbred horses using an open flow system. The horses were trained to run on an inclined treadmill and reached $\dot{V}O_2max$ (130 ± 5.8 ml O_2/kg/min) while trotting. After the physiological experiments the horses were sacrificed and skeletal muscle tissue samples were obtained for ultrastructural morphometry. The entire musculature of the horses was sampled according to a statistical procedure which yields representative results for the volume density of mitochondria, $V_V(mt,f)$, of the whole body skeletal muscle tissue. $V_V(mt,f)$ for the whole musculature of the four horses analyzed was 7.10 ± 0.36%. Assuming that at $\dot{V}O_2max$ all the oxygen is consumed by skeletal muscle tissue, a cm^3 of skeletal muscle mitochondria consumed on average 4.75 ± 0.36 ml O_2/min. This is not significantly different from the value of average whole body mitochondrial oxygen consumption of the European woodmouse exercising at $\dot{V}O_2max$ (4.9 ml O_2/min/cm^3; body mass 20 g). These data suggest that per unit volume, mitochondria consume oxygen at fixed and constant rates and that the high aerobic capacity of horses is thus commensurate with the high mitochondrial content of their musculature.

Index terms: Morphometry; exercise.