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Buffering and Aerobic Capacity in Equine Muscle: Variation and Effect of Training

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Summary

Reductions in intracellular pH are believed to adversely affect the contractile and metabolic processes of skeletal muscle. Skeletal muscle is endowed with an intrinsic buffering system to attenuate the accumulation of protons within the cellular milieu. Experiments were undertaken to determine the buffering and aerobic capacity of equine skeletal muscle, their variabilities in muscle, and their responses to training. Variability was determined from multiple samples obtained from 2 sedentary horses and the training response from 6 Thoroughbred horses which were trained for seven weeks. They performed a standardized treadmill exercise test (STET) consisting of 2 min at 5 m/sec, and 1 min at each of 10, 11, and 12 m/sec on a 10% grade after 4 and 7 week of training. Venous blood (jugular) and muscle (middle gluteal) samples were collected prior to, on cessation of, and 10 min after exercise during each STET. Major variation between biopsy samples existed for aerobic but not buffering capacity. Succinate dehydrogenase activity was 16.2 ± 7.0 before and 26.7 ± 6.4 ($\mu\text{mol}/\text{min}/\text{g}$ wet weight) ($n = 6$) following the training period. Buffering capacity increased from a mean of 57.89 ± 6.51 slykes ($\mu\text{mol}/\text{g}/\text{pH}$) to 92.74 ± 6.95 (mean \pm SEM) ($n = 6$) following 7 weeks training. Buffering capacity was unchanged by the STET throughout the training period. However, muscle and blood lactate were reduced by 30 and 14%, respectively, when samples collected prior to, and on completion of training were compared. This study demonstrates that buffering capacity of equine skeletal muscle is increased by training which may aid in maintaining cellular homeostasis during periods of intense exercise

Index terms: Lactate; adenosine triphosphate; creatine phosphate.

Introduction

Intense physical activity produces an elevation in lactate within muscle. The intracellular proton accumulation and pH decrement associated with this lactate production are believed to adversely affect the contractile and metabolic processes of skeletal muscle.

Since lowered intracellular pH may be involved with the onset of fatigue during intense exercise (Mainwood and Renaud 1985), the ability to buffer the effects of proton accumulation on pH may be an important determinant of maximum performance under circumstances such as those associated with racing. Studies have been reported that indicate differences in buffering capacity exist in muscles from endurance trained, sprint trained, and sedentary people, and rowers (Sahlin and Henriksson 1984; Parkhouse *et al.*, 1985). However, whether buffering capacity can be altered in the horse and to what extent is an unknown, but an important question.

Skeletal muscle is a heterogeneous collection of fibers with different regional populations of fibers of various physiological and biochemical properties (Lexell *et al.*, 1985; Elder *et al.*, 1982; Lexell *et al.*, 1983; Hodgson *et al.*, 1983; Kai, 1984; Bruce and Turek, 1985). Although some investigations have attempted to link the histochemical characteristics of specific limb muscles of the horse to athletic ability (Barlow *et al.*, 1984; Snow and Guy, 1980), such extrapolation must be approached with caution (Gollnick *et al.*, 1980). The most convenient method of obtaining muscle samples for fiber type determination employs a percutaneous needle biopsy technique. Since a small quantity (approximately 2000 fibers or less) of muscle is removed using this technique there is some question as to whether this provides an adequate representation of the muscles being examined. The heterogeneous distribution of fiber types within the middle gluteal muscle of the horse may lead to a spurious interpretation of its characteristics based on biopsy samples. Depending upon the aspect of muscle function critical to a particular study, the variations that can exist in a single biopsy could significantly influence the interpretation of the results.

This paper presents the results of experiments conducted to determine the buffering capacity of equine skeletal muscle in the untrained horse and the effects of training on this property of the gluteus medius muscle. The effects of training were monitored in 6 Thoroughbred horses by determining the succinate dehydrogenase (SDH) activity of muscle samples and by evaluating the muscle and blood lactate (LA) production in response to a standardized treadmill exercise test (STET). Since substantial variation in fiber type within the gluteus medius muscle has been reported (Bruce and Turek, 1985; van den Hoven *et al.*, 1985), multiple samples were collected from 2 additional horses and buffering capacity, citrate synthase (CS), and SDH activity measured.

Materials and Methods

Six untrained Thoroughbred horses, 2 mares and 4 geldings ranging in age from 5 to 8 years, were used. All were maintained on a standard diet of alfalfa cubes and grain.

The horses trained for 7 weeks using a conventional racetrack program in which they galloped at near maximum speed for 600 m, 800 m, or 1000 m every 5th day. On interim days the horses walked, trotted, and cantered for a total distance of approximately 5 km. Each performed a STET prior to, and after 4 and 7 weeks of training. The STET consisted of running 2 min at 5 m/sec and 1 min each at 10, 11, and 12 m/sec on a +10% grade.

Blood from the jugular vein and samples from the middle gluteal muscle were collected with the horses at rest prior to (PRE), within 15 seconds of cessation of (POST), and 10 min after exercise (POST 10) during each STET. All muscle samples were obtained percutaneously using the technique of Bergstrom (1962). Muscle samples were

removed from the biopsy needle, quenched in liquid nitrogen, and stored at -80°C until analyzed.

To determine the effects of the sampling site on buffering capacity, SDH, and CS activity, multiple muscle samples were obtained from 2 untrained horses. One horse was a 2-year-old gelding and the other was a 3-year-old filly. In each instance samples were collected from the middle gluteal muscle immediately following euthanasia. Six samples were removed from 1 horse from locations 6 cm apart along the length of the muscle and at a depth of approximately 8 cm. In the other horse, 10 identical biopsy sites were selected in each middle gluteal muscle. Five areas 5 cm apart along the longitudinal axis of each muscle were selected and 2 biopsies were collected from each area.

Approximately 10 ml of venous blood were collected for determination of the pre- and post-exercise LA concentrations. Blood samples were placed in tubes containing potassium oxalate and retained on ice until centrifuged. Plasma was removed from the centrifuged samples, deproteinized, extracted, and stored at -80°C prior to analysis.

Muscle samples (20–40 mg) for analysis of total creatine (TCr) (TCr = creatine and creatine phosphate), and LA were pulverized at the temperature of liquid nitrogen in a micromortar designed by Pette and Reichmann (1982). Pulverized tissue was placed on frozen 2 N perchloric acid (PCA) and extracted by the method described by Lowry and Passoneau (1972). The samples were then centrifuged (3000 g at $+4^{\circ}\text{C}$ for 10 min) and 200 μl aliquots of supernatant fluid removed and neutralized using 2 N KHCO_3 . The potassium perchlorate precipitate in the neutralized samples was sedimented by centrifugation and any remaining adenylate kinase was denatured by the addition of 6 N NaOH (25 μl). After 10 min, samples were neutralized with 25 μl of 6 N HCl and 100 mM KHCO_3 (pH 7.5) added to yield a final volume of 1.0 ml.

Muscle extracts were measured fluorometrically and expressed in $\mu\text{mol}/\mu\text{mol}$ of TCr. The choice of TCr as a method of expressing metabolite concentrations is based on several factors. Less variation is introduced with TCr as compared to using dry weight, wet weight, or protein as a denominator. The effects of fluid shifts within the cell with exercise have a minimal effect on TCr thereby providing a better reference base for the comparison of pre- and post-exercise samples. Since the concentrations of CR and CP in blood are negligible, increased blood flow in muscle during exercise or blood contamination at the time of sampling should not alter results referenced to TCr. TCr was 35.8 ± 1.0 and 136.0 ± 4.9 $\mu\text{mol}/\text{gm}$ wet and dry weight muscle, respectively. This was not altered by the exercise.

The concentration of CP was determined by the method of Lamprecht *et al.* (1974). The concentration of CR was estimated by the technique described by Harris *et al.*, (1974) with the modification of altering the concentration of ATP and phosphoenolpyruvate to 0.075 mM and 0.25 mM, respectively. Lactate levels were determined by the method described by Lowry and Passoneau (1972).

Three (10–25 mg) portions of each muscle sample were used to determine SDH activity according to the method of Sembrowich *et al.*, (1977). Each portion of the sample was used to perform a separate SDH assay such that 3 assays, all performed in triplicate, were conducted on each muscle biopsy. Similarly, two (10–20 mg) portions were used to measure CS activity. The CS activity was read fluorometrically according to the method described by Shepherd and Garland (1969).

In the determination of buffering capacity frozen skeletal muscle samples (35–70 mg)

were pulverized and added to a solution consisting of KCl 145 mM, NaCl 10 mM, iodoacetic acid, and 2, 4 dinitrofluorobenzene (DNFB) 0.3 mM at 25°C adjusted to pH 7.0. A dilution of 5 ul/mg wet weight of muscle was used. The addition of DNFB to the solution inhibits creatine kinase activity preventing the resynthesis of ATP. The pH of the crushed muscle sample suspension was measured at 25°C using an Orion pH meter and a microelectrode. Buffering capacity was measured as the number of umoles of acid or base required per gram of tissue to produce a change in pH from 6.0–7.0 or from 7.0–6.0 at 25°C.

Plasma samples were deproteinized and extracted in a manner similar to the muscles although alkalization to remove adenylate kinase was not required.

Results

The results of multiple muscle samples collected from the untrained horses indicated the buffering capacity of gluteus medius muscle from 1 horse ranged from 58.38 to 71.59 Slykes (umol/g/pH) with a mean value of 65.31 ± 3.24 (SEM) Slykes. Similarly, in a 2nd horse mean buffering capacity measured in the left and right middle gluteal muscle was 74.48 ± 0.99 and 73.84 ± 2.04 Slykes, respectively. However, there were significant differences in the SDH and CS activity. In the first horse SDH activity ranged from 11.25 to 27.02 umol/g/min, and in the second horse there were significant differences in the SDH activity measurements obtained from similar biopsy sites in the two gluteal muscles (Table 1). There was often a twofold variation in SDH values collected from the same 8 cm² area of each muscle with activities ranging from 6.97 to 19.81 umol/g/min, but less than 4% difference in the values obtained from triplicates of the same biopsy sample. The CS activity varied to a lesser extent with activities ranging from 13.19 to 22.30 umol/g/min (Table 1).

The mean values for SDH activity in samples collected from all horses prior to each STET indicated an elevation in SDH activity following the 7 week training period (Table 2). However, there was no pattern in the results from individual horses to suggest that the overall increase was related to training.

Post-exercise plasma and muscle lactate concentrations were reduced 14% and 30%, respectively, when samples collected prior to and on completion of training were compared (Fig. 1, 2).

Skeletal muscle buffering capacity increased during the 7-week training period (Fig. 3). Comparing the pretraining samples to those collected after 7 weeks training there was an approximately 60% increase in post-exercise buffering capacity from a mean of 57.89 ± 6.51 to 92.74 ± 6.95 Slykes.

Discussion

In the 26 biopsy samples obtained from the middle gluteal muscles of the 2 sedentary horses, there was extensive variation in SDH and CS activity. Therefore, it is not surprising that the SDH activity measured in the 6 Thoroughbred horses prior to training was also quite diverse in spite of the uniform sampling location. This may reflect the variation of skeletal muscle fiber type distribution that exists within muscle in man (Elder *et al.*, 1982; Lexell *et al.*, 1983) and in the horse (Bruce and Turek, 1985, Lindholm and Piehl, 1974; Snow and Guy, 1980). This variation is due to marked regional variation in the fiber populations of the muscles. The complexity of deter-

TABLE 1. Comparison of succinic dehydrogenase (SDH) activity ($\mu\text{mol/g/min}$), citrate synthase (CS) activity ($\mu\text{mol/g/min}$), and buffering capacity (slykes) in muscle from multiple gluteus medius biopsy sites in two untrained horses.

	Sample #	SDH	CS	SLYKES
Horse A				
	1	24.93	22.30	55.60
	2	11.26	16.64	61.59
	3	17.14	18.42	58.38
	4	27.03	17.01	77.15
	5	20.42	13.19	74.55
	6	<u>23.69</u>	<u>21.30</u>	<u>64.62</u>
	MEAN	20.74	18.14	65.31
	SD	5.30	1.23	7.98
Horse B				
(left gluteal)				
	1A	16.18	19.41	69.32
	2A	14.61	17.88	70.15
	3A	18.65	17.90	86.44
	4A	13.33	15.81	70.52
	5A	9.12	13.33	71.69
	6A	14.58	15.56	73.52
	7A	12.65	15.87	71.83
	8A	10.37	14.54	66.07
	9A	18.90	16.68	88.47
	10A	<u>17.54</u>	—	<u>76.83</u>
	MEAN	14.59	16.33	74.48
	SD	3.15	1.14	7.39
Horse B				
(right gluteal)				
	1B	12.36	15.34	65.39
	2B	7.95	—	68.36
	3B	11.22	15.60	73.24
	4B	6.97	13.85	64.73
	5B	8.97	14.43	75.75
	6B	8.58	12.97	70.47
	7B	19.81	16.51	79.55
	8B	19.18	18.48	81.03
	9B	15.72	16.47	74.42
	10B	<u>14.56</u>	<u>18.84</u>	<u>85.32</u>
	MEAN	12.53	15.83	73.83
	SD	4.40	1.18	6.80

mining the types and subtypes of muscle fibers based on histochemical techniques for myofibrillar actomyosin ATPase has been described in detail for skeletal muscle for a number of species including the horse (Matoba et al., 1985). Even within histochemically defined species-specific fiber types, a range of metabolic potentials have been described (Pette, 1984). Thus, considerable variability in both oxidative and glycolytic

TABLE 2 Succinic dehydrogenase (SDH) activity ($\mu\text{mol/g/min}$) in gluteus medius muscle samples collected from 6 Thoroughbred horses prior to each standardized treadmill exercise test at 0, 4, and 7 weeks of training.

Horse no.	PRE EXERCISE SDH ACTIVITY ($\mu\text{mol/g/min}$)		
	Week 0	Week 4	Week 7
1	18.54	10.04	26.90
2	3.23	24.63	31.59
3	18.91	6.30	23.60
4	14.51	22.72	17.50
5	24.04	38.11	35.91
6	<u>17.88</u>	<u>29.98</u>	<u>24.55</u>
MEAN	16.18	21.96	26.67
SD	7.04	12.01	6.44

capacity exists within a single fiber type in the horse (Valberg and Essen-Gustavsson, 1985). This variability in enzyme activity within a fiber type coupled with a diverse percentage of each fiber type throughout the gluteal muscle is probably responsible for the range of SDH activity measured in muscle samples in this study. Based on the

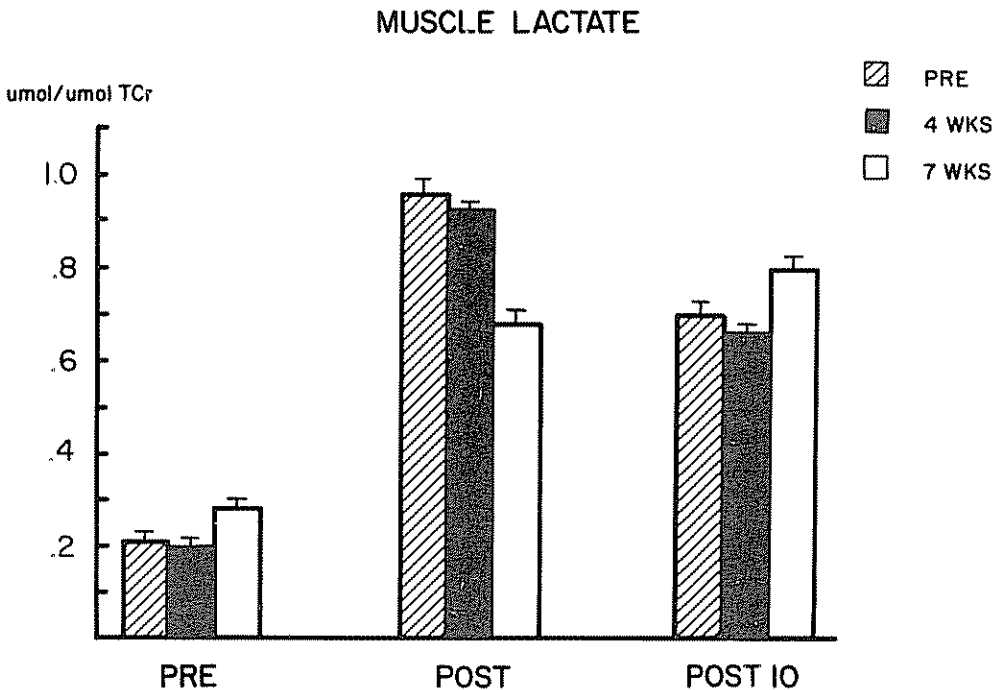


FIGURE 1. Mean lactate concentration in muscle biopsies from 6 Thoroughbred horses. Samples were collected prior to (PRE), immediately after (POST), and 10 min after (POST 10) each standardized treadmill exercise test in a 7 week period of training. ($\bar{X} \pm \text{SEM}$).

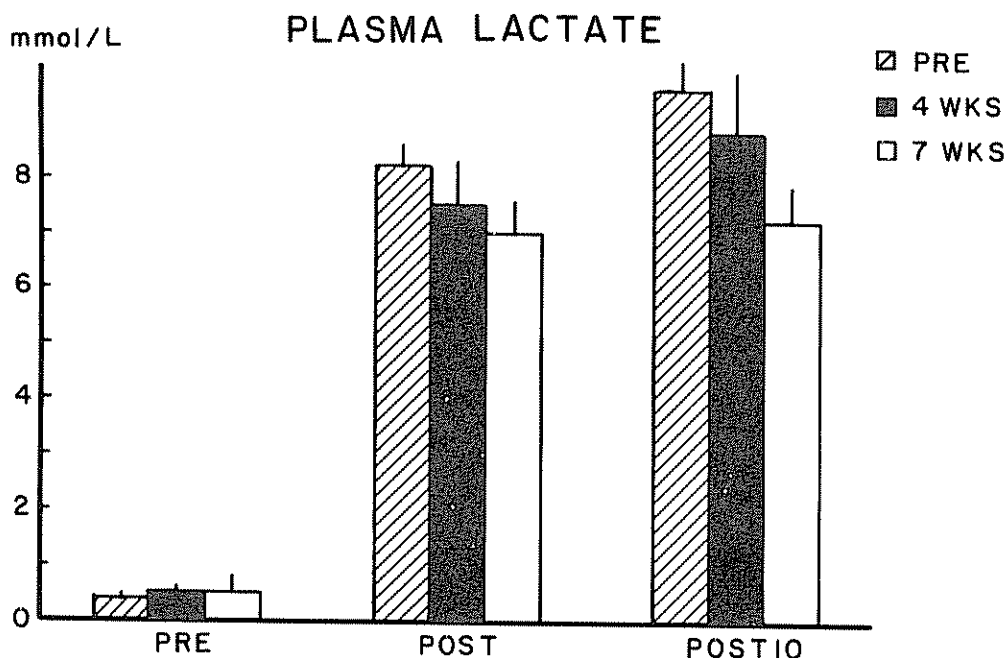


FIGURE 2. Mean plasma lactate concentration in venous blood samples collected prior to (PRE), immediately following (POST), and 10 min after (POST 10) each standardized treadmill exercise test in a 7 week period of training ($\bar{X} \pm \text{SEM}$)

variation within the gluteus medius of the horse, SDH may be difficult to use as a definitive criterion for establishing a training effect.

In spite of the variable SDH activity demonstrated in the 2 nontrained horses, the buffering capacity measured in duplicates of the same muscle biopsy and in the samples collected from the multiple sites was quite consistent and the values were similar to those measured in the 6 Thoroughbred horses prior to training. In these 6 horses initial buffering capacity measurements were distributed over a narrow range and increased over the 7 week period of training. On each occasion samples were collected from the same region of the gluteus medius muscle. The consistency of measurements of buffering capacity from numerous middle gluteal sites in 2 nontrained horses suggests that a single biopsy sample may provide an adequate reflection of the buffering capability throughout the muscle.

In all species examined, there is some heterogeneity recognized in the metabolic (Nemeth *et al.*, 1979) and histochemical properties of muscle fiber types and this heterogeneity is rather pronounced in the horse. The relatively uniform buffering capacity found in the muscle samples of the horses in this study may represent an overlap in this potential among different fiber types sufficient to eliminate variability based on fiber type distribution or it is possible that all fibers in the horse have similar buffering capacity.

The results of this study demonstrate a significant increase in buffering capacity in the horse associated with training. Thus, these results extend with a longitudinal training

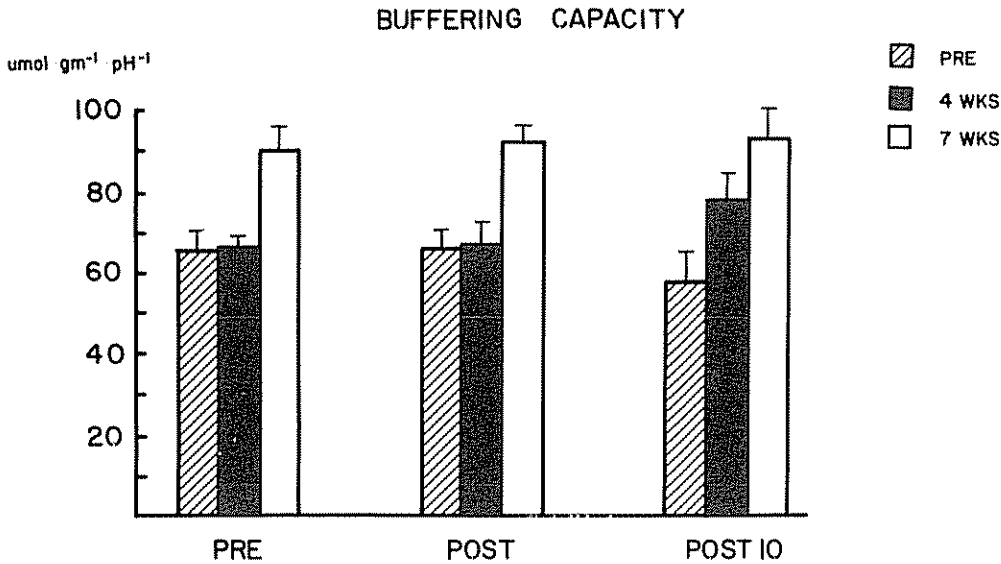


FIGURE 3. Pattern of increase in buffering capacity in response to 7 weeks training measured in middle gluteal muscle biopsies from 6 Thoroughbred horses. Values are means \pm SEM. PRE = prior to; POST = immediately after and POST 10 = 10 min after each standardized treadmill exercise test ($\bar{X} \pm$ SEM).

model the findings from the cross-sectional studies on man in which sprinters and rowers were found to possess elevated buffering capabilities when compared to marathoners and sedentary subjects (Parkhouse *et al.*, 1985).

There was no attempt in this study to determine the relative input of major buffering constituents. Skeletal muscle has three major components contributing to the buffering system including physio-chemical buffering, nonvolatile acid production and consumption, and HCO_3^- buffering. In this experiment the contribution of HCO_3^- buffering was eliminated by conducting the titration of the muscle homogenates in a nitrogen atmosphere to provide a closed system. Of the remaining components, much of the buffering capacity has been attributed to inorganic phosphate, CP, histidine-related compounds and the imidazole-containing compound carnosine (Parkhouse and McKenzie, 1984). A high concentration of these dipeptides in glycolytic tissues has led to the suggestion that they could be suitable and important buffers in the physiological range (Bate Smith, 1938; Davey, 1960). A significant correlation between carnosine levels and buffering capacity in human skeletal muscle has been identified although it is unlikely that this is the only physiological role of these dipeptides (Parkhouse and McKenzie, 1984). Buffering capacity is recognized to vary among species, with a higher inherent capacity identified in marine diving mammals capable of intense short-duration exercise (Castellini and Somero, 1981). An elevated capacity to buffer intracellular fluid would be beneficial in moderating the extent of intracellular pH reduction when large increases in lactate concentration occur in response to training. White skeletal muscle, typically higher in glycolytic enzymes and lower in citric acid cycle enzyme concentrations (Johnston *et al.*, 1977; Somero and Childress, 1980), demonstrates a greater buffering capacity (Castellini and Somero, 1981). In contrast, the higher capillary density rec-

ognized in red muscle (Stevens, 1968) may produce the same result by allowing more rapid removal of accumulated LA and protons by the blood.

The pre-training buffering capacity measurements obtained from the horses in this study are similar to values reported in the literature in other terrestrial mammals (Castellini and Somero, 1981). Based on the samples removed from the middle gluteal muscle, this mixed fiber type muscle of the horse demonstrated the ability to increase buffering capacity in response to training. The pre-training buffering capacity varied little in multiple site measurements despite work to illustrate that the middle gluteal muscle of the horse may vary from less than 10% to greater than 50% slow twitch fibers depending on the location and depth of sampling (Bruce and Turek, 1985), and as much as 30% even when biopsies were collected in a uniform manner at a depth of 8 cm (van den Hoven *et al.*, 1985).

The results of this study indicate the capacity of equine skeletal muscle to buffer the pH decrement associated with proton accumulation accompanying high intensity exercise is enhanced by training and this capacity may provide a significant contribution to the maximum performance ability of the horse.

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