

Skeletal Muscle Buffer Capacity Changes in Standardbred Horses: Effects of Growth and Training

G. FOX, P. HENCKEL, C. JUEL, J. FALK-RØNNE and B. SALTIN
August Krogh Institute, University of Copenhagen, Copenhagen and
Charlottenlund Racetrack Equine Clinic, Charlottenlund, Denmark

Summary

We examined the effect of training and growth on skeletal muscle buffer capacity (β). Middle gluteal muscle biopsies were obtained from 9 young, untrained Standardbred horses over an 18-week period while the horses continued untrained (UT), were exercised regularly without any bouts of intense sprinting (DT), or entered a formal interval training program (IT). Buffer capacity was expressed as the number of $\mu\text{moles HCl}$ required to change the pH one unit from 7.0 to 6.0. The average (β) among all horses increased during the study from 223 ± 14 to 254 ± 21 $\mu\text{moles/g d.w./pH}_{7.0-6.0}$ ($P < 0.01$). The only significant (β) increase was in the IT group, which increased from 220 ± 13 to 263 ± 24 $\mu\text{moles/g d.w./pH}_{7.0-6.0}$ ($P < 0.02$). We conclude that interval training may accentuate the inherent buffer capacity increase in the growing horse.

Index terms: Muscle fiber types; glycolysis; muscle lactate; anaerobic capacity.

Introduction

Most horse racing events place an extremely high emphasis on anaerobic metabolism for energy production primarily because of the relatively short duration of their maximal efforts. The horse in training for such events provides an excellent model for the study of adaptations thought to arise from stresses placed on anaerobic glycolysis and from production of its by-products. When such demands are placed on skeletal muscle the rapid production of ATP is associated with elevated lactate and decreased pH (Hermansen and Osnes, 1972). The accumulation of H^+ has been shown to reduce skeletal muscle contractility (Donaldson, 1983) and thus performance capacity, however the precise mechanism has yet to be resolved.

As the high intensity exercise progresses, lactic acid is generated and the rate of proton efflux cannot match the rate of lactic acid production resulting in proton accumulation within the intracellular compartment. This accumulation of H^+ within the muscle cell maximizes the role of buffers in the muscle, from now on referred to as (β). During a maximal effort it is the activities of these buffers that determine at which point

critical intracellular pH is reached and muscle fatigue ensues. The goal of the present study was to examine the effect of second year growth and interval training on (β) in Standardbred horses with a special interest towards its potential beneficial effects on anaerobic capacity.

Materials and Methods

Seven two-year-old males, one two-year-old female and one three-year-old male Standardbred horses began the study in mid-February. All horses were untrained. Two of the 2-year-old horses were stabled for the entire period with infrequent paddock time during the final weeks, (untrained, UT). After an initial 3-week exercise testing period by the trainer, 4 of the horses were chosen for systematic training geared towards racing beginning in late July, (interval trained, IT). The remaining 3, including the female, were stabled, but exercised regularly without any bouts of intense sprinting, (daily training, DT). The three-week testing program, which began the experiment, included daily "easy" trotting of 4–5 km, 4–5 times per week, with 1–2 test efforts over 1.0 to 1.6 km each week. For those 4 chosen to remain in training, this type of pattern continued with the gradual increase in the frequency, duration and intensity of the intervals without increasing the number of sessions per week. Two of the horses changed trainers and experienced 2 weeks of detraining followed by a similar type of training schedule except with greater frequency of interval training sessions, 2–3 per week.

Muscle biopsies were obtained from the middle gluteal muscle at a depth of 3–4 cm by a technique described by Lindholm and Piehl (1974). Samples were taken before, during and after the experimental period except for the UT group, which was only measured before and after the 18-week period.

The muscle samples were freeze-dried and dissected free of all connective tissue and blood. The mean weight of the final dried muscle fiber fragments was 3.069 mg (± 1.689). These were homogenized in a salt solution (0.1 M HCL/1/pH) containing 145 mmol KCl, 10 mmol NaCl and 5 mmol iodoacetic acid at a dilution of approximately 1:100. The iodoacetic acid was added to inhibit glycolysis (Lundsgaard, 1930). The homogenate was then adjusted to pH 7.0 by addition of .01 N NaOH and titrated to pH 6.0 with 5–25 μ l aliquots of .01 N HCl. The system included a micro magnetic stirring device, a Hamilton precision 25 μ l syringe and an Orion Research pH electrode No. 8103. Muscle buffer capacity was expressed as μ mol HCl per gm d.w. required to change the pH from 7.0 to 6.0. Variability of the (β) measurement technique was $\pm 2.7\%$ based on random samples large enough to measure twice ($n = 5$). One piece of the muscle sample was used for morphological examination with determination of relative fiber composition (type I, 2a and 2b) as well as fiber area (Henckel, 1983).

Results

The average (β) among all the untrained horses was 223 SD ± 14 μ mol/g d.w./pH_{7.0-6.0}, range (197–238) and increased to 254 SD ± 21 μ mol/g d.w./pH_{7.0-6.0}, range (225–289) after the 18-week experimental period. Plots of the individual (β) changes and per cent (β) changes are illustrated in Fig. 1 and 2, respectively. The UT and DT groups both increased their (β) by about 20 μ mol/g d.w./pH_{7.0-6.0}. The IT group showed a significant within group increase which amounted to 43 μ mol/g d.w./pH_{7.0-6.0} ($P < 0.02$) as measured by Sandler's A Test for correlated samples. When looking at the

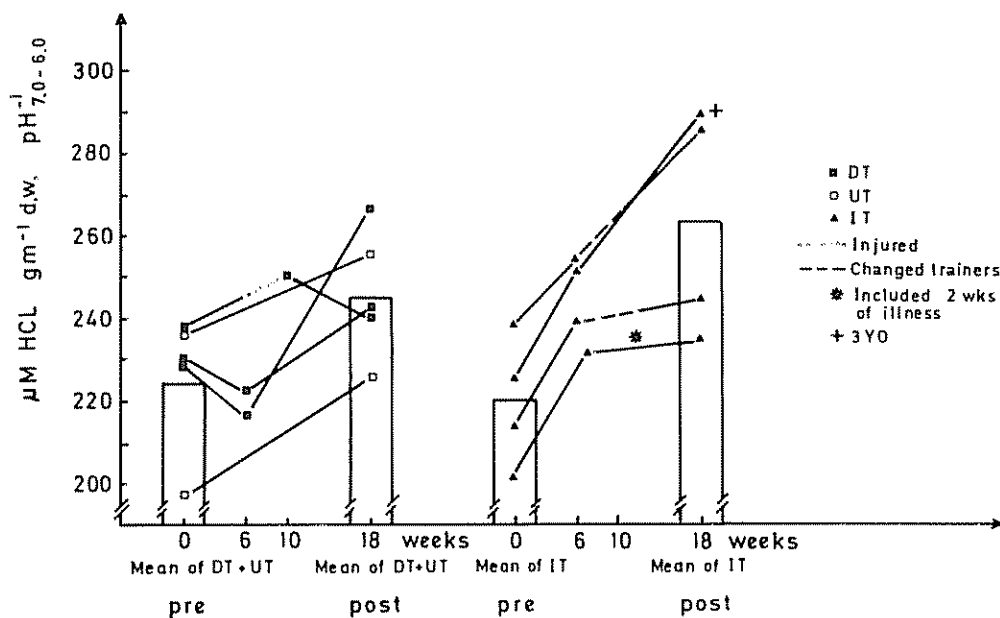


FIGURE 1. Individual and mean values for buffer capacity of horse skeletal muscle before (pre) and after (post) 18 weeks of either no systematic training (DT + UT) or interval training (IT).

group in total, the increase in (β) , about $30 \mu\text{mol/g d.w. /pH}_{7.0-6.0}^{-1}$, shows greater significance ($P < 0.01$). A 3×2 ANOVA was performed and also reflected ($P < 0.01$) significance between pre and post experimental period involving all horses. Table 1 presents data from the individual groups. The results show that (β) changes in the young Standardbred horse.

Relative skeletal muscle fiber composition and mean fiber area were very similar in the horses in the control phase of the study (Table 2). Fiber composition did not change during the 18 weeks of observation in any of the horses whereas the muscle fiber areas did change. This was true for all horses and for the three fiber types.

Discussion

The components of skeletal muscle buffer capacity can be divided into the static or physio-chemical buffers and the dynamic buffers, those that produce or consume acids and bases (Sahlin, 1978). Sahlin presents the relative contributions of the static buffers to be: HCO_3^- , 18% (β); HP_2O_4^- , 11% (β); carnosine, 3–7% (β) (1984); and protein, about 22% (β). He presents the primary dynamic buffering reactions to be creatine phosphate, H^+ and ADP to creatine and ATP, 38% (β) and glutamate, NH_3 and H^+ to glutamine, 11% (β). The (β) measured in this experiment consisted of all of the previously mentioned buffers except the HCO_3^- , which most likely evaporated during the homogenation as CO_2 .

The changes in buffer capacity observed in skeletal muscle could be due to changes in muscle morphology, as type II fibers have higher (β) (Tamaki *et al.*, 1977; McKenzie

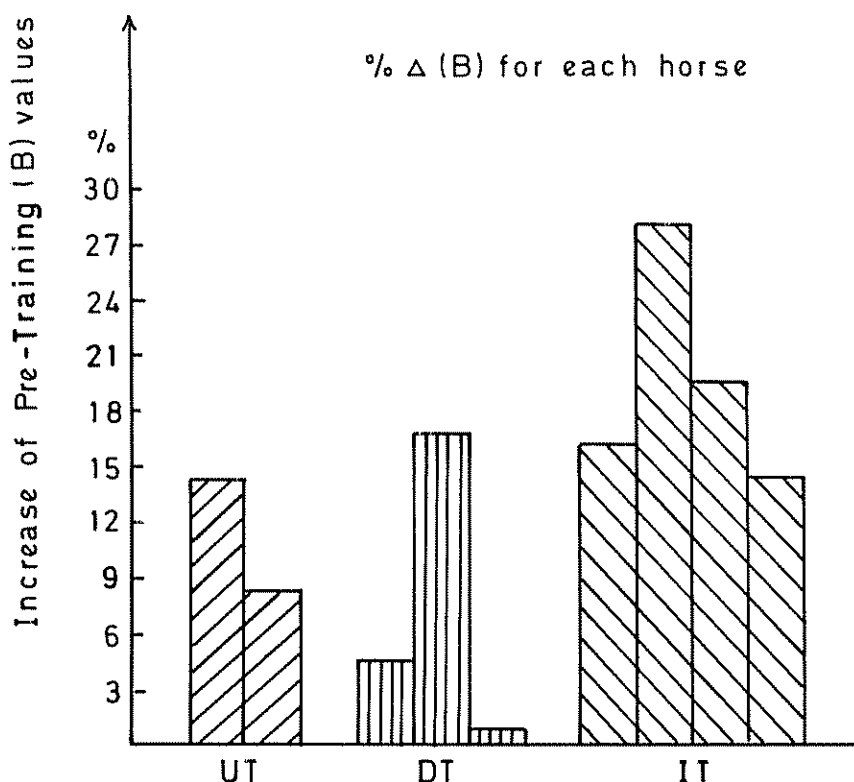


FIGURE 2. Individual values for the relative increase in buffer capacity ($B = \beta$) in the nine horses followed over 18 weeks. UT and DT represent horses without any systematic high speed training and IT included such training

et al., 1983; Castellini and Somero, 1981) or in the composition of buffers in individual fibers or a combination of both. The increase in the relative type II mean fiber area could explain part of the observed enhanced (β). However, the magnitude of the change in relative fiber morphology does not match the magnitude of the (β) increase. Further, the negligible shift in fiber morphology in the IT group would also indicate a qualitative change which has taken place within the muscle fibers of those horses. Several investigators have found a correlation between human athletes trained for anaerobically demanding sports and enhanced (β) without changes in fiber morphology (Sahlin and Henriksson, 1984; Parkhouse *et al.*, 1985). Most recently Sharp *et al.* (1986) found a 37% increase in (β) following a controlled 8-week sprint training program on a bicycle ergometer.

There have been several theories proposed recently for the increase in (β) found in athletes trained for anaerobically demanding sports. Parkhouse *et al.* (1985) revealed a potential source for an increased (β) by demonstrating higher muscle carnosine concentrations in sprinters and rowers as compared to untrained individuals; however, the extent of the increase he found does not match the magnitude of the (β) change and therefore can only partially explain these changes. Since muscle protein, by its incorporated histidine content, is likely the most important intracellular buffer (Sahlin, 1978),

TABLE 1. Change in buffer capacity with training

Group	Pre-training	Post-training	% Change
UT(n = 2)	217 ± 20*	240 ± 15	+10%
DT(n = 3)	232 ± 4	250 ± 12	+8%
IT(n = 4)	220 ± 13	263 ± 24(p < .02)	+20%
Total (n = 9)	223 ± 14	254 ± 21(p < .01)	+14%

*Mean ± SD; ($\mu\text{mol HCL/g d.w. /pH}_{7.0-6.0}$)

it is possible that increased incorporation of protein within the muscle fibers could account for major improvements in (β). This theory has not been tested, however given the training routines utilized in both Sharp *et al.* (1986) and our experiment, increased protein incorporation would be expected (Gordon, 1967; Goldberg *et al.*, 1975). Another theory is that creatine phosphate (CP) concentrations within the muscle cell increases which would increase (β). Sahlin and Henriksson (1984) did not find significant differences, whereas Sharp *et al.* (1986) did find a significant difference between sprint trained and endurance trained subjects. Sharp, however, does not attribute the increase in (β) between the untrained and sprint trained individuals with the increase in CP concentration $\pm 16\%$ (N.S.) because there was no measured difference in the amount of CP breakdown during the exhaustive exercise between the pre and post trained groups.

Because the daily routines and the fiber morphology of the UT and DT groups resemble each other in that neither group experienced any controlled high intensity run-

TABLE 2. Relative frequency of muscle fiber types and fiber area

	DT + UT groups, n = 5:			
	Relative frequency (%)		Area μm^2	
	pre	post	pre	post
Type I:	16	12	1415	1815
Type IIa:	34	38	2045	2650
Type IIb:	50	50	3865	4810
	II group, n = 4:			
	Relative frequency (%)		Area μm^2	
	pre	post	pre	post
Type I:	16	14	1580	1920
Type IIa:	37	39	2305	2700
Type IIb:	47	47	3350	4150

Data were collected before (pre) and after (post) 18 weeks of follow-up of horses in the DT and UT groups. None of the differences in relative fiber composition are significant, whereas all increases in fiber area are significant.

DT = distance trained; UT = untrained

ning and their increase in relative type II, mean fiber areas were similar. We speculate that the increases in (β) were growth related. The (β) values reported in this study are similar in magnitude to humans (58.8 $\mu\text{mol/g w. w./pH}$) (Sahlin and Henriksson 1984), and slightly higher than other terrestrial mammals such as dogs (50.2 $\mu\text{mol/g w. w./pH}$), swine (49.7 $\mu\text{mol/g w. w./pH}$) and cattle (51.9 $\mu\text{mol/g w. w./pH}$) (Castellini and Somero, 1981).

This experiment shows that high-intensity interval training can accentuate the inherent (β) increase in the growing horse. This adaption aids in improving the anaerobic capacity of the horse. An increase in (β) is appropriate in order to maximize the lactate generating capacity of the muscle without reaching critically low pH. A horse with a well developed (β) may find it easier to reach deeply into its anaerobic capacity and thus improve its racing ability.

Acknowledgments

The authors wish to thank the following foundations for their financial support: Protection of the Horse Foundation, Denmark, Danish Warmblood Foundation, Denmark and Radiometer-Copenhagen, Denmark. We would also like to thank Masao Mizuno for his practical assistance, as well as Axel Jacobsen for training and caring for the horses.

References

- Castellini, M. A. and Somero, G. N. (1981). Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. *J. Comp. Physiol.* **143**, 191–198.
- Donaldson, S. B. (1983). Effect of acidosis on maximum force generation of peeled mammalian skeletal muscle fibers. In: *Biochemistry of Exercise*, H. Knuttgen, J. Vogel and J. Poortmans (eds.). Human Kinetics Publishers, Champaign, IL. pp. 126–133.
- Gordon, E. (1967). Anatomical and biochemical adaptations of muscle to different exercises. *JAMA* **201**, 755–758.
- Goldberg, A. L., Etlinger, J. D., Goldspink, D. F. and Jablecki, C. (1975). Mechanism of work-induced hypertrophy of skeletal muscle. *Med. Sci. Sports* **7**, 185–198.
- Henckel, P. (1983). Training and growth induced changes in the middle gluteal muscle of young Standardbred trotters. *Equine Vet. J.* **15**, 134–140.
- Hermansen, L. and Osnes, J.-B. (1972). Blood and muscle pH after maximal exercise in man. *J. Appl. Physiol.* **32**, 304–308.
- 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**, 287–324.
- Lundsgaard, E. (1930). Untersuchungen iiber Muskelkontraktionen ohne Milchsaur-
ebildung. *Biochemische Zeitschrift* **217**, 162–177.
- McKenzie, D. C., Parkhouse, W. S., Rhodes, E. C., Hochochka, P. W., Ovalle, W. K., Mommsen, T. P. and Shinn, S. L. (1983). Skeletal muscle buffering capacity in elite athletes. In: *Biochemistry of Exercise*. Knuttgen, H. G., Vogel, J. A. and Poortmans, J. (eds.). Human Kinetic Publishers, Champaign, IL. pp. 585–589.

- Parkhouse, W. S., McKenzie, D. C., Hochachka, P. W. and Ovale, W. K. (1985). Buffering capacity of deproteinized human vastus lateralis muscle. *J. Appl. Physiol.* **58**, 14–17.
- Sahlin, K. (1978). Intracellular pH and energy metabolism in skeletal muscle of man. With special reference to exercise. *Acta Physiol. Scand.* Suppl. 455.
- Sahlin, K. and Henriksson, J. (1984). Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiol. Scand.* **122**, 331–339.
- Sharp, R. L., Costill, D. L., Fink, W. J. and King, D. S. (1986). Effects of eight weeks of bicycle ergometer sprint training on human muscle buffer capacity. *Int. J. Sports Med.* **7**, 13–17.
- Tamaki, N., Nakamura, M., Harada, M., Kimura, K., Kawano, H. and Hama, T. (1977). Anserine and carnosine contents in muscular tissue of rat and rabbit. *J. Nutr. Sci. Vitaminol.* **23**, 213–219.

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

L. J. McCUTCHEON, T. B. KELSO, L. A. BERTOCCHI,
D. R. HODGSON, W. M. BAYLY, and P. D. GOLLNICK
Departments of Veterinary and Comparative Anatomy, Pharmacology, and
Physiology and Clinical Medicine and Surgery, College of Veterinary
Medicine, Washington State University, Pullman, Washington
99164-6520 USA

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.