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Metabolic Response to Racing Determined in Pools of Type I, II A and II B Fibers

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Summary

Histochemical and biochemical analysis of fiber pools of types I, II A and II B were performed on gluteus medius biopsies obtained, upon standstill after racing, from four Standardbred and four Thoroughbred horses. Within individuals, type I fibers possessed a lower lactate dehydrogenase (LDH) activity and a higher phosphagen level than type II fibers. The citrate synthase (CS) activity of type II B fibers was lower than that of type I and II A fibers but varied in individuals from being much lower than that in type II A fibers to almost similar levels. The lactate concentrations were 2- to 5-fold higher than reported resting concentrations and lactate levels in the three fiber pools of the same horse did not differ markedly. However, lactate, phosphagen and enzyme activities in fiber pools differed markedly between horses. The differences in lactate concentrations between horses for all fiber pools could be explained by the negative correlations between pool lactate levels and both the fiber pool oxidative capacity and the fiber type's capillarization. High lactate levels in fiber pools post race were associated with low ATP levels, particularly in type II B. It is concluded that in both Standardbred and Thoroughbred horses the oxidative capacity and capillarization of fiber types strongly influences the metabolic response to racing. Furthermore, the total number of the various fiber types that are available and subsequently recruited in muscle will influence the metabolic response to exercise. Therefore, with respect to the potential for high lactate and low adenosine triphosphate levels in type II B fibers, the metabolic profile of type II B fibers is especially important during racing when these fibers become recruited.

Index terms: Muscle; horse; fiber types; enzymes; lactate; adenosine triphosphate; creatine phosphate.

Introduction

Skeletal muscle is composed of fibers which possess different contractile and metabolic properties (Saltin and Gollnick, 1983). Functionally, these different fiber types, slow-twitch type I and fast-twitch type II A and II B, are selectively recruited in a specific pattern which varies according to the gait, speed and duration of exercise (Sullivan and Armstrong, 1978). In the horse, as the speed of exercise increases, fibers are recruited in the order, type I through II A to II B (Hodgson *et al.*, 1983; Valberg, 1986).

The fiber type composition of the gluteus muscle in the horse varies greatly between individuals and between breeds (Snow and Guy, 1980, Essén *et al* 1980). Horses with different muscle fiber composition show different metabolic responses to standardised near-maximal exercise (Valberg *et al.*, 1985). This has been interpreted to indicate that there are significant differences in the metabolic properties of the fiber types which are recruited during exercise. Since the type of metabolic response to exercise may be an integral part of the fatigue process during racing, this study was designed to investigate the metabolic response to racing in individual muscle fiber types from both Standardbred and Thoroughbred horses and relate this to the muscle fiber properties of each horse.

Materials and Methods

Horses. Four active Standardbred and four active Thoroughbred racehorses were examined in this study. A description of the horses and their racing performance is provided in Table 1. Since resting muscle samples were not available from the racehorses in this study, 8 resting samples were obtained from other 2-year-old Standardbred and Thoroughbred horses.

Muscle biopsies. Gluteus medius muscle biopsies (Lindholm and Piehl, 1974) were obtained from each horse upon standstill as soon after racing as possible (Table 1). All muscle biopsies were taken by the same person. The site of biopsy was located along a straight line drawn between the highest point of the tuber coxae and the head of the tail. A thumb was placed on the highest point of the tuber coxae and, with the hand in a relaxed position, the site of biopsy was established as the point where the little finger fell upon this line. The window of the biopsy needle was inserted to a depth of about 4 cm. Samples for histochemistry were rolled in talcum powder before being frozen whereas biochemical samples were frozen directly in liquid nitrogen. Biopsies were stored at -80°C until analyzed.

TABLE 1 The sex, age, distance raced, speed, placing in a race and time delay until post-race biopsy for four Standardbred (S) and four Thoroughbred horses (T).

Horse	Sex	Age	Race distance	Speed	Placing	Biopsy time post-race
		(years)	(m)	min:sec/km		(min)
1S	g	5	2140	broke stride	0	6
2S	m	4	2140	1:20	3	6.5
3S	g	7	2140	1:19	4	6.5
4S	s	6	2140	1:17	3	7
1T	s	5	2700	1:07	6	5
2T	g	4	1600	1:03	6	5
3T	m	4	1200	1:06	4	7
4T	g	6	2000	1:18	10	4.5

g = gelding, m = mare, s = stallion

Histochemistry. Samples for histochemistry were serially sectioned 10 μm thick in a cryostat microtome. Sections were preincubated at pH 4.6 for 5 min at room temperature and then stained for myofibrillar adenosine triphosphatase activity (ATPase) at pH of 9.4 in a 37°C waterbath for 30 min (Brooke and Kaiser, 1970). Muscle fiber compositions were determined from the ATPase stain by typing at least 200 fibers. Type I fibers stained darkly, type II A fibers lightly and type II B fibers intermediately in the ATPase stain.

Capillarization was determined from alpha amylase -PAS stainings of 20 μm thick muscle sections (Andersen, 1975). The number of capillaries per fiber were determined visually for approximately 100 fibers and recorded on photomicrographs in which fibers had been identified according to type.

Biochemistry. Resting samples were freeze-dried and dissected free from blood, fat and connective tissue. One to 2 mg of whole muscle samples were used for analyses. From post-race biopsies, single muscle fiber dissections were performed identically on two occasions, once to obtain fibers for enzyme analysis and again to obtain fibers for substrate and metabolite analysis. Samples for single fiber analyses were freeze-dried at -20°C and put under a dissecting microscope in a room where a humidity of 25% and temperature of 20°C was maintained. Blood, fat and connective tissue were dissected free from the biopsies. Approximately 200 single muscle fibers, varying in length between about 3 and 6 mm were teased free from the muscle samples. Two small portions of each fiber fragment were histochemically stained for myofibrillar ATPase activity according to Brooke and Kaiser (1970). A modification was made such that fiber pieces were incubated at 37°C in pH 10.3 for 20 min and in pH 4.6 at the same temperature for 5.5 min. Fibers staining white in pH 10.3 and black in pH 4.6 were classified as type I fibers, fibers staining black in pH 10.3 and brown in pH 4.6 were typed as II A and fragments staining black in both incubations were classified as type II B fibers. For each biopsy, all fiber fragments of one type were pooled and weighed on a Cahn 25 automatic electrobalance. Type I fiber pools ranged in weight from 9.8 to 70.6 μg and type II A and type II B fiber pools ranged from 40.3 to 161.9 μg . The pools of identified fiber fragments were homogenized ultrasonically in an ice-chilled 0.1M phosphate buffer pH 7.3. Citrate synthase (CS) and lactate dehydrogenase (LDH) activities were analysed fluorometrically (Essén Gustavsson and Henriksson, 1984). CS was chosen as a marker for oxidative capacity and LDH was chosen as an indicator of glycolytic capacity. Fiber pools for substrate and metabolite analysis were soaked in an ice-chilled perchloric acid solution (1.5M) and subsequently cold centrifuged. Ice-chilled potassium bicarbonate (2M) was added to the supernatant and samples were allowed to sediment before analysis. Lactate, glucose-6-phosphate (G-6-P), creatine phosphate (CP) and adenosine triphosphate (ATP) were analyzed according to Lowry and Passonneau (1973).

Statistics. Analysis of variance was used to investigate differences in fiber properties. Correlation coefficients were calculated according to the correlations procedure of the Statistical Analysis Systems (SAS Institute 1982).

Results

Table 2 shows the values for fiber composition, lactate, G-6-P, ATP and CP for resting biopsies. Table 3 presents the muscle fiber compositions, enzyme activities in fiber

TABLE 2. The muscle fiber composition and concentrations of lactate, glucose 6 phosphate (G-6-P), adenosine triphosphate (ATP) and creatine phosphate (CP) in biopsies from 5 Standardbred (S) and 3 Thoroughbred (T) horses at rest

Horse	Fiber Composition			Lactate	Resting Muscle		
	I	IIA	IIB		G-6-P	ATP	CP
	(%)				(mmol/kg)		
S	44	15	41	8	—	24	59
S	40	52	8	15	2.5	25	—
S	32	33	35	17	1.9	24	—
S	26	39	35	18	0.5	25	—
S	20	48	32	16	3.1	28	—
T	17	69	14	12	—	25	60
T	6	19	75	35	—	24	59
T	6	17	77	25	—	25	72

pools, the mean number of capillaries per fiber type and the metabolite and substrate concentrations in the fiber pools post-race for the racehorses in this study.

Breed Differences. The Standardbred horses possessed a higher percentage of type I fibers ($P < 0.05$) and a lower percentage of type II B fibers ($P < 0.05$) than the Thoroughbred horses. No absolute difference in enzyme activities or metabolic response to exercise in the three fiber types were obvious between Standardbred and Thoroughbred horses (Table 3). Standardbred horses, similar to Thoroughbred horses, could possess high lactate and low ATP concentrations in fiber pools of both type II A and II B fibers. However, the highest CS activity and lowest G-6-P and lactate concentrations were found in fiber pools of type I, II A and II B fibers from Standardbred horses and the lowest CS activity and ATP concentrations and highest G-6-P and lactate levels were found in fiber pools of type I, II A and II B fibers from Thoroughbred horses.

Enzyme activities and fiber types. Analysis of variance revealed that the glycolytic capacity of type I fibers was significantly lower ($P < 0.01$) than that of type II A fibers and that the CS activity of type I fibers was not significantly greater than that of type II A fibers. Type I fibers also possessed significantly higher CS activity ($P < 0.01$) and lower LDH activity ($P < 0.001$) than type II B fibers. The CS activity of type II A fibers was significantly greater ($P < 0.05$) and the LDH activity significantly lower ($P < 0.05$) than that of type II B fibers.

Within individuals, horses which possessed a high CS activity in type I fibers also possessed a relatively high CS activity in type II A fibers. However, the magnitude of difference in CS activity between type II A and II B fibers could vary greatly such that type II B fibers possessed almost similar levels to type II A fibers in some horses (1T) and half as much CS activity as type II A fibers in other horses (1S). When comparing the enzyme activities of the same fiber type from different horses no characteristic enzyme activity could be attributed to a particular fiber type. For example, the CS activity in type II B fibers from one horse (2S) could be greater than that in the type I fibers in another horse (4T).

The metabolic response of fiber types to racing. A wide range in metabolic response

TABLE 3. Muscle fiber composition, fiber pool enzyme activities, number of capillaries per fiber and post-race concentrations of glucose 6 phosphate (G-6-P), lactate, adenosine triphosphate (ATP) and creatine phosphate (CP) for four Standardbred (S) and four Thoroughbred (T) horses

Horse	Fiber		Enzyme Activity		Cap/fib	G-6-P	Lactate	ATP	CP
	Type	Composition	Fiber Pools ($\mu\text{mol/g/min}$)						
		(%)	CS	LDH			(mmol/kg d. w.)		
1S	I	34	44	305	5.7	0.4	47	23	—
	IIA	52	40	743	6.7	0.7	39	23	46
	IIB	14	22	806	7.1	0.3	40	23	75
2S	I	26	42	616	5.8	4.0	71	22	63
	IIA	41	43	694	6.3	8.9	75	18	74
	IIB	33	35	906	7.1	11.5	74	16	31
3S	I	22	34	367	—	13.3	102	21	47
	IIA	50	24	718	—	12.2	109	15	30
	IIB	28	14	805	—	11.4	112	12	30
4S	I	16	28	722	5.0	4.4	118	19	—
	IIA	49	31	627	6.4	6.8	113	13	93
	IIB	35	20	835	5.5	8.6	116	18	82
1T	I	17	37	640	6.0	12.7	—	29	61
	IIA	51	35	662	7.1	13.5	66	18	41
	IIB	32	33	705	6.7	14.0	56	17	46
2T	I	13	42	—	6.2	—	—	—	—
	IIA	45	31	683	5.6	19.6	121	12	—
	IIB	42	26	725	5.4	17.5	124	11	46
3T	I	6	25	574	—	—	—	—	—
	IIA	43	26	786	—	8.2	100	17	38
	IIB	51	12	1078	—	11.1	113	14	17
4T	I	16	20	640	4.4	15.6	109	17	82
	IIA	42	14	622	5.5	20.3	126	18	63
	IIB	42	11	930	5.4	17.4	143	14	58

was recorded in the fiber types of different horses. Within individuals slight differences in substrate and metabolite concentrations were found between the fiber types. Due to the low percentage of type I fibers in Thoroughbred horses, pools of type I fibers possessed weights which were too low to permit measurement of substrate and metabolite levels in three horses. Lactate and G-6-P levels ranged from very low concentrations post race in some horses to very high concentrations in the fiber type pools of other horses. Within the three fiber types of individual horses, lactate levels tended to be quite similar whereas G-6-P was found in lower concentrations in the type I fiber pools

TABLE 4. Correlation matrix (r values) for enzymes citrate synthase (CS) and lactate dehydrogenase (LDH) and concentrations of lactate, adenosine triphosphate (ATP) and creatine phosphate (CP) after racing

	CS	LDH	G-6-P	Lactate	ATP	CP
Cap/Fib	ns	ns	ns	-.69**	ns	ns
CS		-.54**	-.43*	-.67†	.51**	ns
LDH			ns	ns	-.44*	ns
G-6-P				.67†	-.50*	ns
Lactate					-.71†	ns
ATP						ns

Cap/Fib = capillaries per muscle fiber

*P < 0.05 **P < 0.01 †P < 0.001

Discussion

In agreement with previous histochemical evaluations of fiber type oxidative and glycolytic capacities in the horse, (Essén *et al.*, 1980; Snow, 1983) type I fibers were less glycolytic than type II A fibers within individuals and these two fiber types were found

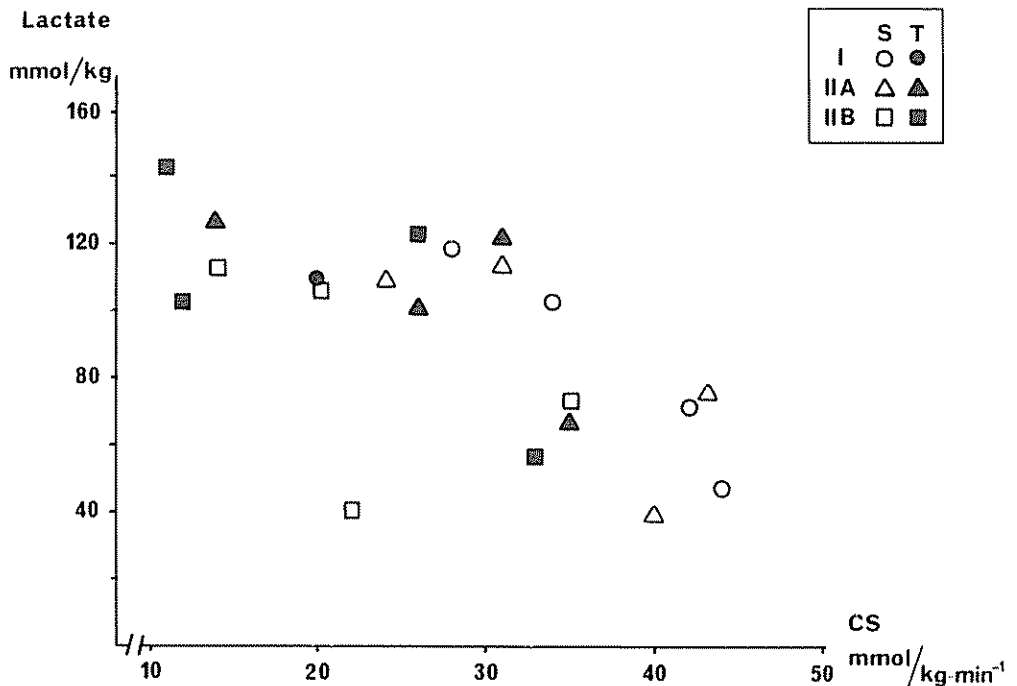


FIGURE 2 Relationship between the citrate synthase (CS) activity in a fiber type pool and the lactate accumulation in that fiber pool for both Standardbred (S) and Thoroughbred (T) horses

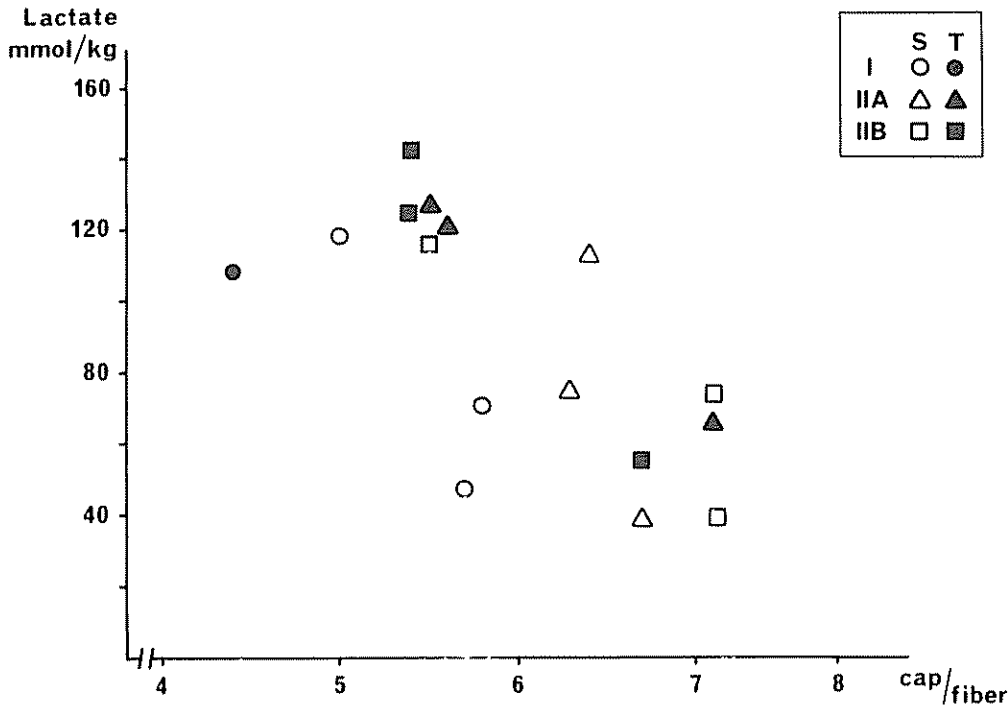


FIGURE 3. Relationship between the mean number of capillaries surrounding a fiber type (cap/fiber) and the lactate accumulation in a fiber type pool for both Standardbred (S) and Thoroughbred (T) horses.

to be more oxidative and less glycolytic than type II B fibers. It is of particular importance to note from this study that the properties of a particular fiber type vary greatly between horses and so could the magnitude of difference in oxidative capacity between the fiber types of individual horses.

Resting samples indicated that CP, ATP and metabolite concentrations are quite similar between fiber types at rest since the percentage of type I fibers could range between 6 and 44%, type II A fibers between 15 and 69% and type II B fibers 8 and 77% without differences being found in the whole muscle phosphagen or metabolite concentrations. After racing, lactate concentrations were higher than resting values in all horses. In spite of differences in metabolic capacity between fiber types, only small differences in lactate accumulation during racing were found between the fiber types of individual horses. One reason for this may be that lactate is a highly diffusible molecule (Danishefsky, 1980) which might therefore have diffused from one fiber type where it was produced into other fiber types. The time delay from the races conclusion to biopsy may have facilitated this process. Another explanation for small differences in metabolic response between fiber types may be that the metabolic response was measured in pools of fibers of the same type and not in individual fibers. This method provides only an average assessment of the metabolic response in a given fiber type. If a proportion of fibers in a fiber pool were not recruited, as may have been the case

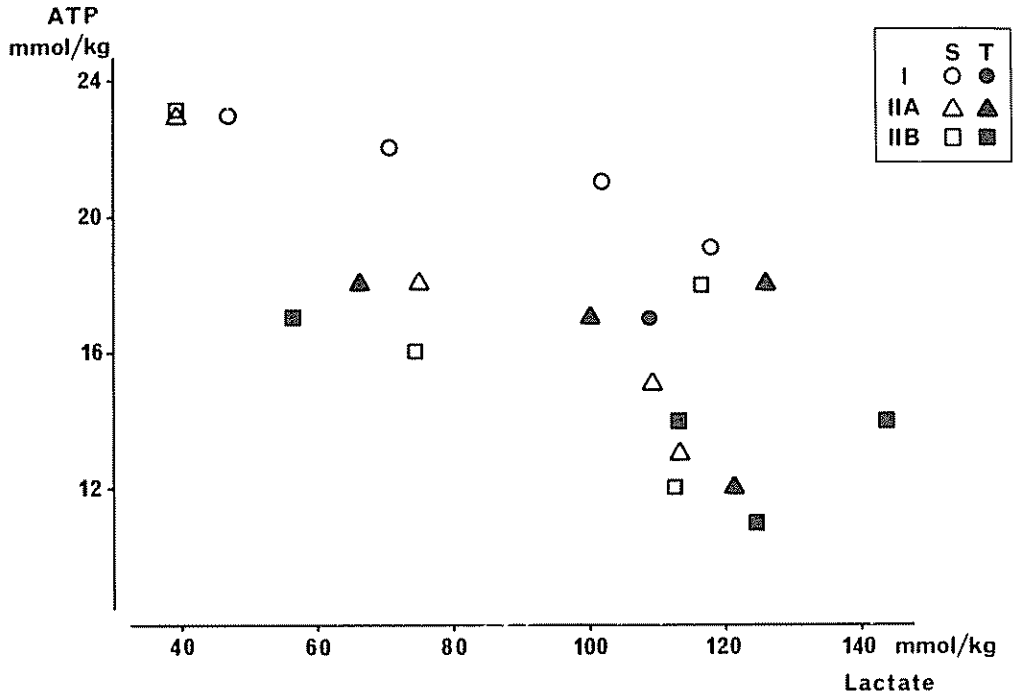


FIGURE 4. Relationship between muscle lactate accumulation in a fiber type pool and the ATP level in those fiber pools for both Standardbred (S) and Thoroughbred (T) horses.

in some type II B fiber pools, then the accumulation of metabolites and utilization of substrates in those fibers which were actually active in the fiber pool may have been underestimated since fiber pools would include fibers which had resting substrate and metabolite levels and fibers which had decreased substrate and increased metabolite levels. This may account for the small differences seen between type II A and II B fiber pools.

After racing, G-6-P concentrations in fiber pools were higher than values found in resting horses and ATP and CP in fiber pools were lower than the reported resting concentrations in all racehorses except 1S (Table 2 and 3). Furthermore, differences were seen in the levels of phosphagen and G-6-P concentrations between fiber types. The lower G-6-P and higher CP and ATP levels in type I fiber pools of most racehorses and the negative correlation between the percentage of type I fibers in a biopsy and the lactate accumulation in all fiber pools (Fig. 1) indicate that, in agreement with their metabolic profile, the rate of glycolysis in type I fibers was likely less than that in type II fibers. Furthermore, the low ATP concentrations in type II B fibers and the positive correlation between the percentage of type II B fibers in a biopsy and the lactate accumulation in all fiber pools indicates that in agreement with their metabolic profile type II B fibers probably had a high rate of glycolysis during racing.

The different metabolic responses to racing found between fiber types and between the muscle fibers of different horses in this study could be accounted for by apparent

differences in recruitment, by differences in the number of capillaries surrounding muscle fibers and by differences in the CS activity of the fiber types.

Horses which possessed many type I and few type II B fibers accumulated very little lactate in fiber pools after racing (Fig. 1). Horse 1S for example, which possessed very few type II B fibers showed very low lactate and G-6-P concentrations in type II B fibers post race in spite of a low oxidative capacity in these fibers (Fig. 2). Thus this horse did not appear to recruit type II B fibers during the race. In contrast, those horses which possessed a high percentage of type II B fibers showed a high lactate and G-6-P accumulation in type II B fibers after racing (Fig. 1) suggesting that they recruited a high proportion of the type II B fibers.

Fibers which possessed a low capillary density (Fig. 3) or a low oxidative capacity (Fig. 2) had high lactate concentrations after racing and in association with high lactate concentrations low ATP levels were found. Both high lactate concentrations and low ATP concentrations are suggested to be factors which cause muscle fatigue (Sahlin, 1983; Harris, 1985). The low ATP concentrations found post race may have been due to a lactic acidosis inhibiting rate limiting enzymes of glycolysis or may have resulted from stimulation of the enzyme AMP deaminase due to a decrease in pH in combination with high concentrations of ADP and AMP, produced with rapid myofibrillar contractions (Snow, *et al.*, 1985). The enzyme AMP deaminase has recently been found to have very high activity in the gluteus muscle of the horse (Cutmore *et al.*, 1986). The deamination of AMP to IMP with ammonia formation depletes the total adenine nucleotide pool in fibers and therefore the ability to replenish ATP (Harris, 1985). In a further study, high muscle ammonia levels were found in horses post race and these levels were positively correlated to the percentage of type II fibers (Essén-Gustavsson and Valberg, 1987). Reamination is a slow process which could also explain the fact that ATP levels were low 7 minutes after racing (Snow *et al.*, 1985).

It appears therefore that the time of onset of lactate accumulation and ATP depletion during the course of a race may be of great importance to a race's outcome. It would obviously be of benefit to delay lactate accumulation and ensuing ATP depletion until the later portion of a race. Horses which possessed a high oxidative capacity in fiber types appeared to maintain high ATP levels throughout the race. The rate of ATP production through oxidative phosphorylation is much less than that through glycolysis (McGilvery, 1975) and therefore it was interesting to note that both Standardbred and Thoroughbred horses possessing a high oxidative capacity were able to race without accumulating lactate.

In conclusion, the oxidative capacity and capillarization of a fiber and the abundance of a fiber type available and subsequently recruited are important for the metabolic response to racing and subsequent fatigue processes. Furthermore, with respect to the low ATP concentrations in type II B fibers and the fact that type II B fibers are recruited at maximum speeds the metabolic profile and time of recruitment of this fiber type must be of crucial importance for racing performance in both Standardbred and Thoroughbred horses.

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Responses to Repeated High Intensity Exercise: Influence on Muscle Metabolism

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Summary

Eight Thoroughbred horses performed four, 600-m exercise bouts at near maximal speeds with 5 min rest between exercise sessions. Blood and muscle samples were collected at rest, before and after each exercise period, and 5 and 10 min following the final exercise bout. Muscle pH decreased from 7.03 at rest to 6.82 after the first exercise and thereafter slowly declined to 6.57 after the final exercise session. Glucose-6-phosphate (G-6-P) concentration increased 3.2-fold after the first exercise session and then declined with additional exercise bouts to 2-fold greater than the pre-exercise value after the final exercise bout. Following the final period of exercise, peak plasma and muscle lactate concentrations were 20- and 9-fold greater than the respective values measured at rest. The change in muscle and plasma lactate and G-6-P suggests that reduced pH caused little or no inhibition of glycolysis during exercise. Pronounced decreases in muscle creatine phosphate (CP), and glycogen, and an increase in creatine occurred after the first exercise bout. Subsequent changes were less. After the fourth exercise period, CP and adenosine triphosphate concentrations were 35 and 32% below the concentrations measured at rest. Although all metabolites, except glycogen, returned toward resting values in the post-exercise period, they had not reached control values by 10 min post-exercise.

Index terms. Muscle metabolites; pH.

Introduction

Horses engage in a variety of physical activities. Some efforts are sustainable for several hours whereas others lead to fatigue in 1 min or less. In all cases, decrements in performance signal the onset of fatigue. During muscular activity the metabolic rate of the active skeletal muscle can increase several hundredfold over that of rest (Åstrand and Rodahl, 1986) in an attempt to maintain a constant concentration of adenosine triphosphate (ATP) required for excitation-contraction coupling. Extensive effort has been devoted to determining factors responsible for skeletal muscle fatigue (Edwards, 1983). Considerable attention has been directed towards understanding the importance of in-