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Effects of Maximal Exercise on Equine Muscle: Changes in Metabolites, pH and Temperature

D. K. LOVELL, T.-A. REID and R. J. ROSE
Department of Veterinary Clinical Studies, University of Sydney,
NSW 2006, Australia

Summary

To study the metabolic response of muscle to different durations of high intensity exercise, five Standardbred horses were exercised on an inclined treadmill at a speed of 12.5 m/sec. Prior to and within 15 sec of the completion of exercise, muscle biopsies were collected from the middle gluteal muscle and immediately frozen in liquid nitrogen. Muscle temperature was measured immediately after biopsy collection and rectal temperature was also measured. Five different exercise durations were given on five separate days, with a period of 72 h between each exercise day. The exercise durations used were 20, 40, 60, 90 and 120 sec. The following metabolite concentrations were measured in freeze dried muscle: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (PCr), creatine, citrate, lactate and nicotine adenine dinucleotide (NAD). Muscle pH was also measured on frozen but not freeze-dried muscle samples.

Significant effects of exercise duration were found for NAD concentration, lactate concentration, muscle temperature and muscle pH. Significant correlations were found between blood lactate and muscle pH ($r = -0.716$), muscle lactate and pH ($r = -0.778$), muscle temperature and muscle lactate ($r = 0.582$) and muscle lactate and exercise duration ($r = 0.618$). No significant changes in ATP were found, but ADP was significantly decreased after 120 sec of exercise. The PCr values had decreased by almost 80% after 20 sec exercise, after which there was little further change with exercise duration.

Index terms: Adenine nucleotides; lactate; muscle pH; muscle temperature; NAD; citrate; creatine phosphate.

Introduction

At the onset of high-intensity exercise, there is an immediate demand for energy to supply the contractile process in muscle. While the energy sources for muscular work are well known: adenosine triphosphate (ATP) hydrolysis, hydrolysis of high energy phosphates, anaerobic fermentations and oxidative metabolism (Hochachka, 1985), the interrelationships of these processes differ between species.

The first study on ATP and phosphorylcreatine (PCr) changes in muscle during exercise was performed in man by Hultman *et al.* (1967). While ATP concentration decreased slightly early in exercise, the concentrations tended to return to resting levels as the exercise continued. However, PCr concentration decreased rapidly and remained low throughout exercise. Subsequent studies in man confirmed these changes, although decreases in ATP concentration of up to 35% have been reported after maximal exercise (Hultman *et al.*, 1967; Karlsson, 1971; Essén, 1978; Cheetham *et al.*, 1985; McCartney *et al.*, 1985).

Few studies have investigated the metabolic response of muscle to exercise in the horse. Lindholm and Saltin (1974) reported a large increase in lactate concentration, together with a small decrease in ATP concentration, following maximal exercise in Standardbred trotters on the racetrack. Nimmo and Snow (1983) found increases in lactate concentration but no change in muscle pyruvate in Thoroughbred horses exercising over distances from 506 to 1600 m. More recently Snow *et al.* (1985) have reported large decreases in ATP and increases in inosine −5′ monophosphate (IMP) concentrations after repeated bouts of maximal exercise in Thoroughbred horses. They associated these changes with exercise fatigue.

With this background, the aim of our study was to examine the metabolic response of muscle to different durations of maximal exercise. In this way, the energy sources at the onset of maximal exercise could be evaluated, and an indication of the metabolic limitations for continued high intensity work could be gained.

Materials and Methods

Five Standardbred horses, 4 geldings and 1 mare, aged between 4 and 9 years, were used in the study. None of the horses had been in training for the three months prior to the investigation. Immediately prior to exercise, a muscle biopsy was collected by the technique of Lindholm and Piehl (1974) using a percutaneous needle (Stille, Stockholm, Sweden). The muscle sample was frozen in liquid nitrogen within 5 sec of collection. Following the biopsy, rectal temperature was measured and muscle temperature was determined with an electronic thermometer (Model 43 TF, Yellow Springs Instruments, Ohio), by inserting a probe to a depth of 7.5 cm into the biopsy site.

The horses were exercised on a high speed treadmill (Equitred, Ipswich, Australia) set at a slope of 6° (10%). To standardize the exercise protocol, the treadmill acceleration, speed and exercise duration were controlled by computer. The program used was 10 sec exercise at 3 m/sec, followed by acceleration to the maximal speed of 12.5 m/sec. The acceleration time required to reach 12.5 m/sec was 20 sec. The timing of exercise duration began when the horse reached maximal speed. At the end of exercise, the computer shut off the treadmill and the emergency stop brought the horse to an immediate standstill. The postexercise muscle biopsy was collected as quickly as possible after exercise from a second pre-prepared site. Muscle and rectal temperature measurements were repeated. Three minutes after exercise, jugular venous blood samples were collected into chilled perchloric acid, for measurement of lactate concentration using the technique of Noll (1966).

Horses were exercised for five different time periods (20, 40, 60, 90 and 120 sec) on five separate days. Exercise bouts were separated by 72 hours.

The frozen muscle from the pre and post exercise muscle biopsies was divided into

three pieces. Two small pieces, 30–60 mg wet weight, were used for duplicate pH measurements and one large piece for metabolite analyses. For pH measurement, a sample was weighed at 0°C and homogenized in a 1 ml glass homogenizer (Wheaton, USA). The homogenization medium consisted of 145 mM KCl, 10 mM NaCl and 5 mM iodoacetate and the homogenate was allowed to stand in ice for 3 min, then brought to 37°C for pH determination with a MI-410 pH microelectrode (Microelectrodes Inc., New Hampshire), connected to a Radiometer pH meter (pH M8, Copenhagen, Denmark). For metabolite assays, a 5–10 mg sample of freeze-dried muscle, dissected free of blood and fibrous tissue was used. After weighing, the sample was homogenized at −10°C in an extraction medium of 3M perchloric acid and centrifuged. The supernatant was neutralized with 3M potassium hydroxide and recentrifuged. The supernatant was frozen in liquid nitrogen for subsequent analyses. The metabolites, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), PCr, creatine, nicotine adenine dinucleotide (NAD), lactate and citrate, were measured fluorimetrically (Perkin-Elmer, Model 1000 M, Illinois) using methods described by Lowry and Passonneau (1972).

Statistical analysis was undertaken by comparing the pre and post exercise values on each experimental day using a paired t test. To assess the effects of duration of exercise, a one-way analysis of variance was performed on pre-exercise samples and where the F values were not significant, a one-way analysis of variance undertaken on the post-exercise values for each metabolite to determine the effect of exercise duration. Correlation coefficients were determined for postexercise muscle and rectal temperature, muscle pH and blood lactate, muscle pH and muscle lactate, muscle temperature and blood lactate, muscle temperature and exercise duration and muscle lactate and exercise duration, using linear regression analysis.

Results

The pre and postexercise values for muscle metabolites, pH, muscle temperature and rectal temperature on the five experimental days are presented in Table 1. The post-exercise blood lactate values are presented in Table 2. The time taken for muscle sample collection, from the time that the treadmill was turned off until the sample was in liquid nitrogen, was 15.2 ± 5.6 sec (mean ± SD). Significant effects of exercise duration were found for NAD, lactate, muscle temperature and muscle pH. The correlation coefficients were as follows: muscle and rectal temperature $r = 0.020$ (ns), muscle temperature and exercise duration $r = 0.839$ ($P < 0.001$), muscle temperature and blood lactate $r = 0.663$ ($P < 0.001$), blood lactate and muscle pH $r = -0.716$ ($P < 0.001$), muscle temperature and muscle lactate $r = 0.582$ ($P < 0.01$), muscle lactate and exercise duration $r = 0.618$ ($P < 0.001$), pH and muscle lactate $r = -0.778$ ($P < 0.001$).

The analysis of variance revealed significant differences in the pre exercise values of the following muscle metabolites: ATP, ADP, AMP, NAD and lactate. Thus a simple post exercise analysis of variance could not be undertaken for these metabolites. The analysis of variance for post exercise values of PCr, creatine and citrate showed no significant effects of exercise duration.

Discussion

Previous studies of the metabolic responses of equine skeletal muscle to maximal exercise have been carried out on horses exercising on the track (Snow *et al.*, 1985;

Lindholm and Saltin, 1974; Nimmo and Snow 1983). Time therefore elapsed, as the horse decelerated and returned for biopsy. Values obtained on the track may not therefore reflect the values in the muscle during the actual work effort. The present trial allowed us to sample as close as possible to the termination of exercise. These samples more accurately reflect the metabolic status of muscle at the end of exercise.

Previous experience with unfit horses had shown that 120 sec of maximal speed on a treadmill with a 10% slope was as much work as the horses could sustain. During the different durations of exercise in this study, considerable urging on the part of the operators was required to encourage the horses to maintain their speed. After 90 sec of maximal exercise our clinical impression was that fatigue began to limit the work capability, since the horses required considerable urging to keep off the restraining bar at the back of the treadmill.

Our results on PCr breakdown during exercise are similar to the findings in man (Hultman *et al.*, 1967; McCartney *et al.*, 1985) and suggest that this reaction occurs very rapidly. However, the PCr reaction has only a limited ability to sustain high intensity exercise. In this study, the depletion of PCr and accumulation of creatine appeared to be almost maximal after 20 sec of exercise. Little further change in concentrations occurred as the duration of work increased and there was no correlation between PCr and exercise duration. Total creatine (PCr + creatine) values in these assays remained quite constant before and after exercise, indicating that the fall in PCr corresponded to the rise in creatine, with no net loss occurring. Decreases in PCr of approximately 50% (Lindholm and Saltin, 1974) and 60–65% (Snow *et al.*, 1985) have been reported after maximal exercise in the horse. The greater percentage decrease (85–90%) in our study is probably the result of obtaining the muscle sample very close to the cessation of the exercise. In other studies, it is likely that some resynthesis of PCr had occurred in the period between the completion of exercise and the collection of samples. In investigations conducted in human subjects (Hultman *et al.*, 1967; McCartney *et al.*, 1985), low levels of PCr in muscle were also found when samples were collected very close to the termination of maximal exercise.

Levels of ATP in the muscle of the horse, although showing a tendency to fall, showed no significant decrease with increasing duration of exercise. This is in direct contrast to the somewhat spectacular decreases reported by Snow *et al.* (1985) who studied repeated exercise bouts. Our results indicate that under conditions of single bout maximal exercise on a treadmill, ATP resynthesis almost complemented usage. This is certainly a very desirable metabolic situation, as continued activity must depend on the maintenance of steady state ATP levels at the muscle motor unit. Falling levels of ATP would imply an inability of metabolism to maintain supply and so would indicate a very demanding work effort. Such a work load would be unlikely to be maintained for any length of time as confirmed by Snow *et al.* (1985). One of the four horses in their study showed little change in ATP and this horse's time for the first heat was slower than the times for the other three horses. However, it was able to improve its performance over the next three heats, while that of the other horses declined sharply. These other horses all showed quite large ATP decreases which even after a 30 min recovery period had not returned to resting values. This emphasizes the very important factor of specificity of work effort and different effects on the body of even slightly different activities. The horses in the study of Snow *et al.* (1985) were galloped on a heavy track with a rider's weight on their backs. Our horses were galloping uphill on a treadmill

TABLE 1 Changes in Muscle Metabolites, pH, Temperature and Rectal Temperature (mean

	Experimental Day/			
Metabolites, (μmol/g D W)	Day 1 20 sec		Day 2 40 sec	
	Pre	Post	Pre	Post
ATP	26.8 ± 2.9	26.7 ± 3.7	25.5 ± 2.6	25.1 ± 2.5
ADP	4.2 ± 0.2	3.7 ± 0.4	4.2 ± 0.5	4.1 ± 0.3
AMP	0.93 ± 0.22	0.69** ± 0.25	0.80 ± 0.13	0.61 ± 0.12
Creatine	55.0 ± 10.0	103.0** ± 7.0	47.0 ± 6.0	95.0† ± 9.0
Creatine P	60.0 ± 6.0	14.0† ± 5.0	52.0 ± 3.0	7.3† ± 3.0
Citrate	0.6 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
Lactate	9.8 ± 1.8	81.0† ± 17.0	7.0 ± 3.0	115.0† ± 26.0
NAD	2.5 ± 0.2	2.3* ± 0.3	1.6 ± 0.2	1.3 ± 0.07
pH (units)	6.90 ± 0.03	6.64 ± 0.06	6.98 ± 0.03	6.63† ± 0.05
Muscle temperature (°C)	37.0 ± 0.3	38.3† ± 0.2	37.2 ± 0.3	38.8† ± 0.5
Rectal temperature (°C)	37.3 ± 0.1	38.5 ± 0.2	37.3 ± 0.2	38.3 ± 0.2

Significant differences when compared to pre-exercise values on same day
$^*P < 0.05$
$^{**}P < 0.01$
$^\dagger P < 0.001$

with no weight on their backs. They were obviously fatigued at the end of the longer exercise durations, but did not show the metabolic exhaustion reported by Snow *et al.* (1985). The maximum speed of our treadmill was 12.5 m/sec, which we had previously found to be the speed limit of untrained Standardbred horses at the same treadmill inclination. From other studies of oxygen consumption in these horses (Evans and Rose, unpublished data), this exercise intensity was equivalent to approximately 130% of maximal oxygen consumption. In the report of Lindholm and Saltin (1974), in horses after a race, there was a decrease in muscle ATP at the sampling time, two min after exercise. These horses had trotted for 2100 m at 11.8 m/sec pulling a sulky and driver under race conditions and had been trained for such an effort. Maximal exhaustive work efforts in humans also produce moderate decreases in ATP (Hultman *et al.*, 1967; Karlsson, 1971; Essén, 1978; McCartney *et al.*, 1985; Cheetham *et al.*, 1985). Hultman *et al.* (1967) and Karlsson (1971) found that slightly lower work efforts failed to produce consistent changes in ATP but that once the effort was maximal, moderate decreases occurred. Whether the decrease in ATP is itself limiting to maximal exercise or whether the limitation is from some other factor needs further elucidation. However, it is clear from the present study that fatigue can occur without associated decreases in muscle ATP concentrations in horses exercising at supramaximal exercise intensities.

Levels of ADP also showed a statistically significant decrease at the longest duration of exercise. However, the extent of the decrease was only small and may have been influenced to some extent by inevitable blood contamination, despite careful dissection of the freeze dried samples. Some day-to-day variations in the resting muscle metabolite concentrations were found. These are most likely to be the result of methodological errors, particularly with measurement of ADP and AMP by fluorimetric techniques. The small changes in fluorescence from these metabolites results in some imprecision in their determination. Overall, our results showed relatively stable levels of muscle

± SD) associated with different durations of maximal exercise.

Exercise Duration					
Day 3 60 sec		Day 4 90 sec		Day 5 120 sec	
Pre	Post	Pre	Post	Pre	Post
20.5 ± 1.8	19.9 ± 2.7	24.1 ± 2.3	22.0 ± 3.0	21.3 ± 2.3	18.1 ± 1.6
5.9 ± 0.8	4.9 ± 0.2	4.6 ± 1.1	3.9 ± 0.7	4.4 ± 0.5	2.9** ± 0.4
1.08 ± 0.25	0.56† ± 0.15	0.75 ± 0.04	0.65 ± 0.11	0.67 ± 0.20	0.61 ± 0.15
50.0 ± 6.0	96.0** ± 14.0	51.5 ± 3.8	106.0† ± 6.0	46.0 ± 8.0	91.0† ± 16.0
59.0 ± 5.0	9.3† ± 2.9	55.0 ± 7.0	11.0† ± 6.0	58.0 ± 7.0	7.5† ± 1.7
0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
7.2 ± 2.7	123.0† ± 31.0	11.7 ± 2.7	119.0** ± 20.0	4.4 ± 1.3	139.0† ± 18.0
1.9 ± 0.2	1.9 ± 0.06	2.7 ± 0.3	2.6 ± 0.3	2.2 ± 0.5	1.3** ± 0.2
6.99 ± 0.03	6.51† ± 0.06	6.96 ± 0.03	6.53† ± 0.07	7.02 ± 0.03	6.50† ± 0.03
37.4 ± 0.4	39.1† ± 0.4	36.9 ± 0.2	39.5† ± 0.4	37.1 ± 0.3	39.8† ± 0.5
37.6 ± 0.2	38.1 ± 0.4	37.2 ± 0.1	38.5 ± 0.2	37.4 ± 0.3	38.1 ± 0.3

AMP, further confirming that changes in adenine nucleotides were not major limiting factors in the horses exercising under these circumstances.

Citrate concentration showed no significant change with the different durations of exercise. Citrate may be important as a metabolic regulator during recovery from exercise. Essén (1978) reported increases in citrate concentration in man during the recovery period between exercise bouts. However, its effect during exercise is less certain.

At the greatest exercise duration, NAD values decreased in muscle. While this may be an artifact associated with blood contamination, similar results have been reported from studies in man (Graham *et al.*, 1978) at both 75 and 100% maximal oxygen uptake. This decrease was thought to be related to an increase in muscle water content. Graham *et al.* (1978) found no correlation between NAD values and lactate. In our study, we were also unable to demonstrate a correlation between lactate and NAD.

The requirement for rapid energy production by skeletal muscle places immediate demands upon glycolysis. The importance of this mechanism, even in the early stages of exercise, is shown by the finding of high levels of lactate in muscle after only 20 sec of maximal work. Further increases in lactate occurred with increasing duration of exercise. Peak lactate levels in both blood and muscle occurred after 120 sec of exercise. At this stage the blood lactate values were similar to those in Thoroughbred

TABLE 2. Mean blood lactate values (±SD) three minutes after different durations of maximal exercise.

Exercise duration (sec)	Blood lactate (mM)
20	10.6 ± 2.3
40	15.9 ± 3.9
60	21.2 ± 1.6
90	22.3 ± 2.2
120	25.6 ± 3.7

horses after maximal exercise over distances of 1000–1600 m (Kubo *et al.*, 1984). The peak muscle lactate values in our study are higher than those previously reported by Nimmo and Snow (1983), Lindholm and Saltin (1974) and Snow *et al.* (1985) after single exercise bouts. The mean lactate values in these studies were in the range 80–110 umol/g dry weight, which were on average 10–40 umol/g below the maximum values in our study. Part of this difference could be due to the quicker sample collection in our study, but there could also be differences due to our horses exercising on an inclined treadmill. Thus, although the exercise speed in our study was similar to that in the report of Nimmo and Snow (1983), the 6° incline considerably increased the workload.

Muscle pH was decreased with the onset of exercise, and was inversely correlated with muscle lactate as has also been reported by Harris *et al.* (1984). It is interesting, however, that there was little change in pH from 90 to 120 sec of exercise, despite the horses' obvious fatigue, demonstrated by difficulty in maintaining treadmill speed. This could bring into question the role of decreasing pH per se as a major limiting factor to high intensity exercise (Terjung *et al.*, 1985). In two different exercise regimes in man, exercise to exhaustion in 2 min resulted in a muscle pH of 6.46 (Hermansen and Osnes, 1972), whereas exhaustive exercise over 6–11 min resulted in a pH of 6.60 (Sahlin *et al.* 1976). It is therefore apparent that some other factor, apart from pH, is responsible for limiting work effort during high intensity exercise. It should be noted, however, that the horses in our study were unfit and it may be that one of the effects of training could be adaptations that allow muscle function to occur at a lower pH. The other possibility is that training induces an improvement in the buffering capacity of muscle (Parkhouse and McKenzie, 1984), so that the pH change induced by a certain increase in lactate would be less. Training may also improve the efficiency of handling of lactate so that its rate of removal may be enhanced, particularly as a substrate for other more aerobic muscle fibers contributing to the work effort.

Considerable heat is produced during muscle contraction (Shepherd, 1982). A strong positive correlation was found between exercise duration and muscle temperature. A similar positive relationship was found between muscle temperature and exercise intensity in Standardbred horses exercising on the racetrack (Lindholm and Saltin, 1974). No correlation was found between muscle and rectal temperature, indicating that rectal temperature is not a good indicator of metabolic heat load, during or after maximal exercise. The highest muscle temperatures in our study are similar to those reported by Lindholm and Saltin (1974) after a 700 m exercise bout at a speed of 12.5 m/sec. With repeated exercise bouts these authors reported that there was a gradual increase in muscle temperature so that after 6 × 700 m intervals of work, the temperature was approximately 43°C. These high temperatures may limit performance, by adverse effects on muscle metabolism (Kozlowski *et al.*, 1985) or via central homeostatic mechanisms involving neuromuscular control (Shepherd, 1982).

This study provides some insight into the adaptations of muscle to sudden onset, intense exercise. The use of a treadmill provides a mechanism whereby samples can be rapidly collected and so metabolites can be preserved. From this study it is apparent that the limitations to continued exercise can be due to a variety of metabolic disturbances. It would seem likely, however, that no one single factor such as ATP depletion, pH decrease or increase in muscle temperature can be implicated in fatigue. Further studies at different exercise intensities and durations, with horses being worked until

they are incapable of maintaining their speed on the treadmill, are necessary to elucidate this problem

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