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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-

transmuscular substrates such as glycogen, ATP, and creatine phosphate (CP), and the delivery of oxygen and substrates by the blood (Karlsson and Saltin, 1971). In addition, the concentrations of a number of other metabolic intermediates and end products have been determined following different forms of exercise.

An inadequate energy source, particularly depletion of the muscle carbohydrate store, is believed to limit work capacity during moderately severe, prolonged exercise (65–85% of the maximal oxygen uptake [$\dot{V}O_{2max}$]) (Saltin and Gollnick, 1983). This form of exercise often results in a decline in muscular CP, whereas ATP and lactate (LA) concentrations may remain relatively unchanged. With short-term maximal exercise there may be increases in the concentration of LA in muscle and blood, and declines in muscular CP and ATP concentrations (Hultman *et al.*, 1967; Karlsson and Saltin, 1971; Snow *et al.*, 1985). Increased LA concentrations in muscle and blood are correlated with reductions in pH which alters a number of the metabolic and contractile properties of muscle (Mainwood and Renaud, 1985). Some enzymes, particularly phosphofructokinase (PFK), may be sensitive to a reduction in pH (Trivedi and Danforth, 1966) and this may inhibit ATP production. Alterations in contractile ability and depletion of ATP could combine to reduce muscular performance.

The purpose of this study was to examine the metabolic response of muscle to repeated bouts of high intensity exercise. Emphasis was placed on assessing factors contributing to the maintenance of ATP concentration of muscle.

Materials and Methods

Exercise protocol. Eight Thoroughbred horses (6 geldings and 2 mares), aged 3 to 7 years, were used in this study. They were fed a diet of alfalfa cubes and grain. All horses had engaged in an exercise program involving regular trotting, cantering, and some galloping for approximately 10 weeks prior to the experiment.

The protocol for the experiments is presented in Fig. 1. Horses walked approximately 300 m from their stables to the track where they were warmed-up with walking and

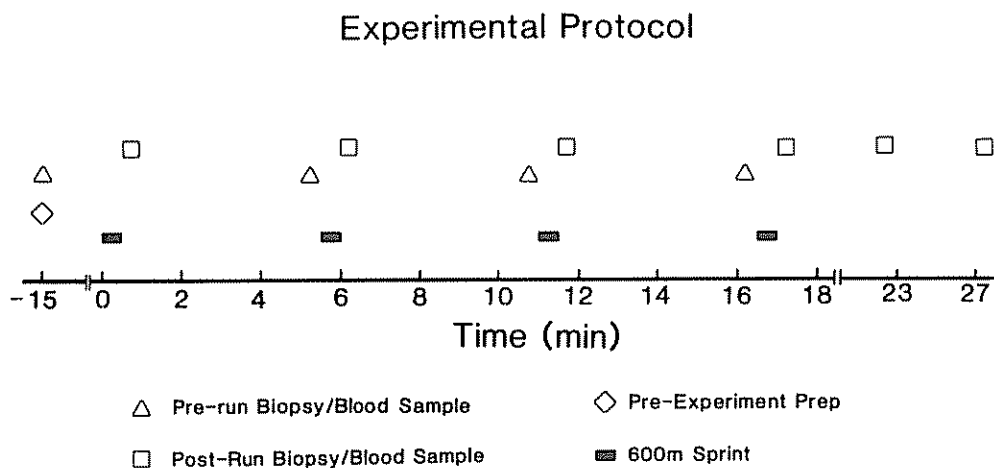


FIGURE 1. The experimental protocol

trotting 1600 m prior to the high speed exercise. Each horse was then exercised four times for 600 m at near near maximal speed by the same rider. After each 600 m run the horses cantered a further 200 m prior to collection of blood and muscle samples. The horses were then walked until prior to the next exercise and the sampling procedure was repeated. Five minute rest periods were allowed between successive exercise bouts. After the fourth exercise period, the horses were walked for 10 min prior to returning to their stables. The experimental design, modeled after Osnes and Hermansen (1972), is similar to that previously used in horses by Snow *et al.* (1985).

Sample Collection. Blood samples (10 ml) were collected from the jugular vein. Muscle samples were collected percutaneously from the middle gluteal muscle using the technique of Bergström (1962) as applied to the horse. This muscle was chosen because of its location and large mass and because it is active at all exercise intensities (Lindholm and Piehl, 1974). Muscle and blood samples were collected at the times indicated in Fig. 1.

Blood samples were transferred to tubes containing potassium oxalate and sodium fluoride and placed on ice. They were subsequently centrifuged and plasma saved. Plasma samples were extracted using perchloric acid (Kelso *et al.*, 1987) and stored at -80°C until analyzed. Muscle samples were rapidly removed from the biopsy needle and within seven seconds quenched in liquid nitrogen. Muscle tissue was stored at -80°C until analyzed.

Analyses. Muscle samples (20–40 mg) were pulverized at the temperature of liquid nitrogen (Pette and Reichmann, 1982) and analyzed for creatine (Cr), ATP, CP, LA, glucose-6-phosphate (G-6-P), and glycogen (Kelso *et al.*, 1987). As exercise changes the water content of muscle, all metabolites were expressed as $\mu\text{mol}/\mu\text{mol}$ of total Cr (CP + Cr). Plasma samples were analyzed for LA using the technique described by Kelso *et al.* (1987) for muscle samples. Muscular pH was estimated on samples of crushed tissue suspended in a medium consisting of 145 mmol potassium chloride, 10 mmol sodium chloride, 5 mmol iodoacetic acid and 0.3 mmol dinitro-fluorobenzene (DNFB) and the pH measured with an Orion model 701A pH meter using a glass electrode (Microelectrodes, Inc. Model MI-410). Data were analyzed by an analysis of variance for repeated measures followed by a Tukey's ω test.

Results

The horses completed the 600-m distance in mean times ranging from a low of 42.2 sec for the first exercise bout, to a high of 45.2 sec for the fourth exercise bout. Horses subsequently sprinted at maximal speed over the same distance in an average of 39.5 sec. Thus, they exercised at between 88 and 93% of their maximal running speed during this experiment.

After the first exercise bout plasma LA increased 14-fold to a mean of 22.0 mmol/l ($P < 0.01$) (Fig. 2). Subsequent increases ($P < 0.05$) in plasma LA were between 5 and 9 mmol/l for each bout, with the smallest increment occurring during the final exercise period. The only decline (about 4%) in running speed occurred during this final exercise bout. There were no decreases in plasma LA between exercise bouts or at the 5 and 10 min sampling periods after the final exercise session.

The largest increase (about 5-fold) ($P < 0.01$) in muscle LA concentration occurred during the first exercise bout (Fig. 3). There was no further change in LA concentration

PLASMA LACTATE (mM)

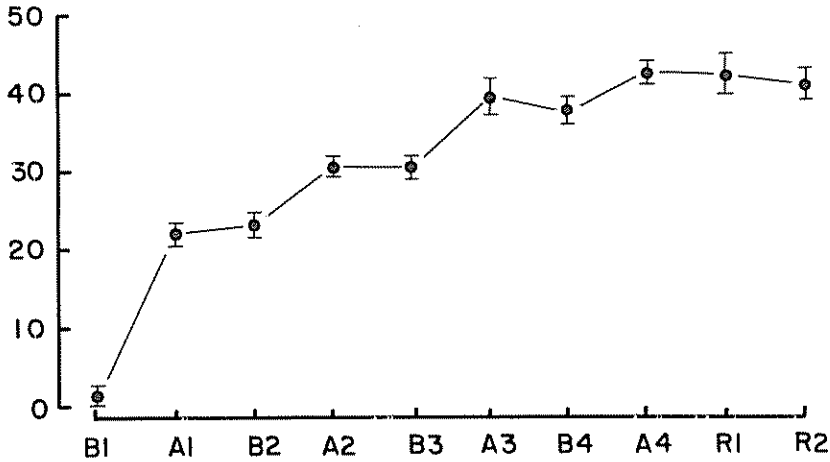


FIGURE 2 Plasma lactate concentration (mmol/L) before (B1-B4) and after (A1-A4) each exercise bout and after 5 (R1) and 10 (R2) min of recovery. Values are means \pm SEM. All lactate concentrations during and after exercise are different ($P < 0.01$) from rest. There were no differences between the post- and pre-exercise concentrations for the individual exercise bouts (e.g. A1 vs B2 etc.). All post-exercise concentrations are greater than those of the previous exercise bouts ($P < 0.05$).

until after the final exercise bout. In contrast to the changes in plasma LA, muscle LA declined 19% ($P < 0.01$) during the 10-min recovery period. The large increases in LA concentration of muscle were paralleled by reductions in intramuscular pH (Fig. 3). However, the association between LA concentration and pH of muscle was less apparent in the post-exercise period.

There was a 42.2% reduction in muscle glycogen concentration during the course of the exercise ($P < 0.01$) (Fig. 4). The largest decline occurred after the first exercise bout with subsequent declines being less. They became disproportionately small when viewed from the standpoint of the increases in blood and muscle LA concentrations. The G-6-P concentration increased 3.2-fold ($P < 0.01$) after the first exercise bout and thereafter declined so that after the fourth exercise bout it was 2.5-fold greater ($P < 0.01$) than the pre-exercise value (Fig. 3). By 10 min post-exercise G-6-P had declined to about double the pre-exercise value.

Muscle ATP concentration remained unchanged until after the fourth sprint. At this point it was 32.5% below ($P < 0.01$) the rest value (Fig. 5). By 10 min post-exercise the ATP concentration had returned to the pre-exercise value. After the initial period of exercise muscle CP concentration declined 33% ($P < 0.01$), however, declines were smaller with subsequent sprints (Fig. 5). There were small increases in CP concentration in the 5 min period between exercise bouts. After 10 min recovery the CP concentration was approximately 80% of the pre-exercise value. The Cr content of muscle increased in direct proportion to the decline in CP. Total Cr was 35.8 ± 1.0 (SEM) and 136.6 ± 4.9 μmol per g wet and dry weight, respectively. These values were not altered by the exercise.

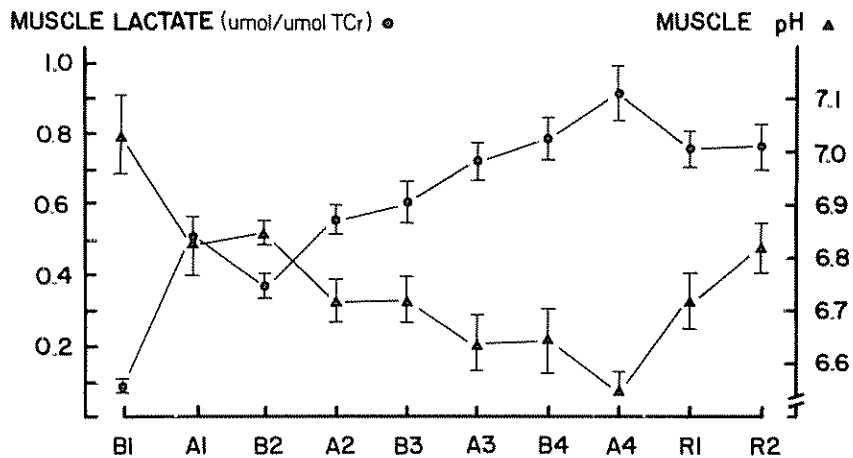


FIGURE 3. Muscle lactate and pH after repeated bouts of exercise. Abbreviations are as in Fig. 2. Concentrations are in $\mu\text{mol}/\mu\text{mol}$ total creatine. Lactate concentration and pH were significantly altered ($P < 0.01$) after the first exercise bout. There was no difference in either variable after the second and third exercise bouts. There were further changes ($P < 0.01$) in these variables after the fourth exercise bout. pH increased during recovery ($P < 0.01$) whereas muscle lactate concentration was unchanged from that immediately after the fourth exercise bout.

Discussion

The large increase in plasma and muscle LA concentrations after the first exercise bout is an indication that although the horses ran at between 88 and 93% of their maximal speed, the exercise was performed at speeds in excess of those required to elicit the $\text{VO}_{2\text{max}}$. The accumulation of LA in blood with exercise reflects the kinetics of its production in muscle and its removal from muscle and blood. In the case of blood, the prime mode of LA removal is via uptake by tissues such as the liver and other muscles. With respect to muscle there can be LA release into the blood as well as oxidation of this substrate by the local oxidative pathways. The large increases in LA concentration at the onset of exercise can be interpreted as being the result of an excessive stimulation of the glycogenolytic pathway. The elevation in the G-6-P concentration of skeletal muscle is consistent with an excessive activation of the Embden-Meyerhof pathway. With subsequent exercise bouts there was a decline in the amount of LA added to the muscle and blood pools and a progressive decline in muscle G-6-P although running speed did not decrease until the final exercise bout. Based on these findings it may be suggested that with continued exercise, activation of the Embden-Meyerhof pathway and the production of pyruvate is more closely matched to the metabolic needs of the muscle than was the case initially. A question which may be addressed is what is responsible for the apparent initial over stimulation of the Embden-Meyerhof pathway leading to the large LA production.

Several mechanisms exist for the activation of the phosphorylase (PHOS), a key enzyme in the regulation of glycogenolysis. At the onset of exercise, Ca^{2+} release into the cytosol is essential for the interaction of the actin and myosin filaments during each

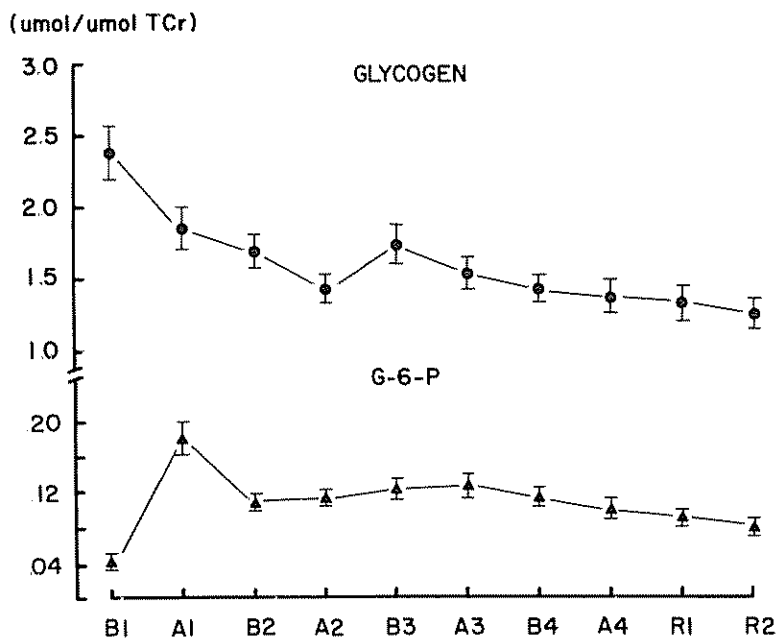


FIGURE 4. Muscle glycogen and G-6-P concentrations at rest and after repeated bouts of exercise. Concentrations are in $\mu\text{mol}/\mu\text{mol}$ total creatine. See Fig. 2 for key to abbreviations. Glycogen declined ($P < 0.01$) after the second exercise bout and was thereafter unchanged. The G-6-P concentration was elevated at all measurement points after rest ($P < 0.01$). After the first exercise bout the G-6-P concentration was greater ($P < 0.01$) than at all subsequent measurement periods.

contraction cycle. In addition, Ca^{2+} activates PHOS *b* kinase which converts PHOS *b* into PHOS *a* which can degrade glycogen to glucose-1-phosphate in the absence of AMP. However, if this activation process is directly linked to the elevation in free Ca^{2+} , why is there a subsequent decline in glycogenolysis? The concentration of Ca^{2+} within the cytosol must remain elevated as the contractile process continues during exercise. This may be explained, at least in part by changes in intracellular pH influencing the PHOS system. At the onset of heavy work there is a temporary increase in muscle pH produced by the degradation of CP, and other mechanisms (MacDonald and Jöbsis, 1976; Molé *et al.*, 1985). This increase in pH potentiates the Ca^{2+} activation of PHOS *b* kinase (Krebs *et al.*, 1959). With continued high intensity exercise pyruvate production exceeds the needs for terminal oxidation and part of it is reduced to LA via a mass action effect. As the LA concentration in muscle increases there is an associated proton accumulation reducing intramuscular pH, which down regulates the effect of free Ca^{2+} on PHOS *b* kinase. In addition, the elevation in the concentration of free glucose within the muscle cell following the onset of contractile activity may also reduce PHOS activity, after an initial intense burst, as free glucose activates PHOS phosphatase, thus reducing the concentration of PHOS *a* within the active muscle. The early LA production and rapid increase in PHOS *a* in muscle have been reported in spontaneous

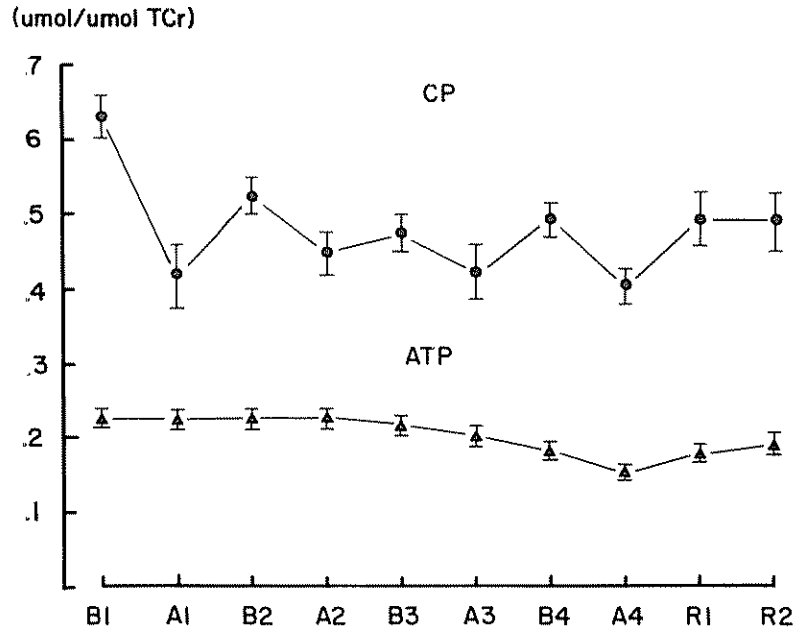


FIGURE 5 The adenosine triphosphate (ATP) and creatine phosphate (CP) concentrations in muscle at rest and after repeated bouts of exercise. Concentrations are in $\mu\text{mol}/\mu\text{mol}$ total creatine. See Fig. 2 for key to abbreviations. The ATP concentration was below ($P < 0.01$) the value measured at rest after the fourth exercise bout and returned to the resting concentration by 10 min of recovery. The CP concentration declined ($P < 0.01$) after the first exercise bout and remained depressed throughout the remainder of the experimental period.

exercise in man and in electrically stimulated isolated muscle preparations (Cartier and Gollnick, 1985).

The large increase in LA production associated with a dramatic decrease in muscle pH agrees with the findings Sahlin *et al.* (1976) and Snow *et al.* (1985). The reduction in pH may be responsible for a decline in metabolic capacity of muscle and in work capacity (Mainwood and Renaud, 1985). Thus, pH may be a major factor associated with metabolic control. It is of interest that the large increase in muscle LA was associated with a pH below that which has been reported to induce an almost complete inhibition of phosphofructokinase (PFK), a key enzyme in the Embden-Meyerhof pathway (Trivedi and Danforth, 1966). If this is true, why was there continued glycogenolysis in the presence of a depressed intramuscular pH? Phosphorylase is unlikely to be affected by physiological acidosis as this enzyme has a pH optimum near 6.1. However, if PHOS were to continue degrading glycogen at a rapid rate in the presence of severely reduced PFK activity there would be concomitant large elevations in the glucose-1-phosphate and G-6-P concentrations of the muscle. What occurred was a progressive decline in G-6-P as exercise continued. Therefore, based on these data, it may be suggested that even under conditions of relatively severe acidosis PFK continues to function at a rate that equals or exceeds that of PHOS. Corsi *et al.* (1969) reached a

similar conclusion for electrically stimulated dog muscle. The report of Dobson *et al.* (1986) also support the contention that the activity of PFK is not depressed under the conditions existing in muscle during intense exercise as had been previously suggested.

The decline in CP concentration of muscle in this study is similar to that reported by others for man and horse in response to heavy exercise. In man, declines in ATP have been reported after heavy exercise (Hultman *et al.*, 1967; Karlsson and Saltin, 1971; Knuttgen and Saltin, 1973). When multiple bouts of very heavy exercise (above the VO_2max) were performed, this decline occurred after the first bout (Karlsson and Saltin, 1971). A similar early decline in ATP has been reported in the horse (Snow *et al.*, 1985) after heavy exercise. In the current experiment a decrease in ATP did not occur until after three bouts of moderately heavy exercise, even though the exercise probably was above VO_2max . The failure of ATP production to keep pace with its utilization is likely to cause the failure (fatigue) of some motor units (Dudley and Terjung, 1986), probably those with low aerobic potential. However, when ATP is measured using the techniques employed in this study the motor units with depressed ATP concentration were averaged with those which retain a normal ATP concentration. As a result, the ATP concentration in some of the motor units with limited oxidative potential is likely to be lower than those reflected by the "averaged" values. The reduced force output by low oxidative fibers may contribute to a decline in performance capacity during high intensity exercise. Although the reason for the decline in ATP is unknown, one possible cause may be that CP declined to a concentration such that it could no longer serve as a high energy phosphate donor.

Even in short periods of intense exercise aerobic ATP production provides the major source of energy and considerable oxygen is consumed (Gollnick *et al.*, 1973) by the working muscle, most likely by the highly oxidative fibers. Thus, all the ATP produced during intense muscular activity does not come from the CP-ADP reaction and/or glycolysis with resultant LA production. The possibility exists that the decline in muscle pH inhibits the oxidative pathways (Bertocci and Gollnick, 1985) resulting in a failure of the mitochondria to maintain an adequate production of ATP.

With high intensity exercise there can be a production of inosine 5'-monophosphate (IMP) and NH_3 . The accumulation of these compounds during exercise indicates that ATP is being produced via the adenylate kinase reaction ($2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$), with the subsequent deamination of AMP to IMP and NH_3 . Activation of this pathway occurs in muscle under heavy exercise conditions (Terjung *et al.*, 1985). Such a process indicates failure of the metabolic pathways to keep pace with metabolic demand. This may be a signal of impending fatigue.

In the animals described in this study, the decline in intramuscular pH resulting from exercise would be likely to have major influence on factors related to metabolic control and the onset of fatigue. Evidence exists associating declines in some of the metabolic and contractile properties of muscle with decrements in intracellular pH. One point of interest in the present study was that in the post-exercise period the tight coupling of intramuscular pH to LA concentration was lost. This is consistent with the observations of Osnes and Hermansen (1972) and of Sahlin *et al.* (1976) that in the post-exercise period protons are more rapidly removed buffered from muscle compared to LA. Benadé and Hesler (1978) have reached similar conclusions when studying isolated muscle preparations. In these cases the equation of Sahlin *et al.* (1976) relating the pH of the muscle to the concentration of LA do not apply. There was some evidence for a rapid

efflux of protons from muscle and a dissociation of the LA-pH relationship even during the 5 min rest period between subsequent exercise trials in the present study. An unresolved question is what protocols are best, following intense exercise, to most effectively produce recovery

Acknowledgments

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

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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.