

blood biochemistry in thoroughbreds. *In: Equine Exercise Physiology*. Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge, pp 389–399.

Wu, M-J., Feldman, B. F., Zinkl, J. G. and Jain, M. C. (1983). Using red blood cell creatine concentration to evaluate the equine erythropoietic response. *Am. J. Vet. Res.*, 44, 1427–1432.

# The Effect of Submaximal Treadmill Training on Heart Rate, Lactate and Ammonia in Quarter Horses

P. A. MILLER and L. M. LAWRENCE

Department of Animal Sciences, University of Illinois,  
Urbana, IL 61801

## Summary

*Seven mature Quarter Horse mares were subjected to an exercise test before and after a seven-week conditioning period on an 11% grade motorized treadmill. The daily workouts during each week consisted of .17 km at 1.4 m/sec followed by 1.1 km at 3.6 m/sec (week 1); 1.7 km at 3.6 m/sec (week 2); 2.2 km at 3.6 m/sec (weeks 3 and 4); 2.2 km at 3.6 m/sec and .5 km at 4.5 m/sec (week 5); 1.1 km at 3.6 m/sec and .5 m/sec at 4.5 m/sec (week 6); 1.1 km at 3.6 m/sec, .17 km at 1.4 m/sec and 1.1 km at 4.5 m/sec (week 7). Each horse was worked 5 d/wk. The exercise test consisted of a 2-min walk at 1.4 m/sec (.17 km), 12 min at 4.5 m/sec (3.2 km) and a one hour recovery period. Each horse carried 27 kg of lead weight. Heart rate, blood lactate, plasma ammonia, alanine, glutamine and glutamate were measured before, during and after exercise. The accumulation of blood lactate during the exercise test was reduced as a result of the conditioning program. In conditioned horses, ammonia increased from  $34.1 \pm 6.1$ – $82.1 \pm 3.3$   $\mu\text{mol/l}$ , which was lower than the accumulation of ammonia from  $36.7 \pm 8.8$ – $113.3 \pm 11.0$   $\mu\text{mol/l}$  in unconditioned horses. Before conditioning, glutamate increased from  $10.4 \pm .9$ – $16.3 \pm .6$   $\mu\text{mol}/100$  ml during the exercise test, and alanine increased from  $38.5 \pm 2.3$ – $79.4 \pm 4.7$   $\mu\text{mol}/100$  ml. After the conditioning period, alanine significantly increased from  $33.3 \pm 2.2$ – $70.7 \pm 4.5$   $\mu\text{mol}/100$  ml, while glutamate and glutamine were not significantly elevated.*

*Index terms: Purine nucleotide cycle; plasma amino acids; exercise test.*

## Introduction

Various metabolic parameters have been investigated to assess the effect of conditioning in the horse. The majority of work has focused on heart rate and lactate as indicators of cardiovascular fitness and oxygen delivery to muscle (Asheim *et al.*, 1970; Milne *et al.*, 1976; Thornton *et al.* 1983; Rodiek *et al.*, 1982). However, many of the physiological pathways that are involved during exercise have not been investigated. One of these pathways is the purine nucleotide cycle that results in the production of ammonia from adenosine monophosphate deamination (Lowenstein, 1972). In humans and horses, blood ammonia increases during exercise (Wilkerson *et al.*, 1977; Miller *et al.*,

1985). In humans, ammonia is removed from muscle by alanine, glutamate and glutamine (Felig, 1975). The purpose of this study was to investigate the effect of conditioning on ammonia, alanine, glutamate and glutamine production in exercised horses.

*Materials and Methods*

Seven Quarter Horse mares with extensive treadmill experience were used in the experiment. The mares were 5–8 years of age and weighed between 472–588 kg. During the experiment, the mares were maintained on good quality grass-alfalfa hay.

For eight months prior to the experiment the horses did not receive any regular exercise except for free movement in pasture. Approximately one week before the first exercise test, the mares were moved to the experimental barn for readjustment to their surroundings and the treadmill. After the adjustment period, each horse performed an exercise test, carrying 27 kg of lead weight. The exercise test was conducted on an 11% grade treadmill (Anamill MLR Design, East Sandusky, OH), and consisted of 2 minutes at 1.4 m/sec (.17 km), 12 minutes at 4.5 m/sec (3.2 km), and a 1 hour recovery period.

After the exercise test, the horses were trained 5 d/wk for 7 weeks on the treadmill set at an 11% grade (Table 1). The conditioning period consisted of increasing work intensity (speed of the treadmill) and work time (time worked per speed). At the conclusion of the training period, the horses worked 2.5 km/day, which consisted of .34 km at 1.4 m/sec, 1.1 km at 3.6 m/sec and 1.1 km at 4.5 m/sec. The mares did not carry any weight during the training program. At the end of 7 weeks, each horse repeated the exercise test.

Approximately 30 minutes before each exercise test, an indwelling catheter was inserted into the left jugular vein. Blood samples for lactate and ammonia were taken prior to exercise, every third minute of exercise and at the first, fifth, fifteenth, thirtieth and sixtieth minute of recovery. Additional blood was collected prior to exercise and during the last minute of exercise for analysis of alanine, glutamate and glutamine. Heart rate was recorded before exercise, every third minute of exercise and at the first and fifth minute of recovery. Heart rate at each time point was measured for at least 20 seconds. Heart rate was monitored using 2 brass electrodes placed behind the withers on the left side and on the sternum and were directly wired to a single-channel recorder.

TABLE 1. Conditioning Program.

Week	Exercise				Distance/ week
	1.4 m/sec	3.6 m/sec	1.4 m/sec	4.5 m/sec	
1	17 km	1.08 km	—	—	6.25 km
2	17 km	1.73 km	—	—	9.50 km
3	17 km	2.16 km	—	—	11.65 km
4	17 km	2.16 km	—	—	11.65 km
5	17 km	2.16 km	—	54 km	14.35 km
6*	17 km	1.08 km	—	54 km	8.95 km
7	17 km	1.08 km	17 km	1.08 km	12.50 km

\*Horses shod during week 6

(Gilson Medical Electronics, Inc., Middleton, WI).

Venous blood for the measurement of lactate was immediately deproteinized in perchloric acid, and plasma for ammonia analysis was immediately centrifuged and frozen in liquid nitrogen. Each sample was assayed spectrophotometrically, in duplicate for lactate and ammonia, using commercially available reagents (Sigma Chemical Co., St. Louis, MO). Plasma for amino acid analysis was deproteinized by the addition of 3.5% 5-sulfasalicylic acid. The protein-free supernatant was frozen until analyzed on a Beckman amino acid analyzer (Beckman Auto-Amino Acid Analyzer, Model 6300, Beckman Instruments, Palo Alto, CA).

Data obtained during each exercise test were analyzed by analysis of variance for repeated measures to compare treatment (unconditioned vs conditioned) differences. To compare observations at each time point, the data were analyzed by t-tests of paired observations between unconditioned and conditioned horses. Pre-exercise values were compared to exercise and recovery values during each exercise test by single degree of freedom contrasts (Freund, 1979).

### *Results*

During the preconditioning exercise test, heart rate increased from  $62.6 \pm 7.3$  at rest to  $206.6 \pm 3.1$  beats per/min (bpm) at the last minute of exercise. After conditioning, heart rate increased from  $69.0 \pm 6.8$  at rest to  $188.5 \pm 3.4$  bpm at the last minute of exercise. Heart rates were higher ( $P < .05$ ) in unconditioned horses at the ninth and twelfth minute of exercise and during the first and fifth minute of recovery (Fig. 1).

Conditioning resulted in lower ( $P < .01$ ) blood lactate during exercise and recovery. Lactate increased in preconditioned horses from  $.63 \pm .08$ – $11.6 \pm 1.0$  mmol/l, and from  $.82 \pm .1$ – $4.8 \pm .5$  mmol/l in conditioned horses. In the unconditioned horses, lactate remained elevated throughout the 60-minute recovery period, while in the conditioned horses, lactate had returned to pre-exercise levels by the sixtieth minute of recovery (Fig. 2).

The exercise test resulted in a significant accumulation of plasma ammonia in both groups (Fig. 3). In unconditioned horses, ammonia increased from  $36.7 \pm 8.8$ – $113.3 \pm 11.0$   $\mu$ mol/l, however significant accumulation did not occur until the sixth minute of exercise. Ammonia levels in conditioned horses were lower ( $P < .01$ ) compared to unconditioned horses. Ammonia increased from  $34.1 \pm 6.1$ – $82.1 \pm 3.3$   $\mu$ mol/l in conditioned horses, and the rise in ammonia was not significant until the twelfth minute of exercise. It is interesting to note that the sharpest increase in ammonia occurred during the last minute of exercise in both groups. Plasma ammonia had returned to pre-exercise values by the sixtieth minute of recovery in both groups.

Plasma glutamate levels were elevated at the last minute of exercise only during the exercise test prior to conditioning. Glutamate levels were lower ( $P < .01$ ) after conditioning. In both exercise tests alanine was elevated ( $P < .01$ ) during the last minute of exercise but there was no effect due to conditioning. Glutamine did not significantly ( $P > .05$ ) increase during either exercise test but levels were lower ( $P < .01$ ) after conditioning (Table 2).

### *Discussion*

During strenuous muscular work in horses, heart rate and lactate will increase above resting levels. At the beginning of work, heart rate will initially overshoot before reach-

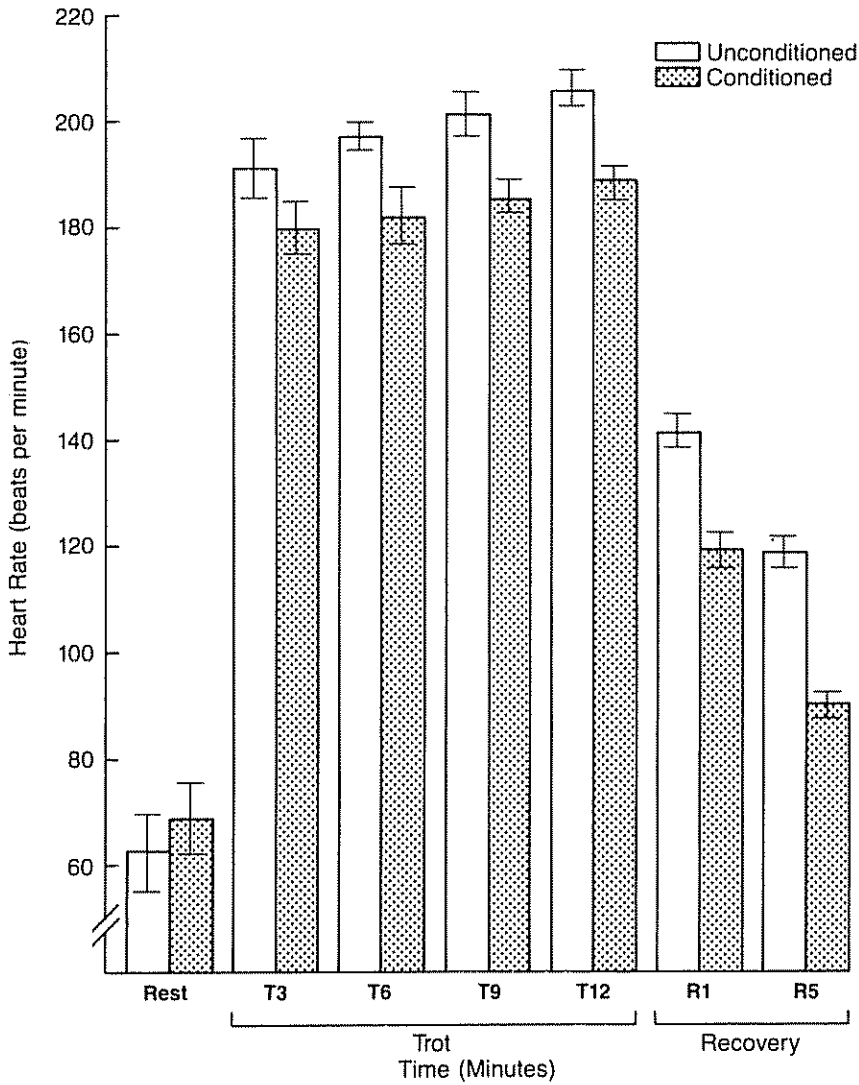


FIGURE 1: Effect of conditioning on heart rate (mean  $\pm$  S E). Heart rates are significantly ( $P < .05$ ) different at T9, T12, R1 and R5.

ing a plateau during steady state work (Fregin and Thomas, 1983). In the present study, the first measurement of heart rate occurred at the third minute of exercise, so any initial overshoot was not recorded. It has been reported that speeds of 7–8 m/sec on a 10% grade are necessary to elicit heart rates between 180–200 bpm (Fregin and Thomas, 1983). The horses in this study had heart rates in the 180–200 bpm range at only 4.5 m/sec on an 11% grade. The added weight probably accounted for a part of the elevation in heart rate. In addition, heart rate data from the naturally longer strided Thoroughbred and Standardbred may not be applicable to Quarter Horses. The heart rates

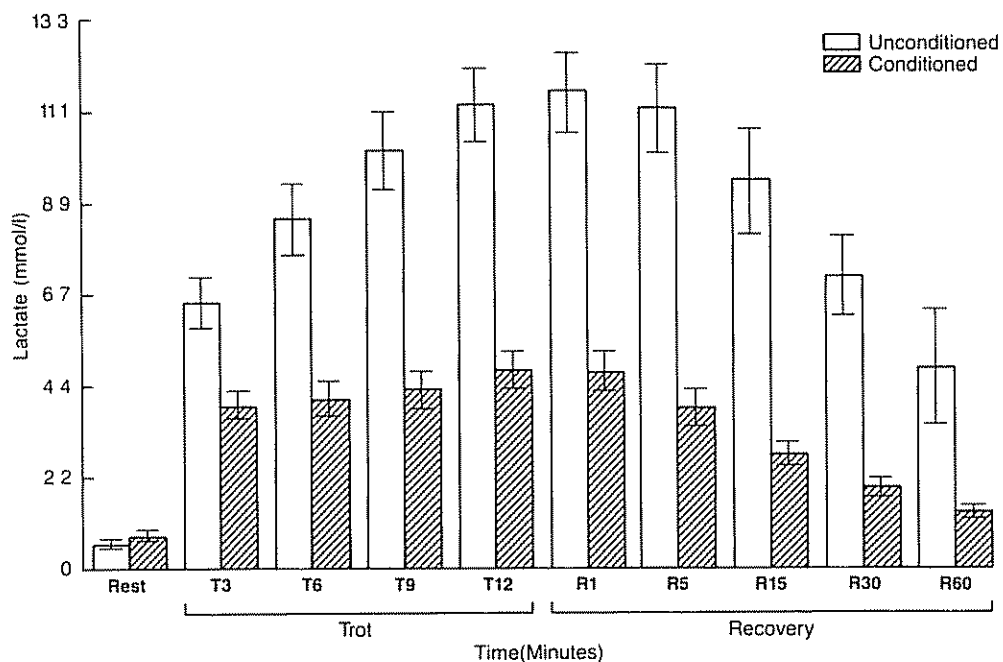


FIGURE 2: Effect of conditioning on lactate (mean  $\pm$  S.E.). Lactate is significantly ( $P < .01$ ) lower after conditioning at all times except rest.

in this study are consistent with those reported for Quarter Horses by Sexton *et al.* (1985).

A decreased heart rate following conditioning has been reported in other papers (Milne *et al.*, 1976; Thornton *et al.*, 1983; Sexton *et al.*, 1985). However, a unique finding in this study was the gradual increase in heart rate during the first exercise test. It is possible that this was related to the fatiguing of the horses during the first test. A number of the horses found it difficult to maintain a consistent gait during the preconditioning exercise test but completed the second exercise test without a problem.

The decreased lactate levels observed in this submaximal exercise test are consistent with submaximal training effects which have been described numerous times in horses (Milne *et al.*, 1976; Thornton *et al.*, 1983). The accumulation of lactate in blood reflects the balance between production and removal. Lower blood lactate levels during submaximal exercise in trained individuals are generally associated with metabolic changes within the muscle fibers that allow for greater oxidative energy production. These changes result in increased fat utilization and decreased glycogenolysis with less lactate production (Matoba and Gollnick, 1984; Gollnick *et al.*, 1986). The training induced changes that alter substrate utilization and lactate production during submaximal exercise may also be responsible for the changes in plasma ammonia observed in this study.

During intense exercise, adenosine monophosphate (AMP) is produced from the myokinase reaction ( $2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$ ). Adenosine monophosphate is then deaminated to inosine monophosphate (IMP) and ammonia by AMP deaminase (Lowenstein, 1972). The removal of AMP by IMP and ammonia formation will drive the myokinase

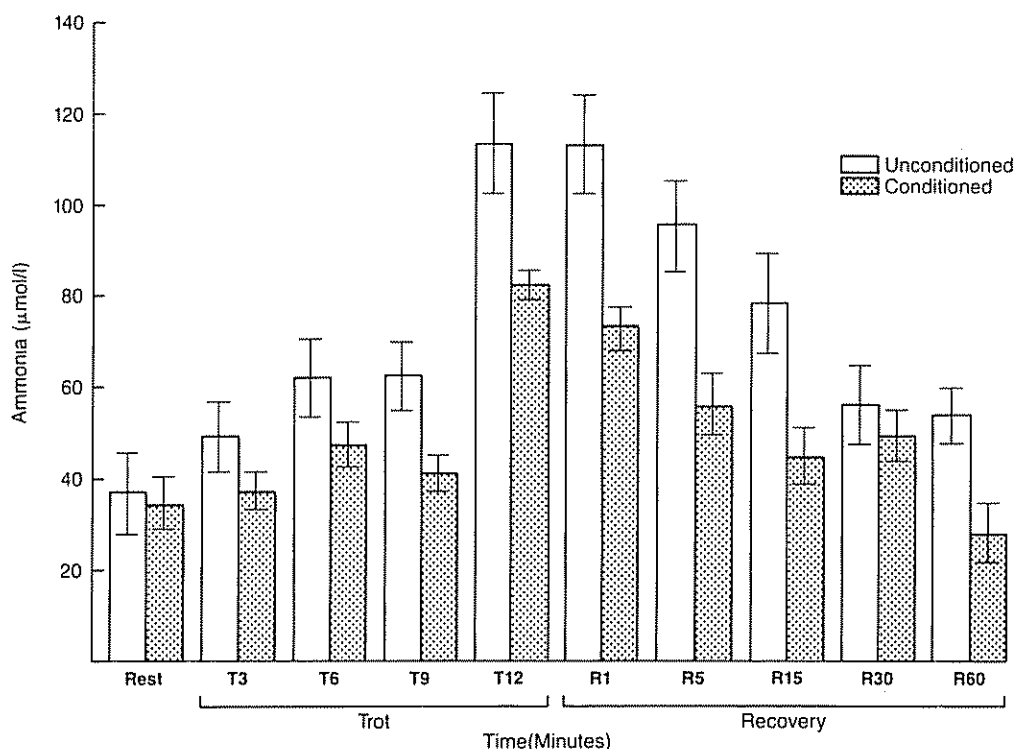


FIGURE 3: Effect of conditioning on plasma ammonia (mean  $\pm$  S.E.) Plasma ammonia was significantly ( $P < .05$ ) lower after conditioning at T12, R1, R5 and R60

reaction towards ATP formation, helping maintain the ATP:ADP ratio in muscle (McMiken, 1983). In horses, intermittent exercise results in a decrease in ATP accompanied by an increase in IMP suggesting activation of AMP deaminase (Snow *et al.*, 1985). Horse muscle has a relatively high activity of AMP deaminase (Cutmore *et al.*, 1986) and the activity increases during exercise (Snow *et al.*, 1985); therefore the rise in plasma ammonia may be related to increased AMP deaminase activity.

Training increases AMP deaminase activity (Cutmore *et al.*, 1986) which could sug-

TABLE 2. Effect of Conditioning on Glutamate, Glutamine and Alanine ( $\mu\text{mol}/100\text{ml}$ )<sup>\*</sup>.

	Unconditioned		Conditioned	
	Rest	T12 <sup>†,††</sup>	Rest	T12 <sup>†,††</sup>
Glutamate <sup>§</sup>	10.4 $\pm$ .9	16.3 <sup>a</sup> $\pm$ .6	9.7 $\pm$ .8	11.4 $\pm$ .9
Glutamine <sup>§</sup>	78.5 $\pm$ 1.8	85.9 $\pm$ 3.3	63.0 $\pm$ 6.5	68.1 $\pm$ 2.9
Alanine	38.5 $\pm$ 2.3	79.4 <sup>a</sup> $\pm$ 4.7	33.3 $\pm$ 2.2	70.7 <sup>a</sup> $\pm$ 4.5

<sup>\*</sup>Mean  $\pm$  standard error.

<sup>†</sup>T12 = trot - 12th minute

<sup>††</sup>Values are significantly ( $P < .001$ ) affected by exercise if followed by <sup>a</sup>.

<sup>§</sup>Significantly ( $P < .05$ ) affected by conditioning

gest that trained horses might have higher IMP and ammonia levels; however, in this study, conditioning resulted in lower plasma ammonia levels. A decrease in ammonia has also been reported in trained rats (Barnes *et al.*, 1964; Dudley and Terjung, 1985). In rat muscle, the decreased ammonia production is attributed to reduced adenylate loss resulting from increased oxidative capacity of the muscle (Dudley and Terjung, 1985). It appears that the conditioned horses in this study were deriving more energy from oxidative means, since lactate levels were greatly reduced. The lower ammonia levels may also reflect an increased dependence on oxidative metabolism.

Another possibility for lower plasma ammonia levels after training is increased removal from muscle by glutamate and glutamine, and increased flux through the glucose-alanine cycle. The unchanged glutamine levels during exercise may support the fact that glutamine synthase is inhibited during contraction (Snell and Duff, 1980) and would not contribute to reduced ammonia levels. Snow *et al.* (1985) reported that during intermittent exercise in horses, muscle alanine increased; however, by the third repeated gallop, alanine decreased and remained low during the fourth gallop. This may be due to inhibition of alanine synthesis as the availability of carbon skeletons become limiting (Chang and Goldberg, 1978). A decrease in the ability to transport ammonia from muscle via alanine may have been the cause of the large increase in ammonia observed in this study during the last three minutes of exercise, while the rise in glutamate in unconditioned horses may have also aided in the transport of ammonia out of muscle.

Conditioning results in an increase in the activity of glutamate pyruvate transaminase, while lactate dehydrogenase remains unchanged in rat gastrocnemius muscle (Mole *et al.*, 1973). Since training increases fat oxidation, which could inhibit pyruvate dehydrogenase, Mole *et al.* (1973) suggested that pyruvate will then be directed towards alanine synthesis with decreased flux towards lactate. This would help prevent the increased lactate levels and associated metabolic acidosis. In the preconditioned horses the ratio of alanine/lactate was .69 at the last minute of exercise, while conditioning increased it to 1.47. This may support the concept that pyruvate is directed towards alanine synthesis in conditioned animals. Therefore, lowered production of ammonia and increased flux towards alanine would result in the lower plasma ammonia levels seen in the conditioned horses.

The effect of an acute load of ammonia on bodily functions is not clear. Ammonia will stimulate phosphofructokinase, the controlling enzyme in glycolysis (Tornheim and Lowenstein, 1975). It may also serve as a buffer during metabolic acidosis. However, ammonia may interfere with oxidative metabolism by inhibiting pyruvate carboxylation (Bryla and Niedzwieka, 1979) and inhibiting isocitrate dehydrogenase (Katunuma *et al.*, 1966). In addition, ammonia will stimulate glutamate and glutamine synthesis resulting in a depletion of  $\alpha$ -ketoglutarate (Katunuma and Okada, 1963). Further studies are needed to evaluate the role of ammonia in oxidative metabolism, because an impairment in the citric acid cycle by depleting  $\alpha$ -ketoglutarate could lead to fatigue. Lastly, ammonia is a neurotoxin and may cause tremors, blurring of vision and convulsions (Martin *et al.*, 1983) which may affect the performance of exercising individuals.

In conclusion, training leads to a reduction in ammonia levels which may be due to improved oxidative capacity and/or decreased AMP formation by the myokinase reaction. In addition, glutamate and alanine transport of ammonia may be improved by training.

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