

# Changes in Muscle Glycogen, Lactate and Pyruvate Concentrations in the Thoroughbred Horse Following Maximal Exercise

M. A. NIMMO<sup>1</sup> and D. H. SNOW<sup>2</sup>

Department of Veterinary Pharmacology, Glasgow University Veterinary School, Bearsden Road, Bearsden, Glasgow.

## Summary

*Six Thoroughbred horses were galloped over 506 m, 1025 m, 1600 m and 3620 m to investigate the effect of maximal exercise on muscle glycogen, lactate and pyruvate. Biopsy samples were taken from the middle gluteal muscle immediately prior to exercise and immediately afterwards.*

*Muscle glycogen utilization/minute was greatest for the 506 m trial ( $149.4 \pm 0.8 \mu\text{mol/g/min.}$ ) and least for the 3620 m trial ( $18.8 \pm 2.6 \mu\text{mol/g/min.}$ ). Utilization decreased with the decrease in intensity of work and with prolonged duration. Lactate accumulation was similar for the three shorter distances (approximately  $80 \mu\text{mol/g}$  dry weight of tissue) but significantly less for the 3620 m trial ( $22 \mu\text{mol/g}$  dry weight of tissue). Resting pyruvate values ( $0.41 \pm 0.04 \mu\text{mol/g}$  dry weight of tissue) did not alter with any exercise régime. It was concluded that the findings were in accord with the energy demands over the distances raced, in that energy production requirements per second were greatest at the faster speeds over the shorter distances.*

## Introduction

Muscle glycogen depletion has been investigated in Thoroughbred horses subjected to an endurance ride (Snow *et al.*, 1981) and in Standardbred trotters exercised over various distances (Lindholm and Saltin, 1974). However, the glycogen depletion associated with short-term maximal performance, requiring energy to be produced by predominantly 'anaerobic' pathways, has not been extensively studied in the Thoroughbred. The breakdown of glycogen under anaerobic conditions produces quantities of intracellular lactate. In man, lactate has been suggested as the major contributor in promoting muscle fatigue during intense short-term exercise (Tesch 1980).

As part of a more extensive study on the effects of an anabolic steroid (Snow *et al.*,

Present address: <sup>1</sup> The Queen's College, 1 Park Drive, Glasgow G3 6LP.

<sup>2</sup> Equine Research Station, Snailwell Road, Newmarket.

1982a), the glycogen depletion and subsequent pyruvate and lactate production within a skeletal muscle of the Thoroughbred horse were investigated over four distances: 506 m, 1025 m, 1600 m and 3620 m.

### *Materials and Methods*

#### *Experimental protocol*

Six clinically normal Thoroughbred geldings aged between three and ten years were used. Prior to the maximal exercise period, each horse underwent a five-week aerobic training period during which it was exercised at the canter or trot in an indoor riding school. The maximal exercise component consisted of weekly gallops over 506 m, 1025 m and 1600 m. The gallops were interspersed by days of easy trotting and cantering. Normally one of the distances each week was run as an experimental trial. The 1025 m gallop was used as a trial in week 1 and in the final week (week 6). In addition, some horses were also tested over 3620 m in the final week. The time lapse between each trial was approximately one week, except in the final week when the time lapse was only two days. When the gallop was an experimental trial, each animal ran individually and was ridden by the same jockey.

The complete training programme was repeated after 15 weeks of detraining. The two training periods were parts of a cross-over study to investigate the effects of anabolic steroids (Snow *et al.*, 1982a). Each horse was therefore tested on two occasions over each distance, once when administered with a placebo and once when administered with an anabolic steroid.

#### *Muscle sampling*

The percutaneous needle biopsy technique was used to obtain samples from the middle gluteal muscle (MG). The area of skin was prepared for biopsy and two small scalpel incisions were made a minimal distance apart, while the horse was still in the box. The pre-exercise sample was taken just prior to the start of the gallop, by which time the animal had trotted and cantered as a 'warm-up'. The post-exercise sample was taken from the second incision within 20 seconds of the cessation of the gallop. Resting samples were taken on a separate occasion with the horses in their boxes. The needle containing the biopsy sample was plunged directly into liquid nitrogen.

#### *Analytical methods*

*Glycogen.* Analysis was carried out on freeze-dried samples (Huijing 1970).

*Lactate and pyruvate.* Tissue handling was extremely important in the samples to be considered for lactate and pyruvate estimations. Samples were powdered under liquid nitrogen, and care was taken to remove any contaminating blood or connective tissue from the samples. After 3 to 6 mg muscle powder had been weighed into small tubes, soluble metabolites were extracted by adding cold 0.5 M perchloric acid containing 1 mmol/l EDTA. Perchloric acid was added at a rate of 1 ml/10 mg powder. This solution was agitated in an ice bath at  $-10^{\circ}\text{C}$  and the supernatant removed. Rigorous cleaning of glassware was carried out as recommended by Lowry and Passonneau (1972). The concentration of lactate and pyruvate was measured by the method of Olsen (1972) and

determined at 340/460 nm on a Perkin Elmer 1000 M fluorimeter. All samples and standards were assayed in triplicate.

#### *Analysis of results*

All results are expressed as mean  $\pm$  SEM. Where there was more than one result for an animal over a given distance, the values were averaged to give a mean value. Biopsy samples taken in the box are referred to as 'resting', those taken at the start of the gallop as 'pre-exercise'. Comparison of the various distances was analyzed using Analysis of Variance followed by a Scheffé test.

#### *Results*

As the muscle samples were frozen while still in the biopsy needle, it was almost impossible to assess whether the sample was of sufficient quantity for analysis. It was necessary that pre-exercise and post-exercise samples were obtained from any one animal, and on some occasions only one sample was satisfactory, resulting in a reduction in sample number.

There was no difference in any of the parameters measured between the horses, whether they had been administered the placebo or the anabolic steroid. Mean results for each animal from the two training periods are therefore presented independent of treatment. In addition, no training effect was noted between the 1025 m trial in week 1 and the 1025 m trial in week 6. These results have also been averaged to give a mean value.

#### *Glycogen*

Resting values varied throughout the training period (Nimmo *et al.*, 1982). The pre-exercise samples showed greater variation than was found in resting animals (Nimmo *et al.*, 1982). The mean of mean pre-exercise value for the six horses was  $542.5 \pm 42.8$   $\mu$ mol/g dry weight of tissue. Varying decreases occurred in the total amount of glycogen utilized by each horse. However, when these values were expressed as a percentage change from pre-exercise levels, the variation in the results was reduced (Fig. 1). In the 506 m trial the horses utilized significantly less glycogen than in the 1025 m ( $p < 0.01$ ) and the 1600 m ( $p < 0.05$ ) trials. In the 3620 m trial the horses used significantly less glycogen than in the 1025 m trial ( $p < 0.05$ ).

#### *Pyruvates*

There was no significant difference between pre-exercise and post-exercise pyruvate values over any distance. The mean of mean value for pre-exercise samples was  $0.41 \pm 0.04$   $\mu$ mol/g dry weight of tissue, while the mean of mean value for post-exercise samples was  $0.45 \pm 0.02$   $\mu$ mol/g dry weight of tissue.

#### *Lactates*

The resting mean value for the six horses was  $8.3 \pm 0.98$   $\mu$ mol/g dry weight of tissue. A mean of mean value for pre-exercise samples was  $16.5 \pm 1.6$ . Lactate accumulation over the 3620 m trial was significantly lower than over all other distances (Fig. 2). No standard errors have been included in Fig. 2 for the 1600 m trial because of the small sample number ( $n = 2$ ).

FIGURE 1. Percentage glycogen utilization in the middle gluteal muscle over various distances. Figures in parentheses are the numbers of horses in each trial.

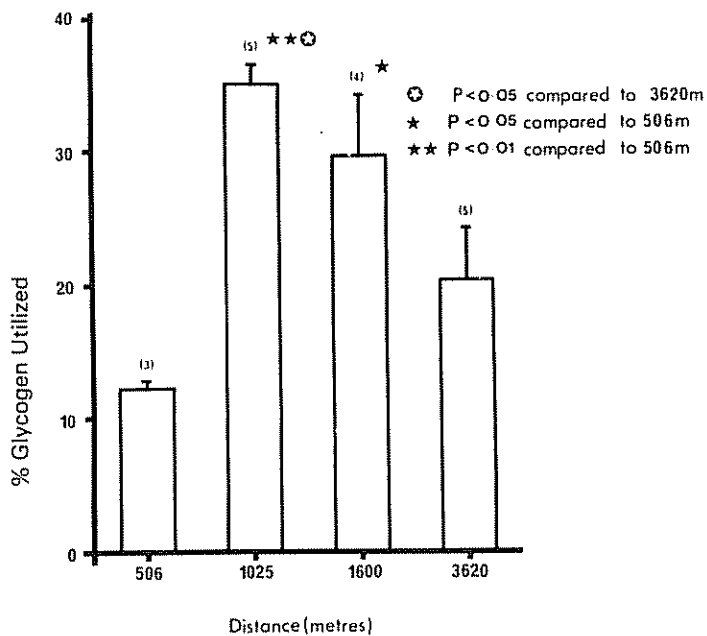
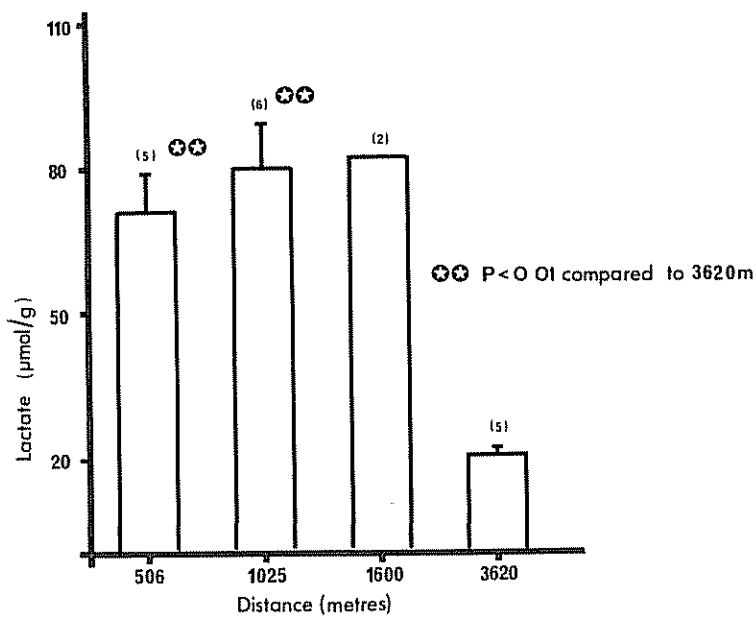


FIGURE 2. Lactate production (  $\mu\text{mol/g}$  dry weight) in the middle gluteal muscle over various distances. Figures in parentheses are the numbers of horses in each trial.



*Times*

Because of practical difficulties one animal was not timed over the 3620 m trial. The speed of the other horses and the glycogen utilization/minute are presented in Table 1.

TABLE 1. Glycogen utilization ( $\mu\text{mol/g/min.}$ ) over various distances (mean  $\pm$  SEM).

Distance (m)	n	Time (s)	Speed (m/s)	Glycogen utilization ( $\mu\text{mol/g/min.}$ )
506	3	34.9 $\pm$ 0.6	14.5 $\pm$ 0.2	149.4 $\pm$ 0.8
1025	5	73.1 $\pm$ 1.7	14.1 $\pm$ 0.3	129.3 $\pm$ 20.0
1600	4	127.2 $\pm$ 9.3	12.6 $\pm$ 0.4	66.5 $\pm$ 8.0
3620	4	316.8 $\pm$ 6.9	11.4 $\pm$ 0.2	18.8 $\pm$ 2.6

*Discussion*

Resting concentrations of lactate and pyruvate are comparable to those in man (Sahlin *et al.*, 1976), and the lactates are also similar to those reported in Standardbred trotters (Lindholm and Saltin, 1974). Variation in 'warm-up' procedure and the possibility of an effect from a previous day's training account for the greater distribution of pre-exercise glycogen levels.

As has been found previously in the horse (Lindholm and Saltin, 1974; Snow *et al.*, 1981), exercise depletes the muscles of glycogen. Direct comparison, in absolute terms, of results in this study with reports on either the horse or man is difficult because of the variety of exercise régimes. It is also complicated by the fact that with repetitive trials over a distance, the initial trial has a higher glycogen utilization than subsequent runs (Bergstrom and Hultman, 1967; Snow *et al.*, 1979).

Extrapolating from published work to give comparable conditions to this study, a 28% decrease in glycogen utilization/minute in the 506 and 1025 m trial is comparable with a previous study in the Thoroughbred horse (Snow *et al.*, 1979) and in man (Karlsson *et al.*, 1971). The decrease is greater than the 19% reported from Standardbred trotters (Lindholm and Saltin, 1974), possibly because the speeds were slower, resulting in a lower energy requirement per second.

The maintained pyruvate concentrations with dynamic exercise are also in agreement with published work in man (Sahlin *et al.*, 1976), although some reports do give significant increases (Karlsson 1971). Lactate levels increased to approximately 80  $\mu\text{mol/g}$  dry weight of tissue in the three shorter distances. Again, comparison with other studies is difficult but approximations show that with a similar test (i.e. supramaximal exercise over a similar period of time) in humans (Karlsson 1971) values were lower than in the horse.

The higher muscle lactates in the horse, compared with man, were anticipated, as higher blood lactates than those in man have previously been reported in the horse. (Snow and MacKenzie, 1977). The reason for the equivalent lactate concentrations after either 506 m or 1025 m, in spite of lower glycogen utilization over the longer distance, is due to the fact that when high-intensity exercise is performed, the relative contribution of the various metabolic pathways to energy transfer depends partly on the duration of exercise. The deficit of oxygen at the start of exercise means that ATP demands which begin

instantaneously at the onset of work must be met from anaerobic mechanisms. Although it was thought that this demand was initially provided from the muscle stores of ATP and by breakdown of phosphorylcreatine (Margaria *et al.*, 1933), producing a lag in lactate production, this now seems unlikely (Rennie and Edwards, 1981), and the anaerobic production of energy at the onset of exercise is also a product of glycolysis.

The prime precursor of lactate formation in skeletal muscle during intense short exercise is intramuscular glycogen. Glucose uptake at the onset of exercise is negligible (Wahren *et al.*, 1971). The fuels used in any type of exercise are dependent on the involvement of the different muscle types. It is now known that during exercise at different intensities not all muscle fibres are used simultaneously and that selective recruitment of different motor units, broadly in line with the size principle of Henneman and Olsen (1965), occurs. During light workloads, i.e. at a low percentage of  $\text{VO}_2 \text{ max}$ , type I and some type II high-oxidative fibres are recruited. As the exercise continues and the originally recruited fibres become depleted of glycogen, fresh type II fibres are recruited to maintain the workload (Snow *et al.*, 1982c). At higher workloads more of the type II fibres are recruited, and this has been demonstrated in the Standardbred trotter (Lindholm and Saltin, 1974) so that, at the highest speeds, the type II low-oxidative fibres are also recruited. In man, Gollnick *et al.* (1974) have shown that when  $\text{VO}_2 \text{ max}$  exceeds 120%, type II fibres become glycogen-depleted before type I fibres do. This may be because these fibres have a lower aerobic capacity than the type I fibres, thus using glycogen less efficiently by producing lactate. The lower aerobic capacity of the type II fibres compared with the type I fibres has been reported by Ingjer (1978). This reduced aerobic capacity is due to both a lower oxidative activity within the muscle fibres and a poorer vascularization. A lower capillary density in type II low-oxidative fibres has been reported in the horse (Nimmo *et al.*, 1982).

The results in the present study illustrate that the greater the speed, the greater the glycogen utilization and lactate production, as the energy demands exceed the aerobic capacity of the muscle fibres involved. Since the speed is reduced over 1600 m, the results suggest that although utilization is markedly reduced, high lactate production is maintained. This may indicate that the alternative energy substrate for the TCA cycle, namely free fatty acids (FFA), is now being utilized. The rôle of FFA in energy production during short maximal exercise in the horse has been suggested by Snow *et al.* (1982b).

Over the longer distance of 3620 m, several factors are probably in operation to reduce lactate production and glycogen utilization. The slower speed indicates that power output by the muscles is reduced. This probably arises from the recruitment of fewer of the type II low-oxidative fibres, a factor which would contribute to a lower lactate production in muscle. The lower energy demand per second also means that less lactate is produced because oxidative metabolism can meet the energy requirements. This decreased glycogen utilization/minute therefore contributes to a decreased lactate production. In addition, it is likely that the uptake and utilization of the blood-borne substrates, glucose and FFA, are in progress over this period of exercise.

It would therefore appear that glycogen was the main substrate for energy over the distances of 506 m, 1025 m and 1600 m. With the recruitment of the low-oxidative type II fibres at these high intensities, relatively high levels of lactate were produced. The 3620 m trial was of a lower intensity and thus the percentage recruitment of the low-oxidative

type II fibres would be less, the majority of activity being in the type I and high-oxidative type II fibres. These fibres are well endowed with mitochondria and capillaries and would therefore produce low lactate concentrations.

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