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Lactate Kinetics, Plasma Ammonia and Performance Following Repeated Bouts of Maximal Exercise

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ABSTRACT. To study the association between increase in plasma lactate and ammonia (NH_3) with performance, 4 horses undertook 4 consecutive maximal gallops each of 700 m. The horses were exercised on an all-weather track with 20 min rest between each gallop. Venous blood samples for determination of plasma lactate, NH_3 and uric acid were taken prior to and 2, 10 and 20 min after each gallop. Average running time over the 4×700 m gallops increased from 47.0 to 53.8 s. Results of 3 of the 4 horses indicated a relationship between the increase in running time over successive gallops and the increase in the pre-gallop plasma lactate concentration (i.e. as a measure of the degree of acidosis in the muscle cells), and also to the sum of the previous rise in plasma NH_3 (i.e. as a measure of muscle adenine nucleotide loss). These trends were not borne out by the results of the fourth horse. No association was found in the comparison of gallop time with the rate of NH_3 accumulation relative to that in lactate. However, it was notable that horse Cp which was fastest over the first gallop showed the highest rate of plasma NH_3 accumulation at each gallop, a finding which parallels recent work on human sprint and middle distance runners.

Key words. Exercise; horses; lactate; ammonia; blood; plasma.

INTRODUCTION

Deamination of adenine nucleotide (AN) to inosine monophosphate (IMP) in muscle is a feature of sustained high intensity exercise in the horse, man and other species[1,10] resulting in a net loss of adenosine triphosphate (ATP). The change in AN content may be as much as 40% after just one gallop over typical flat racing distances.[5] NH_3 released during deamination appears rapidly in plasma whilst over longer periods of time, increases in the plasma concentrations of hypoxanthine and uric acid may also occur due to the further degradation of IMP.[6]

The onset of ATP loss in normal muscle during intense exercise correlates with the development of intracellular acidosis[3] though human patients with myophosphorylase deficiency may show AN loss without evidence of pH decreases.[11] In either case, however, the event of ATP loss appears linked to a reduction in the capacity to rephosphorylate ADP formed with contraction.

In a previous study of maximal intermittent excercise in the Thoroughbred horse, it was found that the decrease in running speed over successive gallops was related to the extent of muscle ATP loss.[10] It was suggested that ATP loss, either on an absolute basis, or the event itself (indicating a decrease in the rate of ADP removal) may be contributory to muscle fatigue. However, changes in ATP in this study could not be separated from changes in lactate.

In the present investigation the relative changes in plasma ammonia (as a marker of ATP loss in the musculature as a whole) and

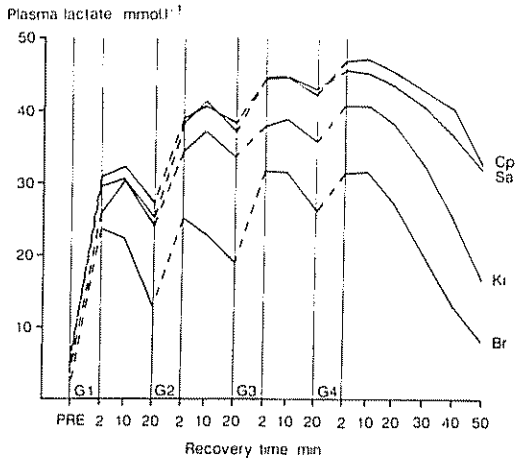

Fig. 1. Individual changes in plasma lactate (mmol l^{-1}) over the 4×700 m gallops and following a 50 min recovery period.

lactate over successive gallops, have been examined using a similar intermittent exercise model.

MATERIALS AND METHODS

Four trained Thoroughbred horses (4–7 years) were used in the study. Horses were first prepared for repeated venous blood sampling by catheterization of the left jugular vein following which they were walked 2 km to the start of the exercise track. This was an all-weather track with a wood-chip surface. Following an initial canter of 200 m, each horse completed four 700 m gallops at maximal pace. A 20 min recovery period was allowed between gallops during which the horses were walked continuously with the jockey in the saddle.

Venous blood samples were collected just prior to the first gallop (pre) and 2, 10 and 20 min after each of the four gallops. Additional samples were taken 30, 40 and 50 min after the final gallop. Ten ml blood samples were collected into tubes containing lithium heparin and stored on ice until centrifuged to harvest plasma. Lactate was assayed in perchloric acid extracts of plasma according to Hohorst.[7] NH_3 and uric acid were assayed directly in plasma using kits from Sigma Diagnostics. A further 3 ml blood sample was collected into 5 ml perchloric acid for determination of whole blood lactate. Relative changes in whole blood lactate were essentially the same as those in plasma lactate and for the purposes of this report only the latter are presented.

RESULTS

Average running time over the 4×700 m gallops increased from 47.0 to 53.8 s. The fastest horse over the first gallop (horse Cp) showed the greatest change in running speed and was the slowest over the fourth.

The individual changes in plasma lactate

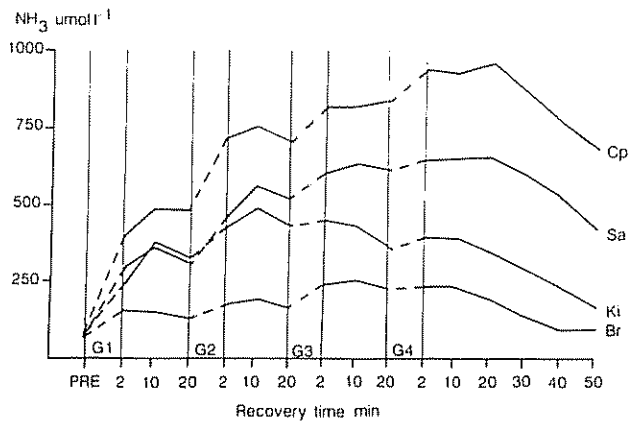

Fig. 2. Individual changes in plasma NH_3 (μmol l^{-1}) over the 4×700 m gallops and following a 50 min recovery period

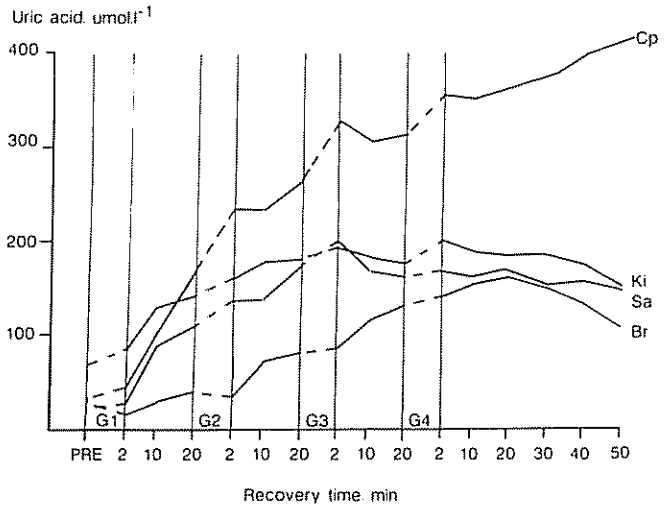

Fig. 3 Individual changes in plasma uric acid (μmol l^{-1}) over the 4×700 m gallops and following a 50 min recovery period.

are shown in Fig. 1. Peak concentrations generally occurred 10 min after the end of exercise. Based on the change in plasma lactate between 10 and 20 min post-exercise, there was a trend towards slower rates of disappearance. Thus from G1 to G4, mean 10 to 20 min recovery disappearance rates were 38.6 (SD 12.0), 23.3 (SD 6.8), 19.1 (SD 9.6), and 15.0 (SD 7.1), mmol l^{-1} h^{-1}, respectively. However, between 30 and 40 min recovery following the end of G4, the mean rate of plasma lactate disappearance was 30.6 (SD 14.2) mmol l^{-1} h^{-1}, similar to the disappearance rate following G1.

Plasma NH_3 peaked approximately 10 min post-exercise exercise and showed progressive increases with each gallop (Fig. 2) suggesting an increasing loss of AN with successive gallops. In horse Cp a maximum concentration of NH_3 close to 1 000 μmol l^{-1} plasma was observed after the fourth gallop. This is the highest value that we have recorded in any study. Further evidence of the extensive loss in AN is provided by the similar changes in plasma uric acid (Fig. 3). The continuing rise in uric acid showed that peak concentrations are usually not reached until 20–40 min post-exercise.

Mean haematocrits (l l^{-1}) prior to the first gallop (pre) and immediately after the 4 gallops were; pre—0.58 (SD 0.04), G1—0.63 (SD 0.05) G2—0.65 (SD 0.06), G3—0.64 (SD 0.06) and G4—0.65 (SD 0.06) l l^{-1}.

DISCUSSION

Based on previous experience of the 4 horses used in the study it was expected that Cp would be the fastest over the first gallop and Br the slowest. As shown in Table 1 this was the case with 6 s difference in running times over G1. What could not have been foreseen from the results of the first gallop was the marked deterioration which occurred in Cp's performance over the next 3 gallops culminating in a 15 s slower running time at G4. This was in contrast to the minimal

Table 1. *Running times (s) over the 4×700 m gallops (G1–G4)*

Horse	G1	G2	G3	G4	Total
Cp	44	46	50	59	199
Ki	47	47	49	50	193
Sa	47	48	51	54	200
Br	50	46	48	52	196
$\bar{X}$	47.0	46.8	49.5	53.8	
SD	3.0	1.0	1.3	3.9	

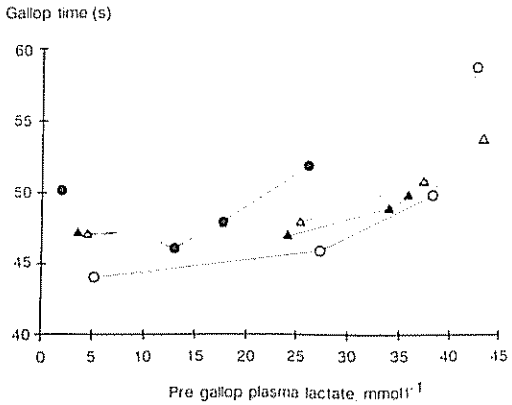

Fig. 4 Comparison of gallop times (s) to the pre-gallop plasma lactate concentration (mmol l^{-1}). Results of Cp, Sa and Ki showed a similar trend and data for these horses are joined, for purposes of illustration, by a continuous line. Data of horse Br showing a different trend are joined by a dashed line. ○, Cp; △, Sa; ●, Br; ▲, Ki

change seen in the performance of horse Br. The relative changes between these horses are similar to those seen in our previous study.[10] It was the intention of the present investigation to examine whether such changes in performance could be explained on the basis of increased muscle acidosis or some measure of AN loss.

Muscle acidosis

In Fig. 4 gallop times have been compared to the pre-gallop plasma lactate concentration as a measure of the degree of acidosis still persisting in the muscle at the start of each exercise. The use of the pre-gallop lactate concentration in this way is justified on the basis of earlier work demonstrating a close relationship between the lactate concentration in intra-muscle cell water and in the plasma water phase during recovery.[8] Secondly, throughout recovery a near linear relationship persists between muscle pH and the lactate content,[4,9] even if the work undertaken is intermittent. The results of 3 horses (Cp, Sa and Ki) showed a similar trend in the relationship between gallop time and plasma lactate, consistent with a deterioration in speed with increasing pre-exercise acidosis. For these 3 horses the relationship of gallop time to pre-gallop lactate could be adequately described by:

$$\text{gallop time (s)} = 45.8 - 0.1603 \times [\text{lactate}] + 0.0086 \times [\text{lactate}]^2$$

$r = 0.88$ (calculated with disregard to the between-horse variance in the relationship).

The results from the fourth horse, however, showed a totally different trend between gallop time and plasma lactate and, as such, question any general expression for these two parameters which would cover all horses. A comparison of gallop time to peak post-gallop lactate, as a measure of the acidosis in the muscles at the end of each gallop, gave a similar plot to that in Fig. 4, with similar conclusions.

Muscle AN loss As a measure of the extent of AN loss which has occurred prior to the start of each gallop we have used the accumulated rise in NH_3 ($\Sigma\Delta NH_3$) calculated over each proceeding gallop; in each case from the pre (for G1) or last 20 min value and the following peak concentration. The use of $\Sigma\Delta NH_3$ in this way is based upon earlier work showing a close correlation be-

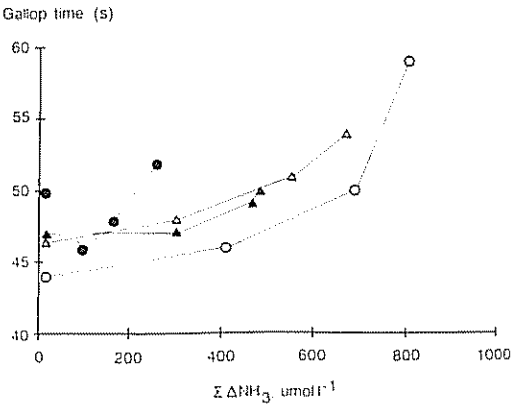

Fig. 5 Comparison of gallop times (s) to $\Sigma\Delta NH_3$ (μmol l^{-1}). Results of Cp, Sa and Ki showed a similar trend and data for these horses are joined, for purposes of illustration, by a continuous line. Data of horse Br, showing a different trend are joined by a dashed line. ○, Cp; △, Sa; ●, Br; ▲, Ki

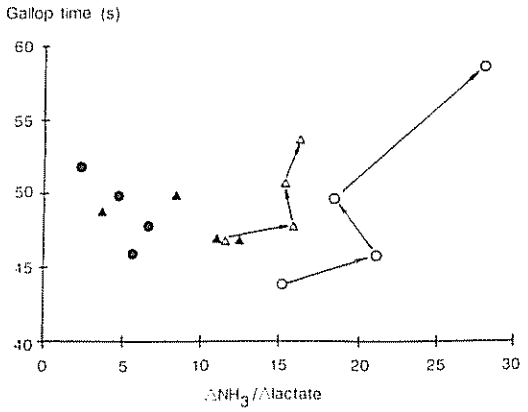

Fig 6 Comparison of gallop times (s) to the change in the plasma NH_3 concentration (μmol l^{-1}) with each gallop, relative to the change in lactate (mmol l^{-1}). Ratios were calculated without change in the units of concentration for NH_3 and lactate. ○, Cp; △, Sa; ●, Br; ▲, Ki. Data points for horses Cp and Sa over the 4 gallops have been joined in sequence.

tween the rise in plasma NH_3 with intense exercise and the loss of AN (to IMP) in the middle gluteal muscle,[6] and presumably therefore in the musculature as a whole. In comparison to the loss of AN, resynthesis from IMP is a slow process.[10]

Comparison of gallop times to $\Sigma\Delta NH_3$ (Fig. 5) showed a similar dispersal of points as in Fig. 4, with the same 3 horses (Cp, Sa and Ki) showing a common trend. For these 3 horses the relationship of gallop time to $\Sigma\Delta NH_3$ could be adequately described by:

$$\text{gallop time (s)} = 46.1 - 0.0057 \times [\Sigma\Delta NH_3] + 0.000024 \times [\Sigma\Delta NH_3]^2$$

$r = 0.91$ (calculated with disregard to the between-horse variance in the relationship).

As with lactate, however, the results of the fourth horse showed a different trend between gallop time and $\Sigma\Delta NH_3$. There was no apparent relationship between gallop time and the immediate post-gallop NH_3 concentration indicating that this in itself is unlikely to be an immediate cause of fatigue, at least over the concentration range observed.

Comparison of gallop times to "metabolic stress" The deamination of AN to IMP with high intensity exercise may be seen as a signal of increasing metabolic stress within the working muscle. However, the event of AN loss and the rate at which it is occurring, is possibly more important than the absolute loss itself. In Fig. 6 gallop times have been compared to the change in the plasma NH_3 concentration relative to that in lactate ($\Delta NH_3/\Delta$ lactate). In either case the change has been calculated from the difference between the immediate pre-gallop concentration and the eventual peak. The quotient is used as an experimental measure of the degree of "metabolic stress"; a rise in the rate of NH_3 accumulation relative to that of lactate indicating that this has increased. As can be seen there was no obvious association between gallop times and $\Delta NH_3/\Delta$ lactate.

General The loss of AN to IMP under conditions of high metabolic stress and high energy demand appears something of a paradox. As indicated earlier, AN loss can amount to as much as 40% of the muscle store in the equine middle gluteal.[6,10] Deamination of AMP to IMP provides an important mechanism for the displacement of the adenylate kinase reaction and through this the maintenance of low ADP concentrations at the site of contraction. This appears to be of greatest importance in fast twitch muscle fibres where the highest levels of AMP-deaminase are found.[12] In sprinting athletes the onset of AN loss appears to be linked to the acidification of the muscles, through lactate production, with possible implications to the fatigue process. The present paper failed to establish any association between the gallop times of successive runs and measures of AN loss or muscle acidosis based on measurements of plasma NH_3 and lactate. However, it was notable that Cp, which was both initially the fastest horse, but also the most fatiguable, showed a higher rate of NH_3 production relative to lactate than any other horse at each gallop. This is illustrated in Fig. 6 where the data points for Cp (and also those of Sa, the nearest to Cp) are joined in

sequence over the 4 gallops. Given that all 4 horses were approximately equally well trained, the overall picture obtained for Cp is that of a sprinter with a greater endowment of low-oxidative fast-twitch muscle fibres. As recently reported for human athletes,[2] the measurement of the plasma NH_3 response to exercise may help in the identification of potential sprinters.

REFERENCES

1 Boobis, L H, Williams, C and Wooton, S A (1982) Human muscle metabolism during brief maximal exercise J Physiol (Lond) 338, 21–22.

2. Hageloch, W, Schneider, S and Weicker, H (1990) Blood ammonia determination in a specific field test as a method supporting talent selection in runners Int J Sport Med 11, Suppl 2, pp 56–61

3 Harris, R C and Hultman, E (1985) Adenine nucleotide depletion in muscle in response to intermittent stimulation in situ J Physiol (Lond) 365, 73P

4. Harris, R C, Katz, A, Sahlin, D and Snow, D H (1989) Effect of freeze-drying on measurements of pH in biopsy samples of the middle gluteal muscle in the horse: comparison of muscle pH to the pyruvate and lactate content. Equine Vet J 21, 45–47.

5 Harris, R C, Marlin, D J and Snow, D H (1987). Metabolic response to maximal exercise of 800 and 2000 m in the Thoroughbred horse J Appl Physiol 63, 12–19

6 Harris, R C, Marlin, D J, Snow, D H and Harkness, R A (1991) Muscle ATP loss and lactate accumulation at different work intensities in the exercising Thoroughbred horse Eur J Appl Physiol 62, 235–244

7 Hohorst, H S (1963) L-(+)-lactate *In* Bergmeyer, H U (ed): *Methods of Enzymatic Analysis* Academic Press, New York, pp 266–270

8 Marlin, D J, Harris, R C, Harman, J and Snow, D H (1987) Influence of post-exercise activity on rates of muscle and blood lactate disappearance in the Thoroughbred horse *In* Gillespie, J R and Robinson, N E (eds): *Equine Exercise Physiology 2* ICEEP Publications, Davis, CA, pp 321–331

9 Sahlin, D, Harris, R C, Hultman, E and Nylind, B (1976) Lactate content and pH in muscle samples obtained after dynamic excercise Pflügers Arch 367, 143–149

10 Snow, D H, Harris, R C and Gash, S P (1985) Metabolic response of equine muscle to intermittent maximal exercise J Appl Physiol 58, 1689–1697

11 Wagenmakers, A J M, Coakley, J H and Edwards, R H T (1990) Metabolism of branched-chain amino acids and ammonia during exercise: Clues from McArdle's disease Int J Sports Med 11, 5101–5113

12 Winder, W W, Terjung, R L, Baldwin, K M and Holloszy, J O (1974) Effect of exercise on AMP deaminase and adenylosuccinase in rat skeletal muscle Am J Physiol 227, 1411–1414