

- Sugden, P. H. and Newsholme, E. A. (1975). Effects of ammonium, inorganic phosphate and potassium ions on the activity of phosphofructokinases from muscle and nervous tissue of vertebrates and invertebrates. *Biochem. J.* **150**, 113–122.
- Valberg S. (1986). Glycogen depletion patterns in the muscle of Standardbred trotters after exercise of varying intensities and durations. *Equine Vet. J.* **18**: 479–484.
- Valberg, S. and Essén-Gustavsson, B. (1986). Metabolic response to racing in pools of type I, IIA and IIB fibres. In: *Equine Exercise Physiology 2*. Gillespie, J. R., and Robinson, N. E. (eds.), ICEEP Publications, Davis CA, pp. 290–301.

Acute Changes in the Water Content and Density of Blood and Plasma in the Thoroughbred Horse During Maximal Exercise: Relevance to the Calculation of Metabolite Concentrations in These Tissues and in Muscle

R. C. HARRIS, J. C. HARMAN, D. J. MARLIN and D. H. SNOW
Physiology Unit, The Animal Health Trust, Balaton Lodge, Snailwell Road,
Newmarket, Suffolk CB8 7DW, England.

Summary

The changes with exercise and recovery in the density (D) and volume water content (Vw) of blood and plasma were studied in four Thoroughbreds galloped at 12 m/s for 2 minutes on a treadmill at 5° incline. Packed cell volume (PCV), D and Vw were measured in samples of venous blood collected throughout the exercise period and during 70 minutes recovery. Total protein (TP), D and Vw were measured in plasma.

TP increased 10.98 ± 3.45 g/l with exercise from a pre-exercise mean of 61.29 ± 3.88 g/l. This was associated with a small decrease in plasma Vw of 15 ± 2 ml/l from a pre-exercise mean of 943 ± 4 ml/l but minimal changes in density. PCV of blood increased 0.208 ± 0.059 l/l from a pre-exercise mean of 0.425 ± 0.065 l/l, recovery taking 50 to 60 minutes. Blood Vw decreased 61 ± 14 ml/l with exercise from 832 ± 81 ml/l whilst D increased 0.01723 ± 0.00542 g/ml from 1.05714 ± 0.00564 g/ml. Plasma Vw and D were significantly correlated to TP, as were blood Vw and D to PCV. The use of appropriate values of Vw and D to minimize errors in the calculation of blood lactate concentration and muscle lactate content are discussed.

Index terms: Blood lactate; muscle metabolites; muscle biopsies.

Introduction

Intense exercise by the horse results in a marked output of red blood cells from the spleen. At maximal work rates this can result in almost a doubling of the hematocrit (Snow *et al.*, 1983). Intense exercise has also been shown to result in increases in the concentration of protein in plasma both in the horse (Poso *et al.*, 1983; Snow *et al.*, 1983) and in man (Delanne *et al.*, 1958; Joyce and Poortmans, 1970). Changes in both hematocrit and plasma proteins will affect the colligative properties of blood and plasma, values of which are frequently employed in the calculation of metabolite concentrations based on assays of perchloric acid extracts. The most important of these are water content and density, which in the absence of specific data on the horse, have previously

been assumed constant. The present study examines the changes in water content and density which occur with exercise in both blood and plasma and illustrates their use in the calculation of metabolite concentrations in plasma, blood and muscle.

Materials and Methods

Experimental protocol. The present findings were obtained from four of the five Thoroughbred geldings used in the study of Marlin, *et al.* (1986) reported elsewhere in these proceedings. These were SM, HR, JW and SL. Horses were fed a commercial cubed diet and allowed free access to water.

Each horse participated in three experimental sessions separated by at least one week. At each session the horse was catheterized for collection of blood during exercise. One ml of 2% lignocaine was placed over a site on the left jugular vein followed by insertion of a 6-inch 14 gauge catheter. The catheter and a 75 cm extension tube were taped into place using adhesive tape. Each horse was harnessed, led onto the treadmill (Sato, Sweden), attached to the overhead safety strap and allowed to stand quietly for 2 to 3 minutes. The exercise consisted of a 4 minute walk (1.6 m/s), 4 minute trot (3.2 m/s) and 50 s of acceleration to 12 m/s followed by a 2 minute gallop (12 m/s). All exercise was done against an incline of 5°.

The 2-minute gallop was followed by one of 3 modes of recovery. In the first mode (T), the treadmill was decelerated to 3.2 m/s (trot) and maintained at this speed for 30 minutes. Thereafter the speed was lowered to 1.6 m/s for a further 40 minutes to give a total recovery time of 70 minutes. In the second mode (W) the recovery speed was maintained at 1.6 m/s for the full 70 minutes and in the third mode (S) the horse was allowed to stand. During recovery the treadmill was lowered to 0° incline.

Sampling. Muscle samples were taken with a 5 mm Bergström-Stille needle as previously described (Snow 1983). One sample was taken just before the horses were led onto the treadmill and others 1, 10, 30 and 70 minutes into the recovery period. Muscle samples were taken during sessions with T and S recoveries.

Ten-ml samples of blood were collected into lithium heparin tubes from which plasma was obtained by centrifugation. Blood samples were taken before exercise and at regular intervals during recovery. For determination of plasma and blood density (P-D and B-D respectively, g/ml) and plasma and blood volume water content (P-Vw and B-Vw respectively, ml/l), 2×250 ul aliquots of well mixed blood or plasma at room temperature were pipetted into weighed 1.5 ml polypropylene tubes using a calibrated Gilson Microman positive displacement pipette. Tubes were reweighed to 0.1 mg and from the difference in weights P-D and B-D were calculated. The 0.5 ml aliquots of blood or plasma were then freeze-dried to constant weight. Total removal of water was checked by further drying of samples in an oven set at 40°C. P-Vw and B-Vw were calculated from the reduction in weight assuming a density of water of 1 g/ml.

For determination of lactate 2 parts of blood or plasma were extracted with 5 parts 1 mol/l perchloric acid. After centrifugation, extracts were assayed for lactate using lactate dehydrogenase (Hohorst 1963). Muscle samples were freeze-dried, extracted and assayed for adenosine triphosphate (ATP), phosphocreatine (PCr), creatine (Cr) and lactate (Harris *et al.*, 1974).

Packed cell volume of blood (B-PCV, l/l) was determined by centrifugation of microhematocrit tubes at 15,000 g for 5 minutes. Plasma total protein (P-TP, g/l) was determined using the Biuret method adapted to the IL Multistat III centrifugal analyzer.

Statistical analysis For the purpose of this study, observations were made on HR during all three recovery modes, from SM during (S) and (T) recoveries, from SL during (S) and (W) recoveries and from JW during (S) recovery. Changes in variables with exercise were assessed using the standard t test for paired data. Where more than one set of data within a horse were available, the mean change for that horse was used. Values are given as means \pm SD.

In the comparisons of P-D and P-Vw with P-TP, and B-D and B-Vw with B-PCV only the data obtained in the sessions with (S) recovery were used. Data from the four horses were analyzed together by simple linear regression analysis, with the assumption that the between-horse variance in the relationship was minimal as indicated by comparison of within-horse regressions. In certain of the comparisons, the data were characterized by a high degree of clustering of values within horses, which precluded the use of other analyses, such as multiple linear regression analysis with a dummy variable for horse, in which no such assumptions are required.

Results

The changes in TP, Vw and D of plasma and of PCV, Vw and D of blood during exercise and recovery were similar in all four horses. The results from two, in either case with recovery modes of (S) and (T) are illustrated in Fig. 1 and 2. As far as could be judged there were no differences in the rates of recovery in any of the parameters with (S), (W) or (T) recoveries. With the exception of P-D, maximum changes from rest in each of the parameters were recorded with the 2-minute post exercise sample. In the case of P-D, maximum change was recorded after 4 minute recovery.

Based on all four horses, mean P-TP increased 10.98 ± 3.45 g/l ($P < 0.01$) with exercise from a mean content at rest of 61.29 ± 3.88 g/l. Recovery of P-TP back to resting values took approximately 50 minutes. In comparison the changes in P-Vw and P-D were small. P-Vw decreased 15 ± 2 ml/l ($P < 0.01$) from a mean at rest of 943 ± 4 ml/l, whilst P-D showed a non-statistically significant increase of 0.00022 ± 0.0050 g/ml from a mean at rest of 1.02478 ± 0.00029 g/ml. Both P-D and P-Vw were significantly correlated to P-TP (Fig. 3):

$$\begin{aligned} \text{P-D (g/ml)} &= 1.00103 + 0.00036[\text{P-TP}] \\ n &= 64, r = 0.74 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{P-Vw (ml/l)} &= 997V168 - 0.921[\text{P-TP}] \\ n &= 64, r = 0.90 \end{aligned} \quad (2)$$

Mean B-PCV showed an increase of 0.208 ± 0.059 l/l ($P < 0.01$) by the end of the 2 minute treadmill exercise at 12 m/s from a mean at rest of 0.426 ± 0.065 l/l. Recovery of B-PCV back to resting values took 50–60 minutes. Changes in B-Vw and B-D of blood were large in comparison to those in plasma. B-Vw decreased 61 ± 14 ml/l ($P < 0.01$) from a mean at rest of 832 ± 81 ml/l, whilst B-D increased 0.01723 ± 0.00542 g/ml ($P < 0.01$) from a mean of 1.05714 ± 0.00564 g/ml. Both B-D and B-Vw were significantly correlated to B-PCV (Fig. 4):

$$\begin{aligned} \text{B-D (g/ml)} &= 1.01926 + 0.08717[\text{B-PCV}] \\ n &= 67, r = 0.96 \end{aligned} \quad (3)$$

$$\begin{aligned} \text{B-Vw (ml/l)} &= 942 - 260[\text{B-PCV}] \\ n &= 67, r = 0.98 \end{aligned} \quad (4)$$

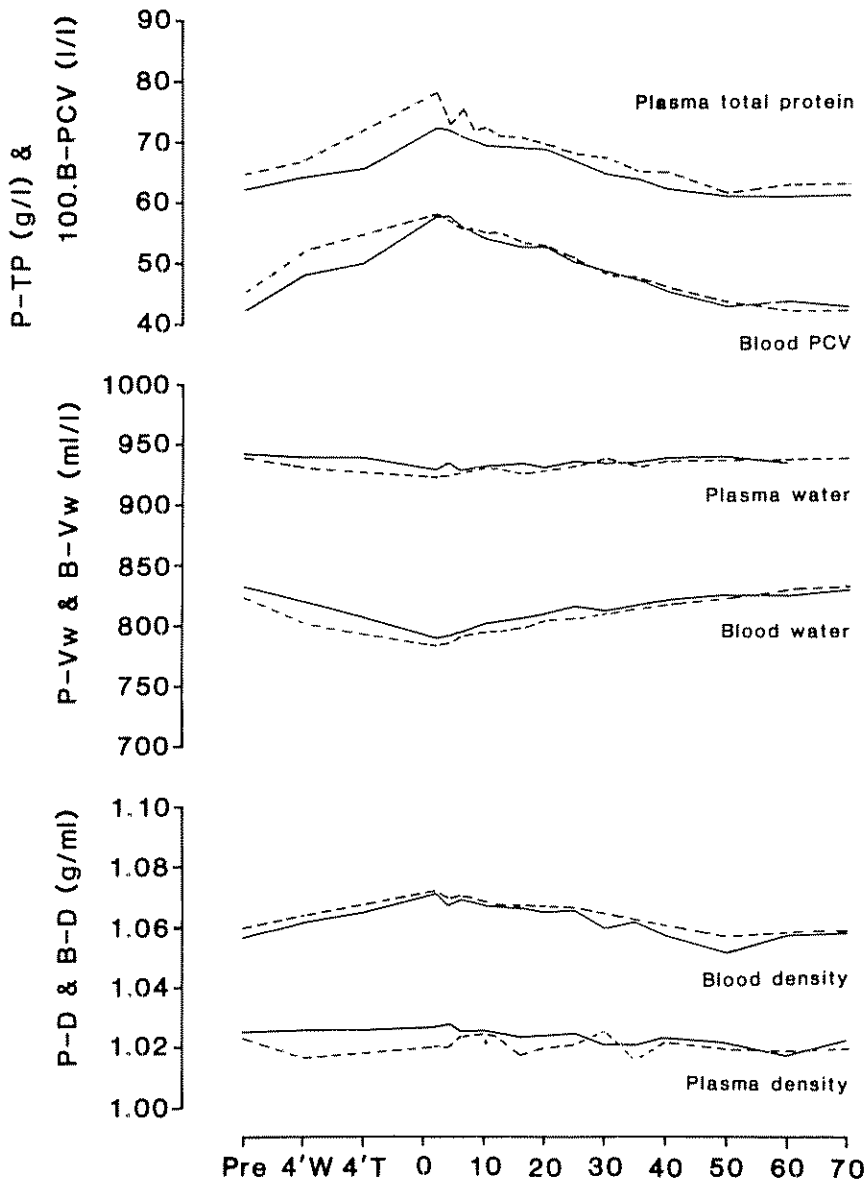


FIGURE 1. Plasma total protein (P-TP), volume water content (P-Vw) and density (P-D), and blood packed cell volume (B-PCV), volume water content (B-Vw) and density (B-D), before exercise (Pre), after 4 minutes walking (4'W) and trotting (4'T) and during 70 minutes recovery from a 2 minute gallop at 12 m/sec. Dashed lines—(T) recovery Continuous lines—(S) recovery Horse HR.

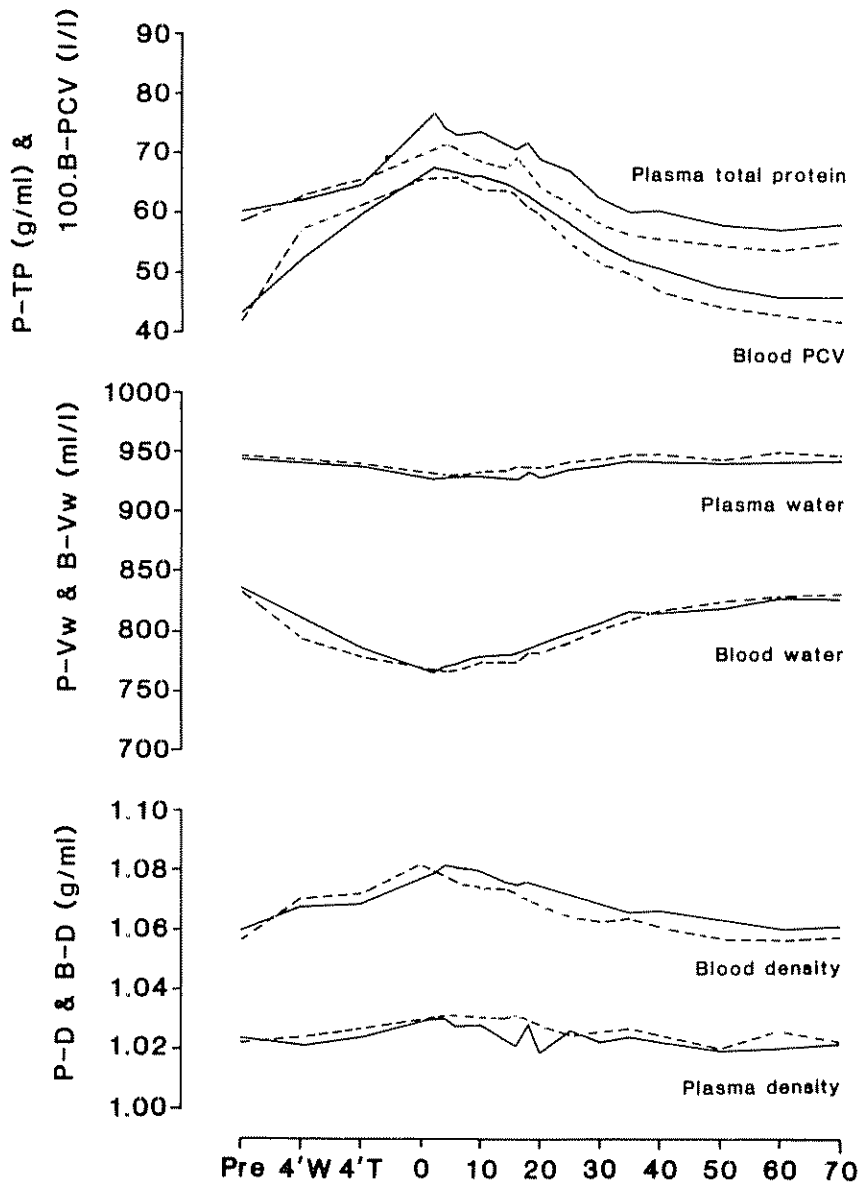


FIGURE 2 Plasma total protein (P-TP), volume water content (P-Vw) and density (P-D), and blood packed cell volume (B-PCV), volume water content (B-Vw) and density (B-D), before exercise (Pre), after 4 minutes walking (4'w) and trotting (4'T) and during 70 minutes recovery from a 2 minute gallop at 12 m/sec. Dashed lines—(T) recovery. Continuous lines—(S) recovery. Horse SM.

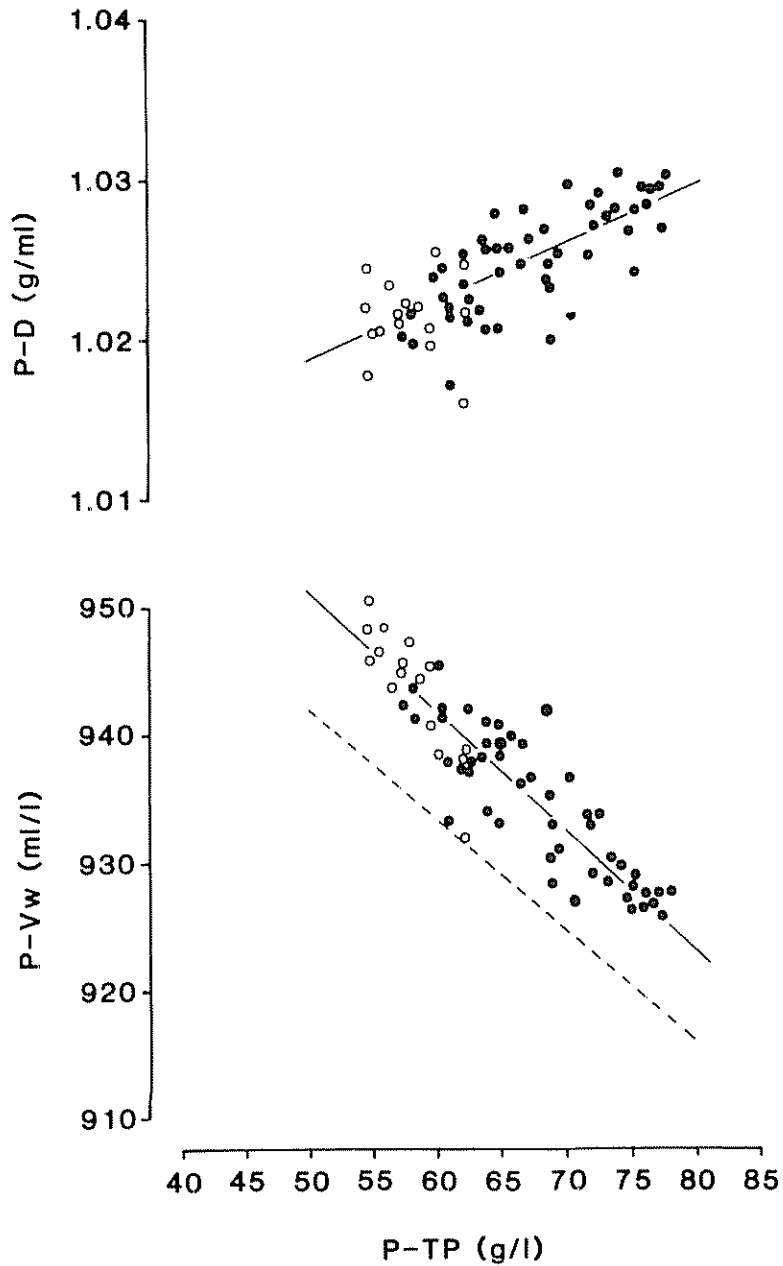


FIGURE 3. Comparison of plasma density (P-D) and volume water content (P-Vw) to plasma total protein (P-TP). Only the four sets of data obtained with (S) recovery are included in the figure. Data from one horse (SL) are shown as open circles to illustrate the degree of clustering which was particularly marked in the comparison of P-D with P-TB. The linear regression lines were calculated from eq. 1 and 2 in the text. The dashed line shows the relationship between P-Vw and P-TP reported in Carlson and Harrold (1977).

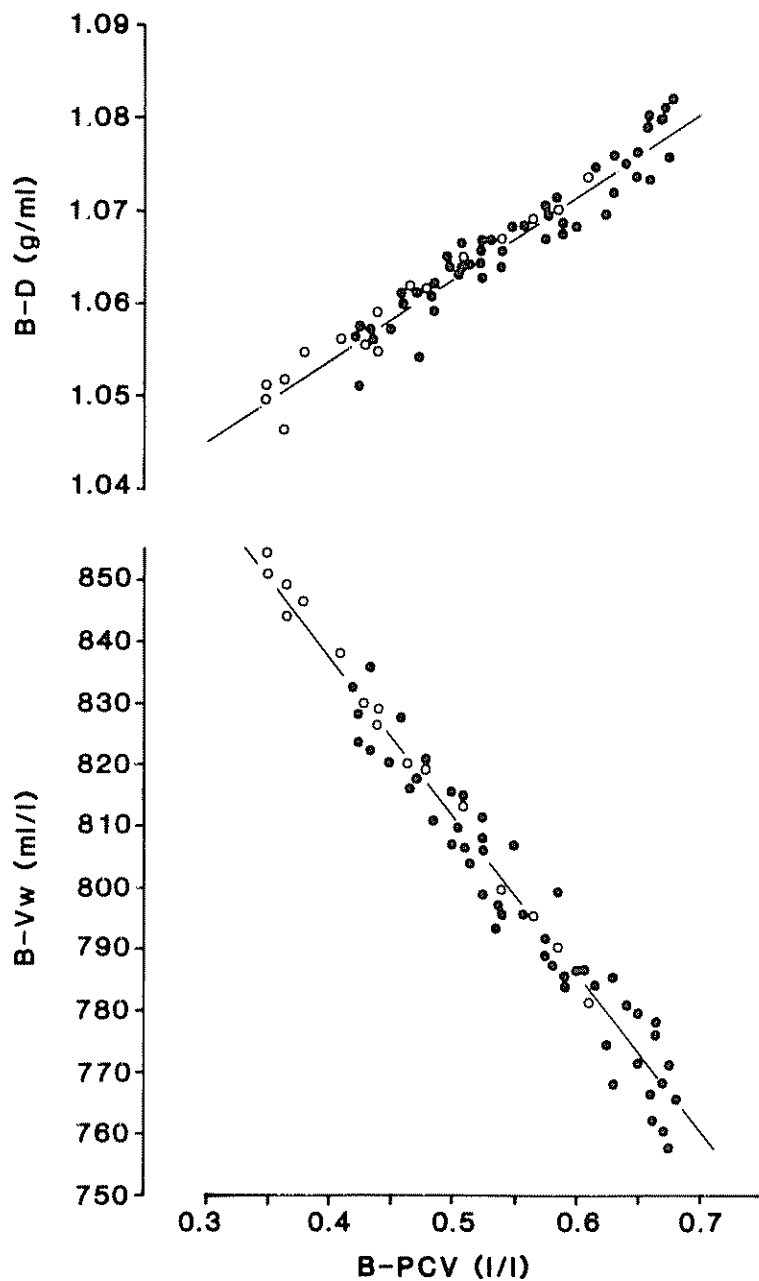


FIGURE 4. Comparison of blood density (B-D) and volume water content (B-Vw) to packed cell volume (B-PCV). Only the four sets of data obtained with (S) recovery are included in the figure. Data from one horse (SL) are shown as open circles. The linear regression lines were calculated from eq. 3 and 4 in the text.

Discussion

The purpose of the present study was to define the changes in density and water content of blood, with acute exercise in horses with normal hydration. Horses dehydrated either through diet or as a result of prolonged activity will likely show different relationships between B-D and B-Vw with B-PCV to those described in Fig. 4, though in the case of plasma, the relationships described in Fig. 3 will probably continue to hold true.

In the comparison of P-Vw with P-TP a slightly different relationship was found to that previously reported by Carlson & Harrold (1977) shown as a dashed line in Fig. 3 and derived from the analysis of 53 horses. Although the slopes of the two regression lines were similar there was a marked difference in intercepts. In the study of Carlson & Harrold (1977) the estimated P-Vw at a protein concentration of 0 g/l was 985 ml/l implying a volume of 15 ml/l for non-protein solids. In this study, the corresponding estimates were 997 ml/l and 3 ml/l. The difference between the two results is unlikely to have arisen as a result of failure to adequately dry samples, the method for which was more extreme in the study of Carlson & Harrold (1977). More likely it reflects differences between laboratories in the protein determinations, and differences in the horses used.

Determination of blood and plasma metabolite concentrations. A procedure commonly employed for the assay of blood samples is to add an approximate volume of blood to a weighed tube (W_1) containing a known volume of perchloric acid (V_{pca}). The tube is reweighed (W_2) and the volume of blood (B-V) is calculated from: $B-V \text{ (ml)} = (W_2 - W_1)/B-D$. The volume of acid extract (V_{ext}) obtained after centrifugation is calculated from: $V_{ext} \text{ (ml)} = (B-V - B-Vw)/1000 + V_{pca}$. In the past, values of 1.04 g/ml for B-D and 800 ml/l for B-Vw based on human data and cited in Bergmeyer (1970), have been used. In Table 1 concentrations of lactate in blood calculated using these estimates for B-D and B-Vw (row a) are shown together with concentrations

TABLE 1. Blood and plasma lactate concentrations before and after 2 minutes treadmill exercise at 12 m/sec and during 70 minutes standing (S) recovery in horse JW.

	Pre	Recovery time (mins)			
		0	10	30	70
B-PCV (l/l)	0.51	0.68	0.66	0.59	0.51
P-TP (g/l)	65.80	76.50	77.30	72.50	63.30
Lactate (mmol/l)					
a) blood	0.68	15.81	21.50	19.10	7.98
b) blood (corrected)	0.69	16.08	21.85	19.43	8.14
c) plasma	0.45	26.76	35.51	27.88	12.06
d) plasma (corrected)	0.45	26.65	35.34	27.81	12.08
e) plasma water (corrected)	0.48	28.75	38.16	29.89	12.87

- a) B-D and B-Vw taken as 1.040 g/ml and 800 ml/l, respectively
 b) B-D and B-Vw estimated from the changes in B-PCV according to eq. 3 and 4.
 c) P-Vw taken as 935 ml/l.
 d) P-Vw estimated from the changes in P-TP according to eq. 2.
 e) Concentrations in d) divided by P-Vw/1000 estimated from P-TP.

calculated using values based on the changes in B-PCV (row b).

A similar approach may be followed in the calculation of plasma lactate concentrations, based on the assay of perchloric acid extracts. However, the practice in our own laboratory is to pipette a measured volume of plasma into the perchloric acid and thus only P-Vw is required for the calculation of acid extract. Because of the very small changes in P-Vw with exercise it is as good to use a constant value for P-Vw (Table 1, row c) as one estimated from the changes in P-TP (row d). The lactate concentration in the plasma water phase (Table 1, row e) is obtained by dividing the plasma concentration by the fractional water content (P-Vw/1000).

Correction of muscle samples for contamination by blood. Contamination of muscle biopsies by varying amounts of blood is a major problem in samples taken after exercise. To some extent this can be minimized by the careful dissection of samples after freeze-drying but even then contamination can account for up to 40% of the sample weight. To allow for such variations in blood contamination, the contents of muscle located metabolites measured in a sequence of biopsies may be referred to a constant value of total creatine (TCr) which acts as an internal reference (Harris *et al.*, 1976). This will additionally correct for errors arising from the variable inclusion in the muscle samples of other non-muscle-cell elements such as connective and fat tissues and variations in muscle glycogen content (an important consideration in endurance studies). Other likely reference bases are NAD (Sabina, *et al.*, 1984) and DNA. Biopsy contents of metabolites which occur in significant amounts in both muscle and blood, such as lactate and glucose, cannot be adjusted by this means until the amount present in the contaminating blood has been eliminated. To do this, a muscle blood content at rest of 10 ml/kg wet muscle was assumed (Dahlberg 1983). Based on a 75% muscle water content the estimated blood content per kg dry muscle (d.m.) was 40 ml which after drying would yield approximately 10 g of dried blood. Although creatine is found in blood, its concentration is so low (Wu, *et al.*, 1983) compared to muscle that its contribution to the apparent TCr content in the biopsy can be ignored, even in the most heavily contaminated biopsies. Thus, the TCr content of bloodless muscle (TCr') is given by $TCr' = TCr/0.99$ where both TCr' and TCr are in mmol/kg dry muscle solids (d.m.s.). TCr' can now be used as a reference base to calculate within a series of muscle biopsies the volume of blood contaminating the sample. For this values of B-D and B-Vw are required.

$$\text{Blood contamination (l/kg d m s)} = 1000(1 - TCr/TCr')/(1000 \cdot B-D - B-Vw) \quad (5)$$

From knowledge of the blood concentration (B-C, as mmol/l) of the metabolite being assessed the amount in the biopsy sample can be calculated. If this is removed from the measured biopsy content (M-C as mmol/kg d m s) and the result multiplied by TCr'/TCr the true muscle content expressed as mmol/kg blood free dry muscle is obtained.

$$\begin{aligned} \text{True muscle content (mmol/kg d m s)} \\ = [M-C - (B-C \cdot 1000) \cdot (1 - TCr/TCr')]/(1000 \cdot B-D - B-Vw) \cdot TCr'/TCr \quad (6) \end{aligned}$$

For metabolites such as ATP where the B-C is very much less than that in muscle eq. 6 reduces to:

$$\text{True muscle content} = M-C \cdot TCr'/TCr.$$

Table 2 lists the muscle contents of TCr, ATP and lactate in horse JW before exercise and during 70 minutes recovery. Raw data show a pronounced drop in TCr in the 30 minute sample indicating heavy contamination by blood. This is reflected also in the ATP content. To compensate for changes in "muscle purity" values in b have been adjusted to a standard TCr content. This is justified by the small within-muscle variance in TCr. This greatly improves the trend in ATP within the series (and also of other metabolites located principally within muscle) but not of lactate. In the latter case both muscle and blood lactate have been affected by the calculation. The estimated blood contents of the individual samples are shown in table 2c. These contents are for biopsy samples which have been dissected free of any obvious blood, but in the case of the 30 minute sample still shows a content equivalent to 1.5 l for every kg of dry muscle solids. The true muscle contents of lactate shown in table 2d were calculated using the blood concentrations given in row b of Table 1. Because of the low concentrations of ATP in blood, no further correction to the muscle ATP contents was made. In table 2d muscle lactate contents clearly show a more orderly progression with recovery than in table 2b. The apparent similarity between lactate contents in table 2a and d is due to the lactate content of dry blood being similar to that of dry muscle, however, this will not always be the case particularly with metabolites such as glucose or glycerol. In the correction of muscle lactate contents it was assumed that the concentration of lactate in venous blood (collected from the jugular vein) can be used to estimate the concentration in blood perfusing the middle gluteal muscle. Only in table 2d are both ATP and lactate correct.

In summary we have demonstrated that when concentrations of metabolites in blood

TABLE 2 Muscle contents of total creatine, adenosine triphosphate, and lactate before and after 2 minutes treadmill exercise at 12 m/sec and during 70 minutes standing (S) recovery in horse JW.

	Pre	Recovery time (mins)			
		0	10	30	70
a) Raw data (mmol/kg d m s.)					
TCr	102.05	91.08	93.35	59.85	103.05
ATP	20.92	9.48	11.72	7.41	18.55
Lactate	9.41	128.17	83.09	63.26	13.58
b) Adjusted to TCr standard of 102.05/0.99 = 103.08 mmol/kg d m					
ATP	21.27	10.72	12.95	12.77	18.56
Lactate	9.51	145.66	91.74	108.96	13.58
c) Blood contamination					
l/kg d m s	0.040	0.372	0.308	1.487	0.000
d) Corrected for blood and adjusted to TCr standard of 103.08 mmol/kg d m.					
ATP	21.22	10.72	12.95	12.77	18.56
Lactate	9.48	138.28	84.32	59.19	13.58

c) and d) were calculated from eq. 5 and 6 using eq. 3 and 4 to estimate B-D and B-Vw from the changes in B-PCV (Table 1). Blood lactate concentrations were taken from row b, of Table 1. The contribution of blood borne ATP and TCr to the muscle contents were considered negligible. ATP = adenosine triphosphate, TCr = total creatine

are low compared to muscle it is sufficient to refer muscle contents to a standard TCr content, e.g. ATP. However, when the concentration of metabolites in blood is high, muscle contents should be corrected using eq. 6. If this is not possible no correction should be applied (though muscle located metabolites such as ATP should still be corrected as in equation 1)

Acknowledgments

The assistance of Pauline Ferrie and Tracey Redworth in caring for the horses and helping during the experiments is greatly appreciated.

References

- Bergmeyer, H. U. (1970). *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim.
- Carlson, G. P. and Harrold, D. R. (1977). Relationship of protein concentration and water content of equine serum and plasma samples. *Vet. Clin. Pathol.* **6**, 18–20.
- Dahlberg, E. (1983). Estimation of blood contamination of tissue extracts. *Analyt. Biochem.* **130**, 108–113.
- DeLanne, R., Barnes, J. R. and Brouha, L. (1958). Changes in concentration of plasma protein fractions during muscular work and recovery. *J. Appl. Physiol.*, **13**, 97–104.
- Harris, R. C., Hultman, E. and Nordesjö, L. O. (1974). Glycogen, glycolytic intermediates and high energy phosphates in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand. J. Clin. Lab. Invest.*, **33**, 109–120.
- Harris, R. C., Edwards, R. H. T., Hultman, E., Nordesjö, L. O., Nylind, B. and Sahlin, K. (1976). The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. *Pflügers Arch.* **367**, 137–142.
- Hohorst, H. S. (1963). L – (+) – lactate. pp 266–270 in Bergmeyer, H. U. (ed). *Methods of Enzymatic analysis*. Academic Press, New York & London.
- Joyce, H. and Poortmans, J. R. (1970). Haematocrit and serum proteins during arm exercise. *Med. Sci. Sports.*, **2**, 187–190.
- Marlin, D. J. Harris, R. C., Harman, J. C. and Snow, D. H. (1986). Influence of post-exercise activity on rates of muscle and blood lactate disappearance in the Thoroughbred horse. *Equine Exercise Physiology 2*. Gillespie, J. R., and Robinson, N. E. (eds.), ICEEP Publications, Davis CA, pp. 321–331.
- Pösö, A. R., Soveri, T. and Oksanen, H. E. (1983). The effect of exercise on blood parameters in Standardbred and Finnish-bred horses. *Acta Vet. Scand.*, **24**, 170–184.
- Sabina, R. L., Swain, J. L., Bradley, W. G. and Holmes, E. W. (1984). Quantitation of metabolites in human skeletal muscle during rest and exercise: a comparison of methods. *Muscle and Nerve*, **7**, 77–82.
- Snow, D. H. (1983). Skeletal muscle adaptations: a review. In: *Equine Exercise Physiology*. Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge, pp 160–183.
- Snow, D. H., Mason, D. K., Ricketts, S. W. and Douglas, T. A. (1983). Posttrace

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 ± 6.1 – 82.1 ± 3.3 $\mu\text{mol/l}$, which was lower than the accumulation of ammonia from 36.7 ± 8.8 – 113.3 ± 11.0 $\mu\text{mol/l}$ in unconditioned horses. Before conditioning, glutamate increased from 10.4 ± 9 – $16.3 \pm .6$ $\mu\text{mol}/100$ ml during the exercise test, and alanine increased from 38.5 ± 2.3 – 79.4 ± 4.7 $\mu\text{mol}/100$ ml. After the conditioning period, alanine significantly increased from 33.3 ± 2.2 – 70.7 ± 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.*,