

- buffer concentration on the efflux of lactate from frog sartorius muscle. *J. Physiol. (London)* **250**, 1–22.
- Milne, D. W., Skarda, R. T., Gabel, A. A., Smith, L. G. and Ault, K. (1976). Effects of training on biochemical values in Standardbred horses. *Am. J. Vet. Res.* **37**, 285–290.
- Sutton, J. R., Jones, N. L. and Toews, C. J. (1981). Effect of pH on muscle glycolysis during exercise. *Clin. Sci.* **61**, 331–338.
- Wilkes, D., Gledhill, N. and Smyth, R. (1983). Effect of acute induced metabolic alkalosis on 800 m racing time. *Med. Sci. Sports Exerc.* **15**, 277–280.

Blood and Muscle Ammonia Concentrations in Horses during Treadmill Work and after Racing

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Summary

Blood ammonia and lactate concentrations were analyzed during treadmill work of different intensities. In addition, muscle biopsies were obtained from 3 horses before and after standardized treadmill work and in 14 horses after racing for analysis of ammonia, lactate and adenosine triphosphate (ATP) concentrations, as well as citrate synthase activity and fiber type composition. An accumulation in blood of ammonia and lactate was only found at higher work intensities. Fiber type composition differed between horses and so did metabolite concentrations after racing. Ammonia concentrations in muscle were increased after exercise and positively correlated to lactate concentrations while a negative correlation was seen with ATP concentrations. Furthermore, the percentage of type II fibers in the biopsy was positively correlated with ammonia concentrations after exercise and negatively correlated to ATP concentrations.

It is concluded that ammonia concentrations increase in both muscle and blood at higher work intensities. Furthermore, the data indicate that during intense exercise adenosine monophosphate deamination may play an important role in type II fibers.

Index terms: Horse; fiber types; lactate; ammonia

Introduction

Skeletal muscle is composed of fibers having slow twitch (type I) or fast twitch (type II) characteristics and during exercise the fibers are recruited in different patterns which depend on the intensity and duration of the performed work (Saltin and Gollnick, 1983; Armstrong and Laughlin, 1985). The immediate source for energy during muscular work is adenosine triphosphate (ATP) which continuously is replenished either through oxidative processes, through glycolysis with lactate formation or through phosphagen breakdown. However, the capacity for ATP supply through oxidative or glycolytic pathways differs between fiber types (Saltin and Gollnick, 1983; Pette, 1985).

In rats when ATP turnover rate is high, such as with intense treadmill work or electrical stimulation, not only lactate accumulates in muscle but also ammonia and IMP (Meyer and Terjung, 1979; Meyer *et al.*, 1980). Activation of the purine nucleotide cycle and deamination of AMP give rise to IMP and ammonia in muscle (Lowenstein,

1972). The AMP deamination is catalyzed by the enzyme AMP deaminase and the activity of this enzyme in the rat is higher in fast twitch than in slow twitch fibers (Meyer and Terjung, 1979). Furthermore, the greatest accumulation of AMP and ammonia occurs in those muscles containing a high percentage of low-oxidative fast twitch fibers (Dudley and Terjung, 1985a).

Thus, with intense exercise where ATP utilization is very high, the type of muscle fibers recruited and the oxidative capacity of the fibers may play an important role for ammonia production. In both rat and man during high intensity work, a relationship exists between blood lactate and blood ammonia levels (Babij *et al.*, 1983; Dudley *et al.*, 1983). In addition, an inverse relationship has been obtained between the percentage of type I fibers in the vastus lateralis muscle of man and the increase in blood ammonia produced by intense bicycle exercise (Dudley *et al.*, 1983).

The purpose of this study of Standardbred trotters was to investigate the relationship between blood lactate and ammonia concentrations during exercise of varying duration and intensity. In addition, it was of interest to elucidate the role of AMP deamination during maximal exercise particularly in relation to muscle fiber type composition.

Material and Methods

Treadmill work. Nine Standardbred trotters, with a mean age of 5 years (range, 2–10), were used in the studies with treadmill exercise. Three horses performed 3 separate exercise sessions on the flat either at 6 m/sec for 105 min or at 10 m/sec for 14 min or a standardized exercise test was performed with speeds increasing from 6 m/sec to 9 m/sec with a 2 min trotting duration at each speed. Each horse had a catheter in the jugular vein and samples were taken at rest and at regular intervals during the exercise sessions. The blood samples were taken after 5, 15, 30, 60, 90 and 105 min at 6 m/sec and after 4, 6, 8, 10, 12 and 14 min at 10 m/sec. During the standardized exercise test, blood samples were taken at the end of each 2 min period.

Six other horses performed a standardized exercise test with incrementally increasing speeds of 6 m/sec to 9 m/sec of 2 min duration on a 6.25% slope. In three of the horses venous blood samples were taken every 2 min while in the other three horses gluteus medius muscle biopsies were taken before and immediately after exercise.

Racing. Fourteen Standardbred racehorses (8 mares, 3 geldings, 3 stallions) with a mean age of 4 years (range, 3–8) were studied in connection with racing. Thirteen of the horses competed over distances of 2140 m whereas one horse raced over 1650 m. Gluteus medius muscle biopsies were obtained as soon as possible after racing. Mean time elapse from the race's conclusion to the time of biopsy was 6 min (range, 5–12).

Blood samples. A portion of the blood sample was put into heparinized tubes, kept on ice and a portion of the blood was immediately deproteinized in chilled perchloric acid. Plasma was collected as soon as possible after centrifugation and stored at -80°C until analyzed for ammonia using a modified enzymatic fluorimetric technique (Kun and Kearney, 1974). Blood lactate was analyzed spectrophotometrically using an enzymatic method (Boehringer Kit Test Combination No. 124842).

Muscle biopsies. Muscle biopsies were taken as previously described by Lindholm and Piehl (1974). One piece of muscle for biochemical analysis was immediately frozen in liquid nitrogen while another piece for histochemical analysis was rolled in talcum powder before being frozen.

Biochemical analyses. The frozen muscle tissue was weighed using a Cahn electro-balance and the tissue was extracted in cold perchloric acid. After neutralization, ammonia, lactate and ATP were analyzed using enzymatic fluorimetric techniques as described by Kun & Kearney (1974) and Lowry and Passonneau (1972). Citrate synthase activity (CS) was analyzed on freeze-dried muscle as described by Essén *et al.* (1980).

Histochemical analyses. Tissue sections (10 µm) were cut in a cryostat and stained for myofibrillar ATPase after acid (pH 4.6) preincubation for 5 min at room temperature (Brooke and Kaiser, 1970).

Photomicrographs were taken of the stained sections and fiber types were identified as type I, IIA and IIB fibers according to staining intensity. Type I fibers stained darkly whereas type IIA fibers were lightly and IIB fibers intermediately stained. At least 200 fibers were identified from each section.

Statistics. Mean ± standard deviation and correlation coefficients were calculated for muscle samples obtained following exercise according to the correlations procedure of the Statistical Analysis Systems (SAS Institute, 1982).

Results

Blood samples. No significant increase in blood lactate and plasma ammonia concentrations occurred during 105 minutes of slow treadmill trotting. However, the plasma concentrations of ammonia and blood lactate increased with increasing exercise intensity on the treadmill (Fig. 1). The magnitude of the increase differed between horses.

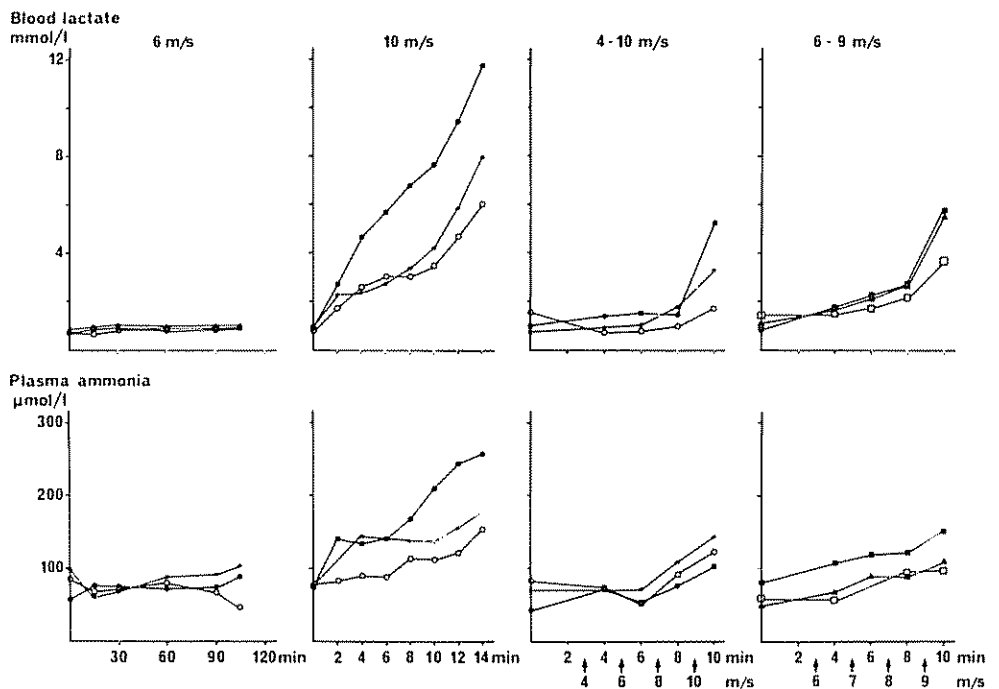


FIGURE 1. Blood lactate and plasma ammonia concentrations of three horses are shown during treadmill work of different intensities and duration.

Significant correlations were found between lactate and ammonia concentrations for each horse at the higher work intensities (10 m/s) on the flat ($r = 0.94$, $r = 0.78$, $r = 0.82$) and during exercise sessions on the slope ($r = 0.97$, $r = 0.94$, $r = 0.84$).

Muscle samples from treadmill work. In the three horses studied before treadmill exercise, concentrations of metabolites were 0.10, 0.23, and 0.30 mmol/kg wet weight for ammonia; 4.4, 5.1, 5.6 mmol/kg wet weight for ATP; and 1.6, 3.1, 3.8 mmol/kg wet weight for lactate. After the standardized work test on the slope values were 0.55, 0.60, 0.95 mmol/kg wet weight for ammonia; 3.8, 4.2, 4.4 mmol/kg wet weight for ATP; and 17.2, 22.2, 24.3 mmol/kg wet weight for lactate.

Muscle samples after racing. The mean fiber type distribution was $24 \pm 9\%$ type I, $47 \pm 4\%$ type II A and $29 \pm 9\%$ type IIB fibers for the fourteen horses. The mean citrate synthase activity was 77 ± 17 mmol/kg dry weight and the mean metabolite concentrations after exercise were; ammonia 1.22 ± 0.47 mmol/kg wet weight, lactate 16.9 ± 6.2 mmol/kg wet weight and ATP 4.2 ± 0.8 mmol/kg wet weight. A positive correlation was found between ammonia and lactate concentration ($r = 0.61$, $P < 0.05$), whereas a negative correlation was found between ammonia and ATP concentrations after racing ($r = -0.68$, $P < 0.01$) (Fig. 2). A positive correlation was found between ammonia concentrations after racing and % type II fibers ($r = 0.67$, $P < 0.01$) whereas a negative correlation was found between ATP concentrations after racing and % type II fibers ($r = -0.73$, $P < 0.01$) (Fig. 3). The % type IIB fibers showed negative correlations to both ATP concentrations after racing ($r = -0.69$, $P < 0.01$) and the CS activity of the muscle ($r = -0.63$, $P < 0.05$).

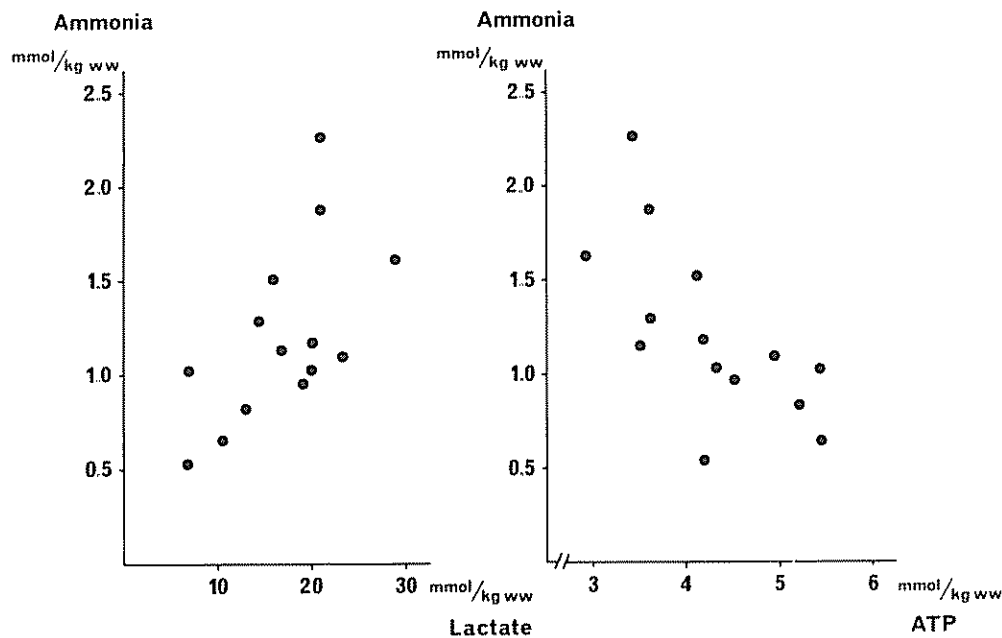


FIGURE 2. Ammonia concentrations in muscle from 14 horses following racing in relation to lactate and adenosine triphosphate (ATP) levels.

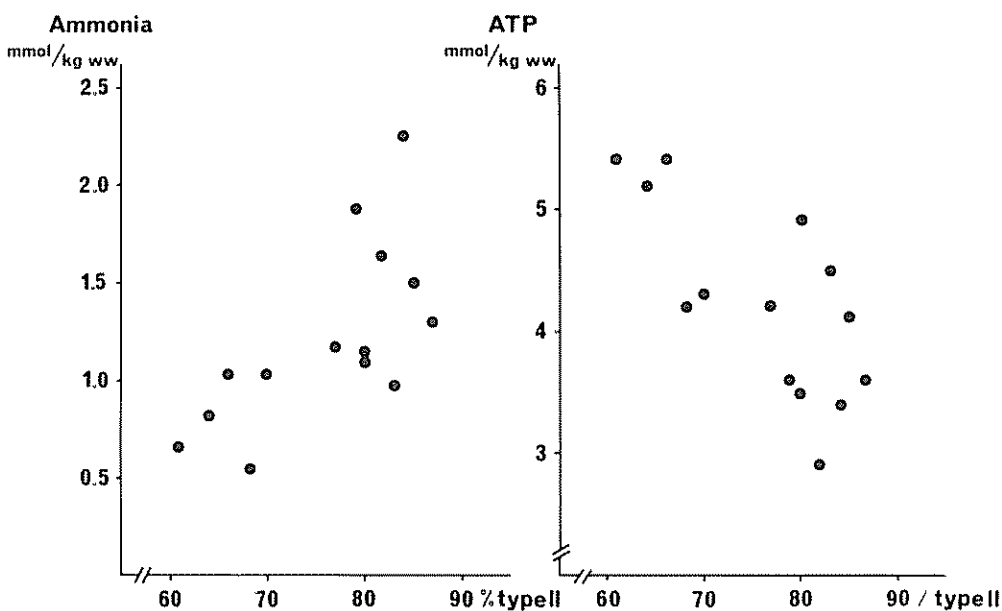


FIGURE 3. Ammonia and adenosine triphosphate (ATP) concentrations in muscle from 14 horses following racing in relation to the percentage of type II fibers.

Discussion

Elevated blood ammonia concentrations were found in this study when horses performed work of high intensity on the treadmill and these elevated concentrations were proportional to blood lactate concentrations. These observations are in agreement with results from studies on man (Babij *et al.*, 1983) and on the rat (Meyer *et al.*, 1980). The origin of both plasma ammonia and lactate appeared to be skeletal muscle since high ammonia concentrations were found in the gluteus muscle after racing in proportion to lactate accumulation. Blood ammonia probably originated from activation of the purine nucleotide cycle in muscle because the enzyme AMP deaminase, which catalyzes AMP deamination to IMP with production of ammonia, has a very high activity in equine skeletal muscle (Cutmore *et al.*, 1986). In a recent study of Thoroughbred horses performing intense exercise, IMP levels increased in muscle in proportion to a decrease in ATP concentrations; also suggesting activation of the purine nucleotide cycle (Snow *et al.*, 1985). In rat and Thoroughbred horses reamination of IMP to AMP is a slow process (Meyer and Terjung 1980; Snow *et al.*, 1985). Thoroughbred horses performing maximal gallops had low ATP concentrations and elevated IMP concentrations up to 30 minutes after exercise. This may explain why low ATP concentrations were found in this study between 5–12 minutes after racing. The IMP can, if not reaminated, also be degraded to uric acid via hypoxanthine. Uric acid concentrations have been found to increase in Thoroughbred horses several minutes after racing (Keenan, 1979).

The AMP deamination may regulate the rate of glycolysis (Lowenstein, 1972). When there is a high rate of glycolysis with increased lactate formation in horses, the intracellular pH decreases (Harris *et al.*, 1984). Lowering of pH stimulates AMP deaminase

activity (Lowenstein, 1972; Dudley and Terjung, 1985b). The formed IMP is an activator of phosphorylase (Aragon *et al.*, 1980) and ammonia activates the rate limiting enzyme of glycolysis, phosphofructokinase (Sugden and Newsholme, 1975). The low ATP concentrations measured after racing, which were inversely related to the increase in lactate and ammonia concentrations, suggests that there is a decrease of the total pool of adenine nucleotides during racing due to AMP deamination.

The data from this study further indicate that AMP deamination in Standardbred horses originates specifically from type II fibers during intense exercise. A correlation was found between ammonia concentrations following racing and the percentage of this fiber type. With increasing speed, more type IIA and IIB fibers become recruited and during racing it is likely that all fiber types are recruited (Valberg 1986). In contrast, during treadmill work at low speeds, mainly type I and IIA fibers are recruited (Valberg, 1986). Since these fiber types have a high oxidative capacity, energy can mainly be provided by oxidative processes and this may explain why there was no ammonia or lactate accumulation when horses in this study performed treadmill work at 6 m/s. The influence on oxidative metabolism of AMP deamination is also indicated by studies on rats in which the most significant changes in ammonia, ATP and IMP with intense work occur in muscles which contain either a high percentage of fast twitch low oxidative fibers or fast twitch oxidative fibers which have an occluded blood supply (Meyer and Terjung, 1979; Dudley and Terjung, 1985a). The greatest phosphagen breakdown and AMP deamination may occur when type IIB fibers are recruited during racing since the percentage of type IIB fibers was negatively correlated to the CS activity and positively correlated to the low ATP levels. A recent study in which analyses were made on pools of different fiber types, confirms that ATP levels post race are low particularly in type IIB fibers and that the lowest oxidative capacity also is found in these fibers (Valberg and Essén-Gustavsson, 1986). Although there was a significant correlation between the percentage of type II fibers and ammonia levels following racing in this study, no correlation was found specifically to type IIB fibers as could have been expected. This may be explained by the fact that ammonia, like lactate, had diffused into other fibers and into blood.

In conclusion this study show that ammonia concentrations increase in both muscle and blood at higher work intensities. Furthermore, the data indicate that the extent of ammonia formation in muscle may be greater in type II than type I fibers.

Acknowledgment

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References

- Aragon, J. J., Tornheim, K. and Lowenstein, J. M. (1980). On a possible role of IMP in the regulation of phosphorylase activity in skeletal muscle. *Febs. Lett. Suppl.* **117**:56–64.
- Armstrong, R. B. and Laughlin, (1985). Metabolic indicators of fibre recruitment in mammalian muscles during locomotion. *J. Exp. Biol.* **115**, 201–213.

- Babji, P., Matthews, S. M. and Rennie, M. J. (1983). Changes in blood ammonia, lactate, and amino acids in relation to workload during bicycle ergometer exercise in man. *Eur. J. Appl. Physiol.* **50**:405–411.
- Brooke, M. H. and Kaiser, K. K. (1970). Muscle fibre types: How many and what kind? *Arch. Neurol.* **23**, 369–379.
- Cutmore, C. M. M., Snow, D. H. and Newsholme, E. A. (1986). Effects of training on enzyme activities involved in purine nucleotide metabolism in Thoroughbred horses. *Equine Vet. J.* **18**, 72–73.
- Dudley, G. A., Staron, R. S., Murray, T. F., Hagerman, R. C. and Luginbueh, A. (1983). Muscle fiber composition and blood ammonia levels after intense exercise in humans. *J. Appl. Physiol.* **54**, 582–586.
- Dudley, G. A. and Terjung, R. L. (1985a). Influence of aerobic metabolism on IMP accumulation in fast-twitch muscle. *Am. J. Physiol.* **24B**, C37–C42.
- Dudley, G. A. and Terjung, R. L. (1985b). Influence of acidosis on AMP deaminase activity in contracting fast-twitch muscle. *Am. J. Physiol.* **24B**, C43–C50.
- Essén, B., Lindholm, A. and Thornton, J. (1980). Histochemical properties of muscle fibre types and enzyme activities in skeletal muscles of Standardbred trotters of different ages. *Equine Vet. J.* **12**, 175–180.
- Harris, R. C., Katz, A., Sahlin, K. and Snow, D. H. (1984). Measurement of muscle pH in the horse and its relation to lactate content. *J. Physiol.* **357**, 110p.
- Keenan, D. M. (1979). Changes of blood metabolites in horses after racing with particular reference to uric acid. *Aust. Vet. J.* **55**, 54–57.
- Kun, E. and Kearney, E. B. (1974). Ammonia. In: *Methods of Enzymatic Analysis*, H. U. Bergmeyer (ed.) Academic Press, New York, p. 1802–1805.
- Lindholm, A. and Piehl, K. (1974). Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of standardbred horses. *Acta Vet. Scand.* **15**, 287–309.
- Lowenstein, J. M. (1972). Ammonia production in muscle and other tissues: The purine nucleotide cycle. *Physiol. Rev.* **52**, 382–414.
- Lowry, O. H., and Passonneau J. V. (1972). *A Flexible System of Enzymatic Analysis*. Academic Press, New York.
- Meyer, R. A. and Terjung, R. L. (1979). Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. *Am. J. Physiol.* **237**, C111–C118.
- Meyer, R. A., Dudley, G. A. and Terjung, R. L. (1980). Ammonia and IMP in different skeletal muscle fibers after exercise in rats. *J. Appl. Physiol.* **49**, 1037–1041.
- Meyer, R. A. and Terjung, R. L. (1980). AMP deamination and IMP reamination in working skeletal muscle. *Am. J. Physiol.* **239**, C32–C38.
- Pette, D. (1985). Metabolic heterogeneity of muscle fibres. *J. Exp. Biol.* **115**, 179–189.
- SAS Institute (1980) SAS Users Guide: Statistics. A. Ray (ed.) Statistical Analysis Systems Inc Gary N.C.
- Saltin, B. and Gollnick, P. D. (1983). Skeletal muscle adaptability: Significance for metabolism and performance. In: *Handbook of Physiology: Skeletal Muscle*. L. D. Pedchy, R. H. Adrian and S. R. Geiger (eds.) Williams and Wilkins, Baltimore. pp 555–631.
- Snow, D. H., Harris, R. C. and Gash, S. P. (1985). Metabolic response of equine skeletal muscle to intermittent maximal exercise. *J. Appl. Physiol.* **58**, 1689–1697.

- 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

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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