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Muscle Metabolic Responses to Maximal Exercise in Standardbred Trotters and Effects of Creatine and Bicarbonate Administration

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Abstract

The principal aim of this investigation was to introduce a protocol for standardised maximal treadmill exercise for the horse that would induce an anaerobic metabolic response and to find reproducible markers in plasma for anaerobic metabolism, specifically for adenine nucleotide degradation. A further aim was to determine whether the muscle metabolic response during exercise influences the locomotion pattern of the horse. This test was also used to examine the effects of administration of creatine and sodium bicarbonate on muscle metabolism and/or performance. A study was also performed to find out whether plasma total carbon dioxide can be measured under post-race conditions as an indicator of pre-race bicarbonate administration.

Horses performed an incremental maximal treadmill exercise test in one-minute steps until they no longer could keep pace with the treadmill. Biopsy specimens were collected from the gluteus medius muscle at rest, immediately post-exercise and after 15 minutes of recovery. Blood samples were collected at rest, during exercise, immediately post-exercise and at frequent intervals during recovery. The exercise test induced an anaerobic metabolic response, as ATP, creatine phosphate (CP) and glycogen decreased and ADP, AMP, inosine monophosphate (IMP) and lactate concentrations increased in muscle after termination of exercise. Plasma lactate concentrations increased during exercise and early recovery and plasma hypoxanthine, xanthine and uric acid concentrations increased during recovery. The test was found to give reproducible results. There was a negative correlation between the increase in muscle ADP post-exercise and the stride frequency close to fatigue. Plasma uric acid post-exercise was related to adenine nucleotide degradation in muscle during exercise. Duration of exercise was related to increase in muscle IMP, AMP and lactate and to change in peak plasma uric acid, indicating the importance of anaerobic metabolism and adenine nucleotide degradation for maximal performance. Neither administration of bicarbonate nor of creatine was found to influence muscle metabolism. Results from a simulated race showed that in all horses given bicarbonate pre-race the total carbon dioxide concentration was above 37 mmol/l 3 hours post-race.

In conclusion, the duration of exercise during a maximal test seems to be dependent on the anaerobic metabolic response, and the degradation of adenine nucleotides can be estimated by measuring plasma uric acid during recovery. Neither administration of bicarbonate nor of creatine affects the muscle metabolic response during the maximal

exercise test. Post-race plasma carbon dioxide can be used as an indicator of bicarbonate administration pre-race.

Key words: horse, exercise, anaerobic metabolism, creatine, bicarbonate

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Contents

Appendix

Papers I-V

The present thesis is based on the papers listed below, which will be referred to in the following by their Roman numerals.

I: Schuback, K. and Essén-Gustavsson B. (1998) Muscle anaerobic response to a maximal treadmill exercise test in Standardbred trotters. *Equine Vet. J.* **30**, 504-510.

II: Schuback, K., Essén-Gustavsson, B. and Persson, S.G.B. (2000) Effect of creatine supplementation on muscle metabolic response to a maximal treadmill exercise test in Standardbred trotters. *Equine Vet. J.* In press.

III: Schuback, K., Kallings, P., Bondesson, U., Essén-Gustavsson, B. and Persson, S.G.B. (2000) Effect of sodium bicarbonate treatment on anaerobic metabolism and total carbon dioxide in plasma during post exercise recovery. *Proceedings 12th Internat. Conference of Racing Analysts and Veterinarians.* pp 14-19.

IV: Schuback, K., Essén-Gustavsson, B. and Persson, S.G.B. (2000) Effect of sodium bicarbonate administration on metabolic responses to maximal treadmill exercise in Standardbred trotters. Submitted.

V: Schuback, K., Essén-Gustavsson, B. and Persson, S.G.B. (1999) Incremental treadmill exercise until onset of fatigue and its relationship to metabolic response and locomotion pattern. *Equine Vet. J. Suppl.* **30**, 337-341.

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Abbreviations

ATP	Adenosine triphosphate.
ADP	Adenosine diphosphate.
AMP	Adenosine monophosphate.
IMP	Inosine monophosphate.
CP	Creatine phosphate.
PLa	Plasma lactate.
TCO_2	Total carbon dioxide.

Introduction

What characteristics does a Standardbred trotter need to possess to become a winner?
From the time when man first began to use the horse for racing, attempts have been made to find the answer to this question with the aim of breeding the optimal horse for this purpose. With this objective in mind, exercise physiologists began to study the physiological responses to exercise in draught horses (Zuntz and Hageman 1898; Proctor *et al.* 1934). In the 1930s, studies were undertaken to determine the concentrations of blood glucose and lactate in horses at rest and after exercise (Solun 1930; Solun 1935; Bogdanow *et al.* 1935). It was found that exercise increased the blood lactate concentration to varying degrees in different individual horses. The invention of the high-speed treadmill made it possible to evaluate performance under more standardised conditions, and Persson (1967) was the first to introduce standardised exercise protocols for Standardbred trotters. These studies showed a relation between the performance capacity of the horse and the heart rate and blood lactate responses during submaximal and maximal exercise, as well as the total blood volume (Persson 1967; Persson and Ullberg 1974).

During exercise, energy is produced both by aerobic (in the presence of oxygen) and anaerobic (without oxygen) pathways, but the aerobic pathway is believed to be of greater importance in most equine athletic activities (racing, three-day event, endurance). Most exercise tests have therefore been focused on measurements of the cardiovascular and respiratory responses to exercise in order to evaluate the capacity of these systems to provide working skeletal muscle with oxygen (aerobic capacity). To be able to assess the cardiovascular capacity of the horse, the heart rate is measured during a submaximal incremental treadmill exercise test in two-minute steps (Persson 1983). From this test the parameter V_{200}, i.e. the speed at which the horse reaches a pulse rate of 200 beats /minute, can be obtained. The reason for using a pulse of 200 as a reference point is that it is usually at this pulse rate that the anaerobic threshold is reached (when the rapid accumulation of lactate begins) and anaerobic metabolism becomes more important. V_{200} has been shown to be positively correlated with the red cell volume both during track and treadmill exercise (Persson and Ullberg 1974; Persson 1983). Another parameter that provides information about the aerobic capacity of the horse is the maximal oxygen uptake (VO_{2max}). Oxygen uptake increases with speed and VO_{2max} is defined as the point at which no further increase in oxygen uptake occurs despite an increase in work load (Åstrand and Rodahl 1986). A correlation between V_{200} and VO_{2max} has been established, but those advocating the use of VO_{2max} as an indicator of aerobic capacity consider this parameter to be more reliable, as it actually measures oxygen uptake at maximal speeds, whereas V_{200} is calculated from submaximal exercise intensities (Evans and Rose 1987). VO_{2max} is

thought to reflect the maximal aerobic capacity of the horse and is regarded as the best parameter for athletic performance in human beings (Sutton 1992). Results from different exercise studies have shown that the horse is a superior athlete with a maximal oxygen uptake (Thoroughbreds: 140-187 ml/kg/min) and a maximal stroke index (Thoroughbreds: 2.5-2.7 ml/kg) that are far above those of humans (69-85 ml/kg/min and 1.5 ml/kg, respectively) (Ganong 1985; Physick-Sheard 1985; Evans and Rose 1987; Rose *et al.* 1988; Rose *et al.* 1990; Seeherman and Morris 1990; Derman and Noakes 1994).

At very high exercise intensities, the anaerobic energy supply becomes more important, and parameters that measure the contribution from the anaerobic pathways are therefore necessary. The peak blood lactate in response to a maximal treadmill exercise to fatigue has been found to be higher in horses with a well developed anaerobic capacity than in those with a lower anaerobic capacity (Eaton *et al.* 1992). This indicates that peak blood lactate can be used as a marker for anaerobic capacity. Blood lactate, however, can also be used as an indicator for aerobic energy supply during exercise, using the parameter V_{La4}, which is the speed at which a lactate level of 4 mmol/l is reached (anaerobic threshold) (Persson 1983). This can be determined using the standardised submaximal exercise test described by Persson (1983). The horse has the capacity to endure a much higher lactate accumulation (around 30 mmol/l) during intensive exercise (1,600 or 2,000 m race) than man (around 15 mmol/l) (Snow *et al.* 1985; Harris *et al.* 1987; Derman and Noakes 1994; Ronéus *et al.* 1999). Measurement of lactate in the blood or plasma is an indirect method of determining the anaerobic capacity, as the production of lactate takes place within the muscle. A close correlation between muscle lactate and blood lactate accumulation has been found after exercise (Valberg *et al.* 1985). The development of the needle biopsy technique has made it possible to study the muscle metabolic response to exercise more directly (Lindholm and Piehl 1974). This is of advantage, as one of the major causes of fatigue at high exercise intensities is thought to be an impairment of muscle fibre function. This can be due to a number of factors, such as depletion of substrates for energy production, interference with ATP production caused by a change in the internal environment of the muscle fibre, and a decrease in blood flow and increase in muscle temperature (Snow and Valberg 1994). Factors that might impair muscle fibre function are changes in the electrolyte gradients, causing alterations in the irritability of the neuromuscular system and also in calcium ion uptake by the sarcoplasmic reticulum. One of the factors thought to impair muscle fibre function in man is the degradation of adenine nucleotides that occurs in muscle in connection with exhaustive exercise (Sahlin 1986).

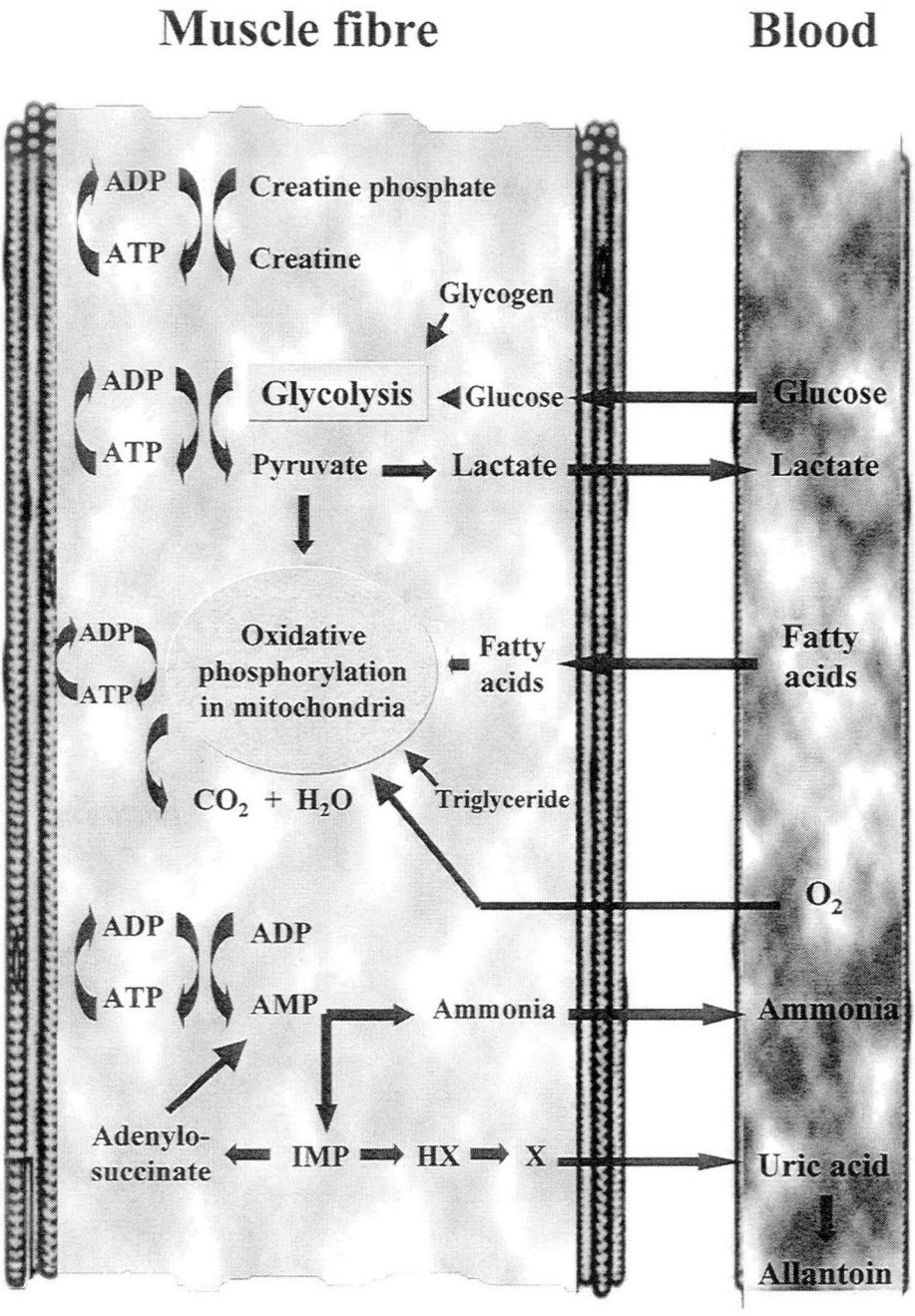

Fig. 1
Substrate sources and metabolic pathways for aerobic and anerobic metabolism.

Muscle metabolism during exercise (Fig. 1)

Skeletal muscle fibres consist of a number of longitudinally running myofibrils. These have functional contractile units called sarcomeres, which contain two types of filaments, thick (myosin) and thin (actin). The contraction process is initiated by a release of Ca^{2+} ions from the sarcoplasmic reticulum that enables the actin to bind to myosin. During contraction the sarcomeres are shortened as the actin filaments slide over the myosin filaments. The contraction process demands energy in the form of adenosine triphosphate (ATP), which is stored in the muscle. The store of available ATP is very small, however, and only sufficient to provide the muscle with energy for a few seconds of exercise. Consequently, ATP must be replenished for exercise to be continued. This can be achieved in two ways, through the aerobic or the anaerobic pathway (Fig.1). During low-intensity exercise, when the supply of oxygen is sufficient, ATP is mainly produced through aerobic processes in the mitochondria, by utilisation of blood glucose, free fatty acids and muscle glycogen. The aerobic process is highly advantageous, as it yields more ATP per molecule of substrate than the anaerobic pathway. The disadvantage of this process, however, is that it is slow, and during intensive exercise a rapid replenishment of ATP is required. This need is mainly fulfilled by the anaerobic pathway. The anaerobic replenishment of ATP can be achieved both by breakdown of creatine phosphate (CP) and by anaerobic glycolysis. The breakdown of CP leads to rapid restoration of ATP through rephosphorylation of adenosine diphosphate (ADP), with concomitant release of free creatine. It is mainly during the initial part of exercise that energy is supplied by this process, as the stores of intramuscular CP rapidly decline as exercise continues. ATP is then mainly produced by anaerobic glycolysis, which metabolises blood glucose or muscle glycogen, resulting in lactate, with a concomitant accumulation of H^+ ions. This H^+ accumulation causes a drop in the pH, i.e. acidosis, which can inhibit important glycolytic enzymes, such as phosphorylase and phosphofructokinase (Trivedi and Danforth 1966; Newsholme and Leech 1983). In vitro studies have shown that the acidosis can also have a direct inhibitory effect on muscle contraction, as it inhibits the release of Ca^{2+} ions from the sarcoplasmic reticulum (Nakamuru and Schwartz 1970; Nakamuru and Schwartz 1972; Fabiato and Fabiato 1978). The buffering capacity of muscle is therefore of great importance for the possibility of continuing exercise and delaying the onset of fatigue. The buffering of H^+ ions occurs through different buffering systems, of which one of the more important is blood bicarbonate. The rephosphorylation of ADP via CP also acts as an intramuscular buffering system, as this process contributes to the elimination of the H^+ ions produced by anaerobic glycolysis (Sahlin 1986).

During extremely intensive exercise, when the demand for ATP is higher than the production (from anaerobic glycolysis), there is an accumulation of ADP and adenosine monophosphate (AMP) in skeletal muscle. The increased

accumulation of ADP in the muscle activates the enzyme myokinase, which in turn catalyses the formation of one molecule of ATP and one molecule of AMP from two molecules of ADP. During this type of exercise there is a marked acidosis, which increases the activity of another enzyme called AMP deaminase, which catalyses the formation of inosine monophosphate (IMP), from AMP, with the production of ammonia (Newsholme and Leech 1983; Dudley and Terjung 1985b; Sahlin 1986). It is well known that in horses AMP-deaminase is highly active (Cutmore *et al.* 1978). This allows the AMP concentration to be kept low, which is needed to drive the myokinase reaction towards ATP production. Previous studies on horses performing intensive exercise on a track or treadmill have shown that they exhibit a marked decrease in ATP concomitantly with an almost equal increase in IMP in muscle immediately post-exercise (Snow *et al.* 1985; Sewell and Harris 1992; Sewell *et al.* 1992; Essén-Gustavsson *et al.* 1997). IMP may undergo further degradation to uric acid via the formation of inosine, hypoxanthine and xanthine. In non-primate animals such as horses, uric acid can be further metabolised to allantoin. The concentrations of plasma uric acid and alloantoin have been found to increase after strenuous exercise in horses (Keenan 1978, 1979; Räsänen *et al.* 1993). The presence of these metabolites in the blood indicates that the utilisation of ATP in the muscle has exceeded the ATP turnover rate and that a total loss of adenine nucleotides has occurred.

As the racing competition has become harder, the use of different drugs to improve the performance capacity of the horses has become more common. Many of these substances are regarded as illegal, as they have the potential to influence the performance of the horse during competition. Today there is a wide range of drugs and substances that can be inflicted on the horse to improve performance, and new techniques for detecting administration of illegal substances to horses are constantly under development. As the welfare of the horse could be at stake, these substances are often prohibited, even when their actual effects on performance are unclear. Two substances, creatine which is not regarded as a doping agent in Sweden, and bicarbonate, which is, are believed to have an influence on the metabolic response during exercise. Both creatine and bicarbonate are thought to have a beneficial effect under anaerobic conditions, when degradation of creatine phosphate and adenine nucleotides occurs and there is a breakdown of glycogen with consequent production of lactate.

Creatine supplementation

The depletion of ATP is one factor that is believed to promote the onset of fatigue in both humans and horses (Sahlin 1986; Snow *et al.* 1985; Harris *et al.* 1991). Maintenance of high muscle ATP and CP concentrations is therefore probably beneficial in preventing or delaying the onset of fatigue. High resting concentrations of CP are believed to improve performance by delaying the depletion of ATP, and hence by providing effective maintenance

of the ATP concentration in muscle during exercise and more rapid resynthesis during recovery (Hultman *et al.* 1967; Balsom *et al.* 1993a; Greenhaff *et al.* 1995; Casey *et al.* 1996). Marked increases in the total muscle creatine concentration (TCr) have been observed in humans after supplementation with 20 g creatine monohydrate daily for 5 or 6 days (Harris *et al.* 1992; Greenhaff *et al.* 1994; Balsom *et al.* 1995; Casey *et al.* 1996; Green *et al.* 1996; Hultman *et al.* 1996). An increased body weight has also been found after creatine supplementation, possibly due to increased retention of water (Balsom *et al.* 1993a,b; Balsom *et al.* 1995; Green *et al.* 1996; Cooke and Barnes 1997; Dentkowskii *et al.* 1997; Engelhardt *et al.* 1998). Not in all studies, however, has an increase in body weight been found in connection with creatine supplementation (Prevost *et al.* 1997; Terrillion *et al.* 1997). Studies on human beings have also shown that creatine supplementation can have a beneficial effect on performance, especially during short-term high intensity exercise carried out in repeated bouts (Greenhaff *et al.* 1993; Birch *et al.* 1994; Dentkowski *et al.* 1997). More rapid resynthesis of CP and diminished ATP depletion have been observed after creatine supplementation, despite an increase in work load (Greenhaff *et al.* 1993; Greenhaff *et al.* 1994; Casey *et al.* 1996). There have also been reports on reduced post-exercise concentrations of muscle lactate and/or plasma lactate, hypoxanthine and ammonia after creatine supplementation (Greenhaff *et al.* 1993; Balsom *et al.* 1995; Prevost *et al.* 1997). These findings indicate that creatine supplementation can influence the anaerobic muscle metabolism during intensive exercise. Other studies on humans, however, have failed to show any effect of creatine administration on the performance or metabolic response (Cooke *et al.* 1995; Febbrario *et al.* 1995; Mujika *et al.* 1996; Redondo *et al.* 1996; Cooke and Barnes 1997; Odland *et al.* 1997; Snow *et al.* 1998). In these studies the exercise was mostly performed with submaximal work loads or in single bouts on a bicycle.

Little attention has been paid to the uptake of creatine in horses. In the two studies that have focused on this question, one on Standardbreds and one on Thoroughbreds, no significant changes in the concentrations of muscle total creatine and creatine in the blood were found after prolonged supplementation with creatine in the water or feed (Essén-Gustavsson *et al.* 1994; Sewell and Harris 1995). In addition, supplementation with a daily creatine dose of 100 g for 6.5 days showed no effect on muscle metabolism in Standardbred horses that performed a submaximal exercise test on a treadmill (Essén-Gustavsson *et al.* 1994).

Bicarbonate administration

Metabolic alkalosis has been found to enhance the efflux of lactate and H^+ ions from muscle to blood in isolated frog muscle (Hirche 1975; Mainwood and Worsley-Brown 1975). Administration of bicarbonate has been shown to cause a metabolic alkalosis both in human beings and in horses and is

therefore thought to be beneficial during high-intensity exercise when the lactate production is high (Kelso *et al.* 1987; Lawrence *et al.* 1987; Horswill *et al.* 1988; Greenhaff *et al.* 1990; Lawrence *et al.* 1990; Harkins and Kamerling 1992; Lloyd *et al.* 1993). Results regarding the effects of bicarbonate loading on work performance and metabolic response to exercise are conflicting both in humans and horses. In most studies on horses performing heavy track or treadmill exercise, administration of bicarbonate has shown no influence on performance (Kelso *et al.* 1987; Lawrence *et al.* 1987; Lawrence *et al.* 1990; Greenhaff *et al.* 1991a; Harkins and Kamerling 1992; Lloyd and Rose 1995). There has also been a report of metabolic changes such as decreased accumulation of plasma ammonia, an increased blood lactate concentration and decreased depletion of muscle ATP in horses performing intensive treadmill exercise after bicarbonate administration (Greenhaff *et al.* 1991b). Despite the conflicting reports on the effects of bicarbonate on the performance capacity of the horse, administration of bicarbonate before a race is prohibited, on the grounds that bicarbonate may interfere with the excretion of other doping agents (Rose and Lloyd 1992; Snow 1994). The mode of administration (nasogastric intubation) also involves a risk of aspiration pneumonia if the tube is inserted incorrectly.

Muscle fibre composition and locomotion

The aerobic and anaerobic capacities of the horse are of great importance for the athletic performance, but one should not forget that this is also influenced by other factors, such as muscle fibre composition, running technique, locomotion pattern and the horse's motivation. The muscle consists of a number of muscle fibres, which can be histochemically classified into three types on the basis of the myosin ATPase activity, namely slow-twitch type I and fast-twitch type IIA and IIB fibres (Brooke and Kaiser 1970). Fibres of types I and IIA have a high oxidative capacity, whereas the oxidative capacity of type IIB fibres varies considerably between horses (Essén *et al.* 1980; Valberg and Essén-Gustavsson 1987). The characteristics of these fibres change with age and training. Foals are born with a large proportion of type IIB fibres, but with age and training there is an increase in the type IIA/IIB ratio and in the oxidative capacity of type IIB fibres (Essén *et al.* 1980; Nimmo *et al.* 1982; Henckel 1983; Hodgson *et al.* 1986; Hodgson and Rose 1987; Ronéus and Lindholm 1991; Ronéus *et al.* 1992; Ronéus *et al.* 1994). By studying the glycogen depletion patterns of the different fibre types during exercise, it has been shown that the different fibre types are recruited in a certain manner, starting with type I fibres at a low exercise intensity and followed by type IIA and IIB fibres as the intensity of the exercise increases (Lindholm *et al.* 1974; Snow *et al.* 1981; Hodgson *et al.* 1983; Essén-Gustavsson *et al.* 1984; Valberg 1986; White and Snow 1987). The production of lactate during exercise in the horse has been shown to be correlated to the muscle fibre composition and mainly to the proportion of low-oxidative type IIB fibres (Valberg *et al.* 1985; Valberg 1987; Ronéus *et al.* 1995). After

racing, ATP concentrations have been reported to be lower in pools of type IIB fibres than in types I and IIA (Valberg and Essén-Gustavsson 1987). In exercising rats, IMP is mainly found within the type IIB fibres, indicating a greater loss of ATP in these fibres (Meyer *et al.* 1980). The muscle fibre composition, the oxidative capacity of the fibres, the oxygen transport capacity and the training status of the horse have also been found to influence the locomotion pattern (Persson *et al.* 1991). During exercise, speed is increased by an increase both in stride length and in stride frequency. It has been suggested that the increase in speed during exercise near the anaerobic threshold is mainly achieved by an increase in stride length (Persson *et al.* 1991). In that study the stride length during exercise at submaximal intensities was shown to be negatively related to the proportion of type IIB fibres. This is supported by findings in another study in which young Standardbred trotters performed a track test covering 1,000 m at close to maximal speed, where it was observed that the horses with a low percentage of type IIB fibres performed maximal trotting with a low lactate production and a long stride length and stance time (time when the hoof is in contact with the ground) (Ronéus *et al.* 1995). The stride length can only increase to a certain limit during exercise, after which the stride frequency must be increased in order to increase speed (Dusek *et al.* 1970). The ability to increase the stride frequency (during high speeds) is therefore considered important for the performance at high speeds (Bayer 1973). A relationship between stride frequency and metabolic cost of locomotion has also been suggested (Taylor 1985; Heglund and Taylor 1988).

Aims

The aims of the present investigation were:

1) to study the anaerobic muscle metabolic response, especially the adenine nucleotide degradation, in Standardbred trotters performing a standardised incremental maximal exercise test;

2) to find markers in plasma for the anaerobic muscle metabolic response, and to assess the reproducibility of the measured values, in Standardbred trotters performing a standardised incremental maximal exercise test;

3) to determine whether administration of creatine could influence the muscle anaerobic metabolic response during a standardised incremental maximal exercise test;

4) to determine whether administration of sodium bicarbonate could influence the muscle anaerobic metabolic response and the duration of exercise during a standardised incremental maximal exercise test;

5) to determine whether administration of sodium bicarbonate could influence performance and affect plasma markers for muscle anaerobic metabolic response in horses performing a simulated race on a track;

6) to find out whether pre-race administration of sodium bicarbonate could be detected by analysing the total carbon dioxide (TCO_2) concentration in plasma post-race;

7) to determine whether the duration of exercise and the locomotion pattern are influenced by the muscle anaerobic metabolic response in horses performing a standardised incremental maximal exercise test.

Materials

Horses

Study I: Six Standardbred trotters, five geldings and one mare, of ages 4 – 6 years, weighing between 420 and 525 kg.

Study II: Six Standardbred trotters, all geldings, of ages 4–6 years, weighing 492–553 kg.

Study III: Six Standardbred trotters, five geldings and one mare, aged 3–5 years, weighing 450–500 kg.

Study IV: Five Standardbred trotters, four geldings and one mare, of ages 4–9 years, weighing 431–555 kg.

Study V: Nine Standardbred trotters, eight geldings and one mare, 4-6 years old, weighing 420–553 kg. Six of the horses in this study were also included in study I and three of them in study II.

The horses in study III were all in professional training and privately owned. The informed concent of the owners was obtained prior to the horses´ entry into the study. During the test period of one week these horses were kept at the Department of Large Animal Clinical Sciences, Uppsala. All other horses used in this investigation were owned by the department, but had all been in training and all, except one, had raced before arriving at the clinic. All horses were fed the same standard diet consisting of grain and hay. They were all accustomed to treadmill exercise and were regularly trained on the treadmill.

The horses in this investigation were all considered to be healthy on the basis of a thorough clinical examination, routine haematology, ECG and endoscopy at rest. All horses in studies I, II, IV and V also performed a standardised submaximal exercise test as described by Persson (1967, 1983) before entering the investigation, to ensure that they had a normal physiological response to exercise and a blood volume within normal limits.

Methods

Experimental protocol

In study I the horses performed a standardised incremental maximal exercise test on a high-speed treadmill (Säto, Sweden). After a warm-up period of 10 minutes (5 min walk and 5 min trot), the treadmill incline was increased from a horizontal level to an incline of 4.7° (8.2 %) and the speed was set to 7 m/s. The velocity of the treadmill was then increased by 1 m/s every 60 seconds until the horses could not keep pace with the treadmill despite mild encouragement. At this point the treadmill was lowered and the horses were allowed to walk during a recovery period of 15 minutes, after which the treadmill was stopped. The heart rate was monitored at rest and during the last 15 seconds of each exercise step. Blood samples for measurements of plasma lactate (PLa), hypoxanthine, xanthine and uric acid concentrations were collected at rest, at the end of the warming-up period, during the last 15 seconds of each exercise step, immediately post exercise and at 2, 5, 10, 15, 20 and 30 minutes of recovery. Biopsy specimens for measurement of the ATP, ADP, AMP, IMP, CP, glycogen and lactate concentrations were collected from the gluteus medius muscle at rest, immediately post-exercise and after 15 minutes of recovery. In study I, the standardised incremental maximal exercise test was performed on two occasions (1a and 1b), with at least one week between the tests, to check the reproducibility of the measured values.

In study II the influence of creatine supplementation on the muscle metabolic response was investigated with a cross-over design. The horses performed the same standardised incremental maximal exercise test as in study I without any treatment (baseline). After this baseline test they were given creatine monohydrate (25 g) twice daily or the same dose of lactose (placebo). The supplementation period was 6.5 days, after which the horses performed the standardised incremental maximal exercise test again. A wash-out period of 14 days was allowed before treatments were switched between horses and a new supplementation period was started. After this period the standardised incremental maximal exercise test was performed a third time. In the baseline test the horses exercised on the treadmill until they showed signs of fatigue (could not keep pace with the treadmill), and in the following two tests the horses were stopped after performing exercise for the same length of time as in the baseline test. The heart rate was measured at rest and during the last 15 s of each exercise bout. Blood samples for measurement of plasma lactate, creatine, creatinine, hypoxanthine, xanthine and uric acid were collected at the same intervals as in study I, but for a longer period during recovery (60 and 120 min). The total blood volume was determined after exercise. Muscle biopsy specimens were taken at rest, immediately post-exercise and after 15 minutes of recovery and analysed for concentrations of ATP, ADP, AMP, IMP, CP, total creatine, glycogen and lactate.

In studies III and IV the effects of sodium bicarbonate administration on performance, blood acid-base balance and the muscle metabolic response were studied by administering 0.6 g /kg b.w. of sodium bicarbonate dissolved in 2 litres of water or 2 litres of water alone (placebo) by nasogastric intubation 4 hours prior to a simulated race covering 2,000 m on a track (study III) or the same standardised incremental maximal exercise test on the treadmill (study IV) as was used in study I. The order of administration was randomised in a blind cross-over manner. In study III blood samples were collected at rest and 90 and 235 minutes after administration, and after the race at frequent intervals during recovery (5, 30, 60, 90, 120, 180 and 240 min). These samples were analysed to determine the concentrations of bicarbonate in the blood, the pH, and the plasma TCO_2, lactate and uric acid concentrations. In study IV the heart rate was monitored at rest and during the last 15 seconds of each speed step. Blood samples were collected at rest and also at 90 and 235 minutes after administration, at the end of each exercise step and at 5, 15, 30, 60, 120, 180 and 240 minutes and also 24h, after termination of exercsie. These samples were analysed for the concentrations of plasma lactate, hypoxanthine, xanthine and uric acid. Muscle biopsy specimens for assays of ATP, ADP, AMP, IMP, CP, glycogen and lactate concentrations were collected from the gluteus medius at rest, immediately post-exercise and after 15 minutes of recovery. In study IV the total blood volume was determined.

In study V, the stride length and stride frequency were determined for each exercise step. Stride frequency was calculated by timing 25 paces at each speed, and stride length was calculated from stride frequency and treadmill velocity. Oxygen uptake was measured at each exercise step in six of the nine horses. Blood samples for determination of plasma lactate and uric acid concentrations were collected at rest, at the end of each exercise step, at the end of the exercise test and after 30 minutes of recovery. Muscle biopsy specimens were collected at rest and immediately after exercise and analysed for the concentrations of ATP, ADP, AMP, IMP, CP, glycogen and lactate.

Heart rate

The heart rate (HR) was monitored with an electrocardiograph (Siemens-Elema Mingograph 804).

Oxygen uptake

Oxygen uptake was determined by analysis of gases collected from the expired air, using a gas analyser (Servomex, Sussex, UK, integrated into an Oximeter 3200, Isler Bioengineering, AG, Zürich, Switzerland), and the bias flow rate was measured with an industrial calibrated flow-meter (013GL160, Fluid Inventor, Stockholm, Sweden).

Catheterisation

Blood samples were collected from a venous catheter (Hémocath 60, 14G, 600mm, Vygon, Écouen, France) that was introduced into the right jugular vein under local anaesthesia. In study III samples were drawn with a vacutainer technique before exercise. After the race, samples were collected through a venous catheter (Intranule, 14G, 105mm, Vygon, Écouen, France) inserted in the jugular vein.

Blood volume

The total blood volume was calculated from the Evans Blue dye dilution space technique (corresponding to the plasma volume) and the post-exercise haematocrit (Persson 1967). The total red cell volume was calculated as the difference between total blood volume and plasma volume.

Plasma parameters

The blood samples were centrifuged and the plasma was frozen at -80°C. For determination of plasma lactate concentrations a lactate analyser (Analox, GM-7, Analox Instruments Ltd, London) was used. The plasma concentrations of creatine, creatinine, hypoxanthine, xanthine and uric acid were assayed by a modified HPLC technique using a C:18 (250 x 4.6, 5 µm) column (Sellevold *et al.* 1986; Dunnett *et al.* 1991).

Acid-base status

An EL-ISE analyser (Beckman Auto Analyser) was used for analysis of TCO_2. Blood bicarbonate (HCO_3) and pH were determined with a blood-gas analyser (ABL-5, Radiometer, Copenhagen, DK).

Muscle biopsies

Biopsy specimens were collected from the gluteus medius muscle according to the technique described by Lindholm and Piehl (1974). All samples were frozen immediately in liquid nitrogen and stored at -80°C. The samples were freeze-dried and dissected free from blood, connective tissue and fat, and then weighed and extracted in perchloric acid before being neutralised with potassium hydroxide. The concentrations of ATP, ADP, AMP and IMP were determined by a modified HPLC technique (Sellevold *et al.* 1986). Creatine phosphate and creatine concentrations were measured with an HPLC technique (Dunnett *et al.* 1991). Muscle lactate and glycogen concentrations were assayed by fluorometric methods (Lowry and Passoneau 1973).

Statistics

The results are expressed as mean ± standard deviation (s.d.). The Wilcoxon signed-rank test was used to compare differences in concentrations of plasma and muscle metabolites both within and between tests. Differences were

regarded as significant at $p<0.05$. Linear regression analysis was used to calculate the correlations. The standard error of measurement was calculated as:

$S= \sqrt{\sum d^2/2N}$

Results

Study I

Heart rate

The heart rate increased with speed in all horses and a mean peak heart rate of 211±8 beats/min was recorded in test 1a and of 211±9 beats/min in test 1b during the last speed step. The standard error of measurement was 1 beat/min.

Duration of exercise

Most horses completed four speed steps (7, 8, 9, 10 m/s) on both occasions, but the duration of exercise differed between some horses in the last speed step (10-15s). The standard error of measurement was 7.4 seconds.

Plasma lactate, hypoxanthine, xanthine and uric acid concentrations

The lactate concentrations increased significantly during exercise and the peak plasma lactate concentrations were reached within 5 minutes of recovery in all horses. The mean peak plasma lactate concentration was 27.3±4.5 mmol/l in test 1a and 27.0±5.6 mmol/l in test 1b. Individual peak plasma lactate concentrations in relation to duration of exercise are shown in Fig. 2a. The plasma hypoxanthine and xanthine concentrations remained unchanged throughout the exercise period (hypoxanthine: 1a, 3.4±1.4 µmol/l, 1b, 2.7±0.8 µmol/l; xanthine: 1a, 0.1±0.1 µmol/l, 1b, 0.2±0.4 µmol/l). Small but significant increases were seen in the hypoxanthine (1a: 4.0±1.5 µmol/l, 1b: 3.5±1.0 µmol/l) and xanthine (1a: 0.6±0.2 µmol/l, 1b: 1.3±0.8 µmol/l) concentrations within 5 minutes of recovery. The plasma uric acid concentrations were low at rest and during exercise but increased significantly during recovery and reached a peak concentration at 20-30 minutes of recovery. The mean peak uric acid concentration was 129.5±69.9 µmol/l in test 1a and 113.4±64.6 µmol/l in test 1b. Individual peak plasma uric acid concentrations in relation to duration of exercise are shown in fig 2b. The standard error of measurement was 4.0 mmol/L for peak plasma lactate, 1.8 µmol/l for peak hypoxanthine, 2.9 µmol/l for peak xanthine and 15.8 µmol/l for peak uric acid.

Muscle metabolic response (Fig. 3a, b)

Significant decreases in muscle ATP, CP and glycogen concentrations were observed immediately after termination of exercise. Concomitantly, muscle IMP and lactate concentrations were significantly increased.

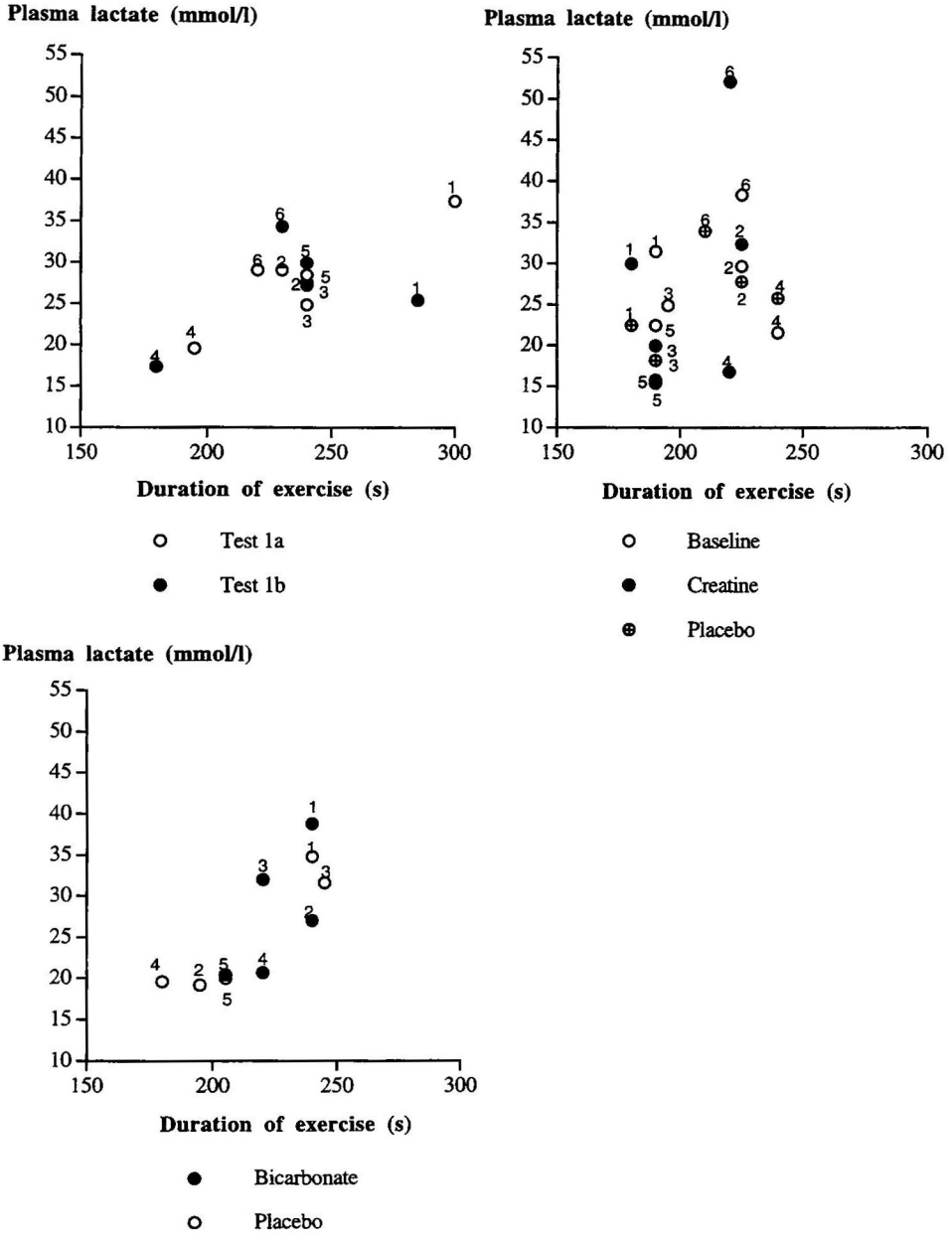

Fig. 2a
Peak plasma lactate (mmol/l) in relation to duration of exercise (s) in all horses in studies I (Test 1a, 1b), II (Baseline, Creatine, Placebo) and IV (Bicarbonate, Placebo).

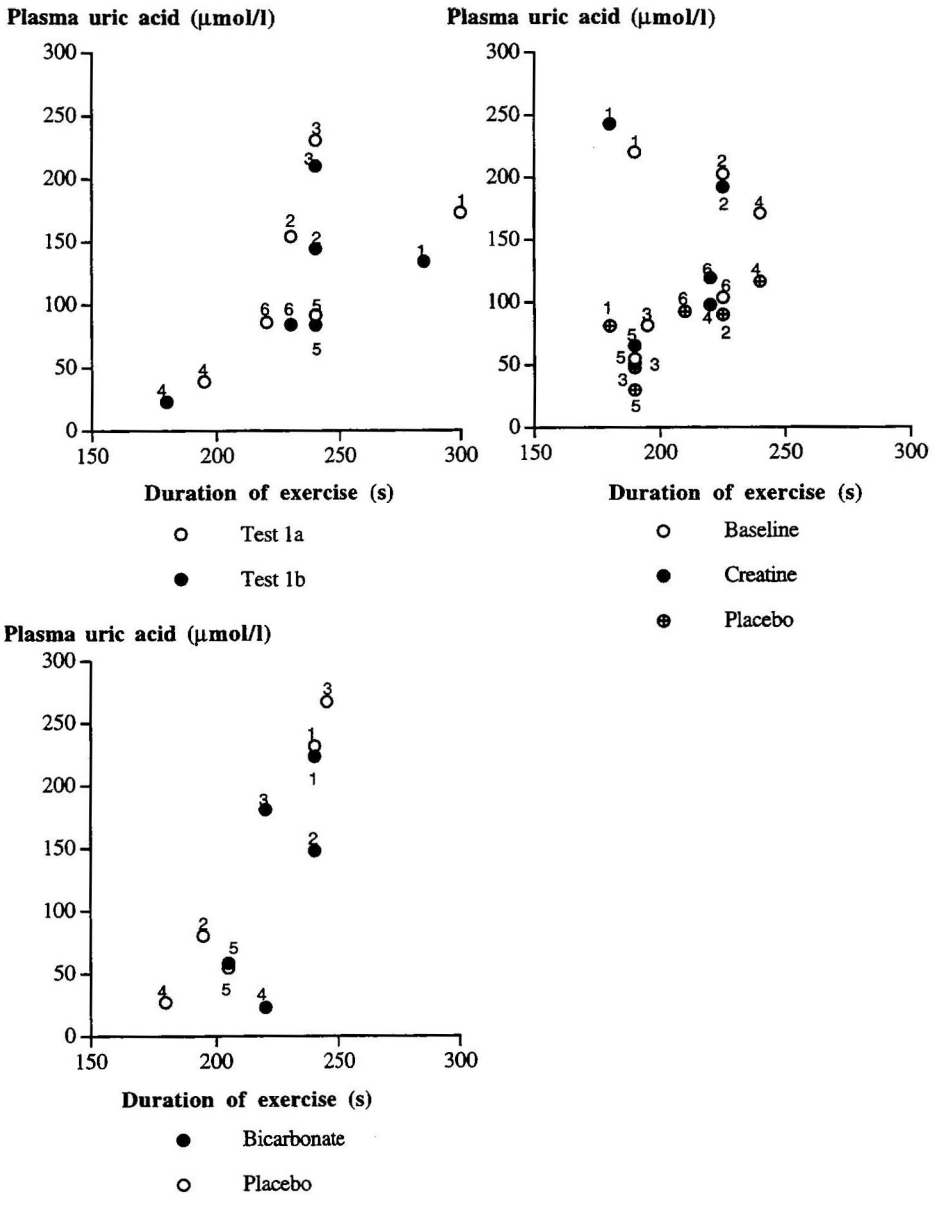

Fig. 2b
Peak plasma uric acid concentrations (µmol/l) in relation to duration of exercise (s) in all horses in studies I (Test 1a, Ib), II (Baseline, Creatine, Placebo) and IV (Bicarbonate, Placebo).

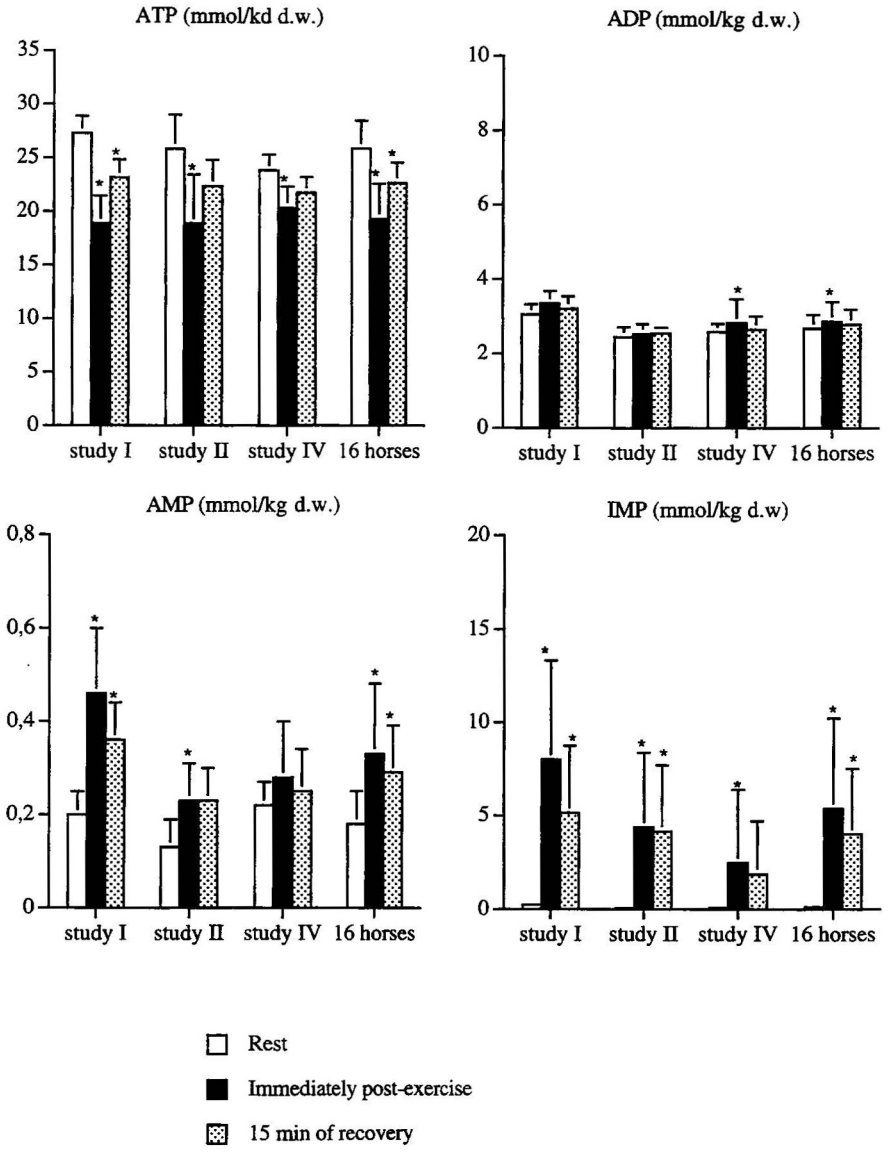

Fig. 3a
Mean concentrations of muscle ATP, ADP, AMP and IMP (mmol/kg d.w) at rest, immediately post-exercise and at 15 minutes of recovey in horses from studies I (test 1a), II (baseline) and IV (placebo), and in 16 horses combined from these studies.*=significantly different from rest ($p < 0.05$).

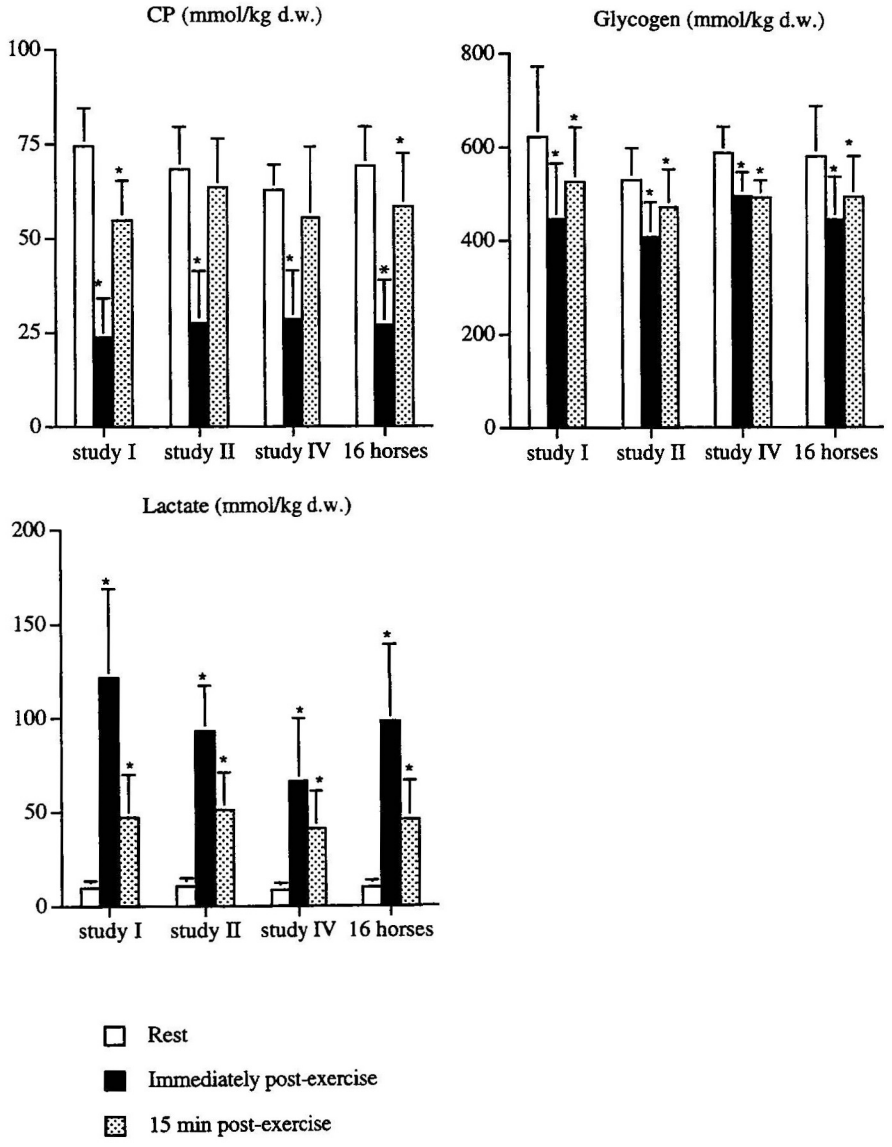

Fig. 3b
Mean concentrations of muscle CP, glycogen and lactate (mmol/kg d.w.) at rest, immediately post-exercise and at 15 minutes of recovery in horses from studies I (test 1a), II (baseline) and IV (placebo), and in 16 horses combined from these studies *=significantly different from rest ($p < 0.05$).

The resting concentrations of ADP (1a: 3.04±0.28 mmol/kg, 1b: 3.19±0.47 mmol/kg) and AMP (1a: 0.20±0.05 mmol/kg, 1b: 0.38±0.10 mmol/kg) were low and no changes occurred in the ADP concentrations after termination of exercise. The AMP concentrations were significantly increased in test 1a but not in test 1b. After 15 minutes of recovery the ATP, CP and glycogen concentrations were significantly increased compared to the values immediately post-exercise, but had not yet returned to the resting levels. The IMP and lactate concentrations were still elevated in comparison with the resting values. The standard error of measurement immediately post-exercise was 2.7 mmol/kg for ATP, 0.57 mmol/kg for ADP, 0.13 mmol/kg for AMP, 3.61 mmol/kg for IMP, 5.5 mmol/kg for CP, 33.8 mmol/kg for lactate and 48 mmol/kg for glycogen.

Correlations

The plasma uric acid concentration at 30 minutes of recovery showed correlations with the muscle ATP ($r=-0.62$), IMP ($r=0.63$) and lactate ($r=0.67$) concentrations immediately post-exercise. An almost significant correlation was also found between peak change in uric acid and decrease in ATP content ($r=0.56$, $p<0.07$).

Study II

Heart rate

The heart rate increased significantly with speed and reached a peak of 213±9 beats/min at baseline, 208±7 beats/min after creatine supplementation and 210±7 beats/min after administration of placebo. The heart rate did not differ between treatments.

Duration of exercise

The duration of exercise varied between horses but most of them performed four speed steps (7, 8 , 9, 10 m/s). With the test design used the duration of exercise should not differ between test occasions. Three of the horses were not able to complete the same duration of exercise as in the baseline test.

Plasma lactate, hypoxanthine, xanthine and uric acid concentrations

The plasma lactate concentrations increased significantly during exercise and reached peak values within 5 minutes of recovery. The mean peak plasma lactate concentration was 28.1±6.4 mmol/l at baseline, 27.8±13.8 mmol/l, after creatine supplementation and 23.5±6.4 mmol/l after administration of placebo. Individual peak plasma lactate concentrations in relation to duration of exercise are shown in Fig. 2a. The mean resting concentrations of hypoxanthine under the same conditions were 1.7±1.1 µmol/l, 1.8±0.8 µmol/l and 1.5±0.7 µmol/l respectively. The mean resting concentration of xanthine was 0.3±0.1 µmol/l at baseline, and 0.4±0.1 µmol/l in both the creatine and

placebo situations. No changes in the plasma hypoxanthine and xanthine concentrations were found either during exercise or recovery. The plasma uric acid concentrations were low during exercise but increased significantly and reached peak values at 10 to 30 minutes of recovery. The mean peak uric acid concentration was 139.2±68.5 μmol/l at baseline, 76.3±31.9 μmol/l after creatine supplementation and 109.5±69.8 μmol/l after administration of placebo. Individual peak plasma uric acid concentrations in relation to duration of exercise are shown in Fig. 2b. No differences were seen in any of these metabolites (plasma lactate, hypoxanthine, xanthine and uric acid) between the different treatments.

Plasma creatine and creatinine concentrations

No changes were found in the plasma creatine or creatinine concentrations after creatine supplementation. These concentrations increased significantly during exercise and were higher at the end of exercise in all situations. The mean post-exercise creatine concentration was 171±58 μmol/l at baseline, 211±40 μmol/l after creatine supplementation and 188±45 μmol/l after administration of placebo. The corresponding mean creatinine concentrations post-exercise were 203±14, 204±19 and 200±14 μmol/l respectively.

Muscle metabolic response (Fig. 3a,b)

Exercise caused significant reductions in the muscle ATP, CP, glycogen and total creatine (not shown in figure) concentrations. The muscle IMP and lactate concentrations were significantly increased. No changes were seen in ADP (resting values: baseline 2.4±0.3 mmol/kg, creatine 2.5±0.3 mmol/kg, placebo 2.6±0.7 mmol/kg) or AMP (resting values: baseline 0.12±0.06 mmol/kg, creatine 0.20±0.06 mmol/kg, placebo 0.15±0.04 mmol/kg) concentrations after termination of exercise except in the baseline test, where the AMP concentration had significantly increased immediately post-exercise. After 15 minutes of recovery ATP had returned to the resting concentrations, while CP and glycogen were still significantly decreased in all groups except in the baseline group for CP and in the placebo group for glycogen. The muscle IMP and lactate concentrations were still elevated compared to the resting values. There was no change in the metabolic response after administration of creatine in comparison with the baseline and placebo situations.

Total blood volume

The total blood volumes were similar between treatments. The mean total blood volume was 132.9±11.8 ml/kg b.w. at baseline, 136.6±12.1 ml/kg b.w after creatine supplementation and 131.9±13.2 ml/kg b.w. after administration of placebo.

Study III

Speed

No differences in speed or placement in the simulated race on the track were evident after sodium bicarbonate administration in comparison with placebo (range 82-85 s/km).

Acid-base balance

The total carbon dioxide concentrations were significantly higher on all sampling occasions after administration of sodium bicarbonate in comparison to placebo (Fig.4). After bicarbonate loading the mean TCO_2 concentration exceeded 37 mmol/l both at 3 hours (40.5±2.5 mmol/l) and at 4 hours (41.0±3.4 mmol/l) post-exercise. After treatment with bicarbonate the TCO_2 concentrations invariably exceeded 37 mmol/l at 3 h post-exercise, and 36 mmol/l at 2, 3, and 4 hours post-exercise. At no time did any of the horses show TCO_2 concentrations above 36 mmol/l after administration of placebo. Blood bicarbonate and pH were significantly higher on all sampling occasions after administration of sodium bicarbonate.

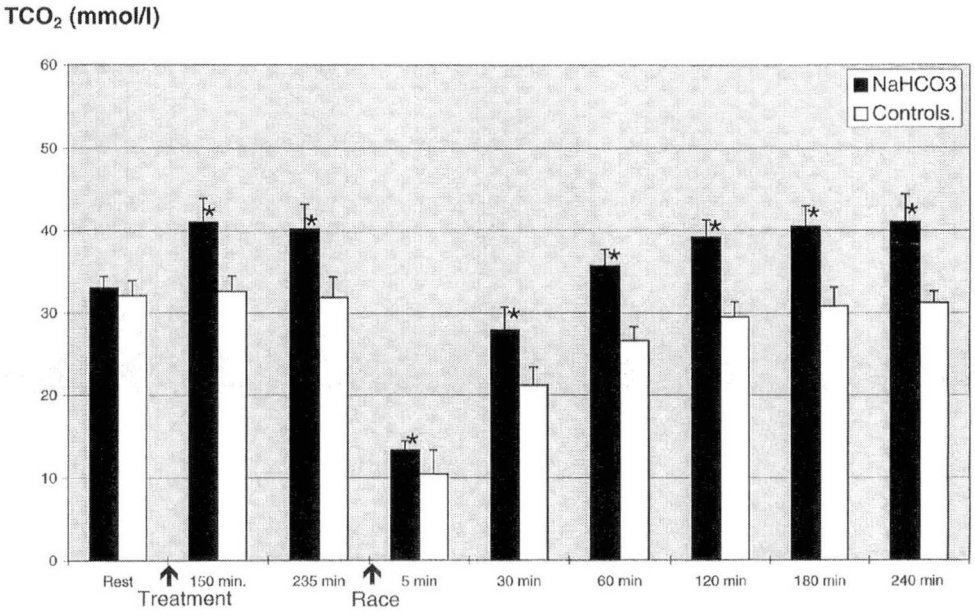

Fig. 4
Plasma total carbon dioxide (TCO_2) concentrations (mmol/l) before and after administration of sodium bicarbonate or water, and during repeated intervals post-exercise. *=significantly different between treatments ($p < 0.05$).

Plasma lactate and uric acid concentrations

The highest plasma lactate concentrations were recorded after 5 minutes of recovery and the mean PLa concentration at that time was 32.6±4.3 mmol/l after bicarbonate loading horses and 29.8±7.0 mmol/l after administration of placebo. The plasma uric acid concentrations increased during recovery and the highest concentrations were seen at 30 minutes of recovery. The mean peak uric acid concentration was 119.0±64.0 µmol/l after bicarbonate administration and 88.0± 69.0 µmol/l after administration of placebo. These differences between treatments were not significant.

Study IV

Heart rate

The highest heart rates were recorded during the last speed step and the mean peak heart rate was 213±3 beats/min after bicarbonate loading and 214±5 beats/min after administration of placebo. No significant difference was found in the heart rate response between the two treatments.

Duration of exercise

Most of the horses in the study performed exercise at four speed steps (7, 8, 9, 10 m/s), but the duration of the last speed step varied between horses. No significant difference was found in the duration of exercise, however, between the two treatments, but in two of the horses the duration of performance was longer (by 45 and 40s) when they had been given bicarbonate than when they had received placebo. One horse continued to run for 25s more when given placebo. Owing to a misunderstanding in this case, the treadmill was slowed down after 10 s on the last speed step, and was then accelerated again, and the horse ran for an additional 15s at 10 m/s.

Acid-base balance

Exercise-induced reductions in pH, blood HCO_3^- concentration and base excess were observed in both treatment groups. The pH, blood HCO_3^- and base excess were significantly higher after bicarbonate loading than after administration of placebo (Fig. 5a,b). The blood HCO_3^- concentrations and base excess were higher at 30, 60, 120 minutes of recovery and the pH at 30, 60, 120 and 180 minutes of recovery after treatment with bicarbonate compared with placebo.

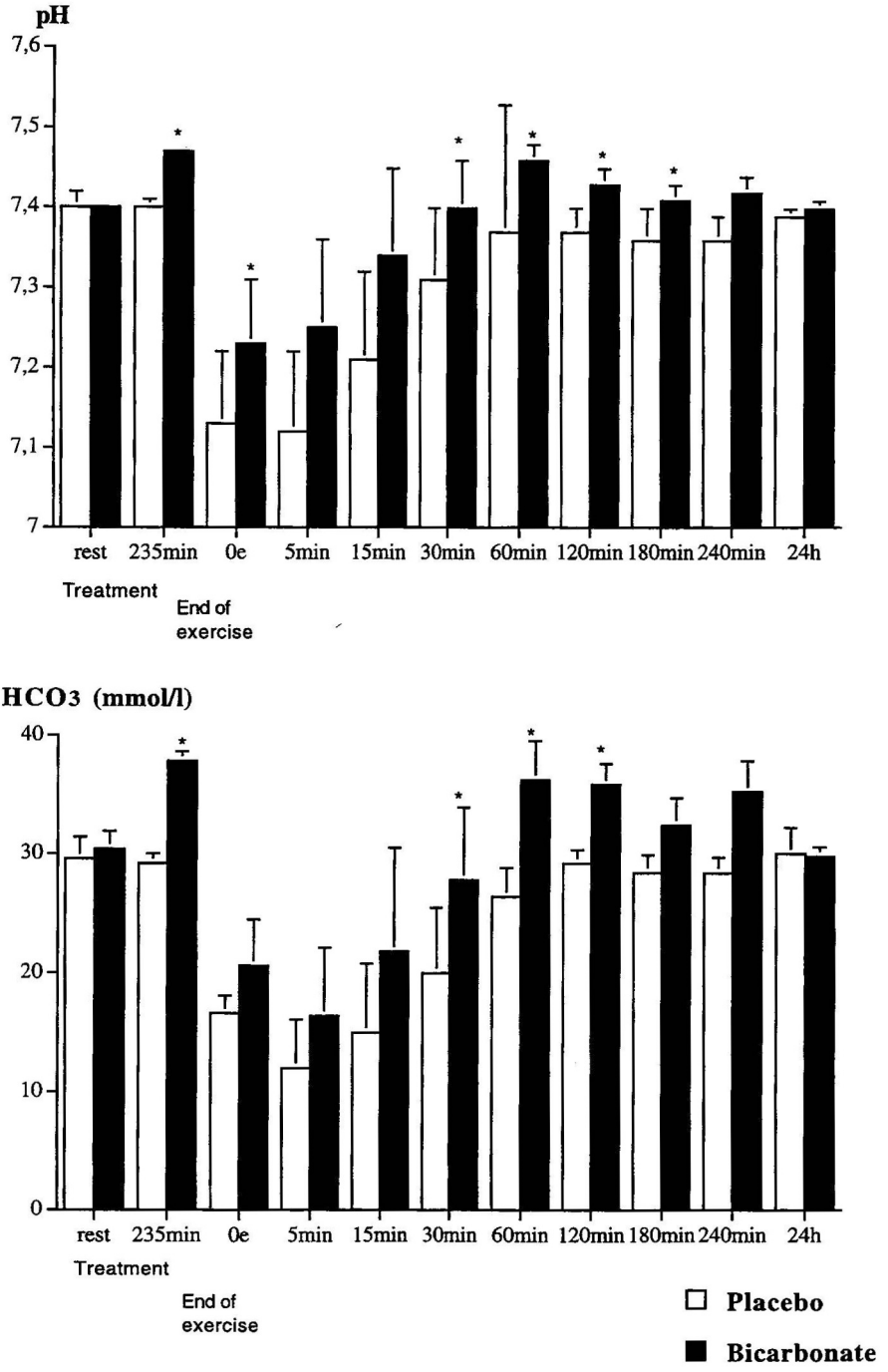

Fig. 5a
Blood HCO_3^- (mmol/l) and pH before and after administration of sodium bicarbonate or water, and during repeated intervals post-exercise. *=significant difference between treatments ($p < 0.05$).

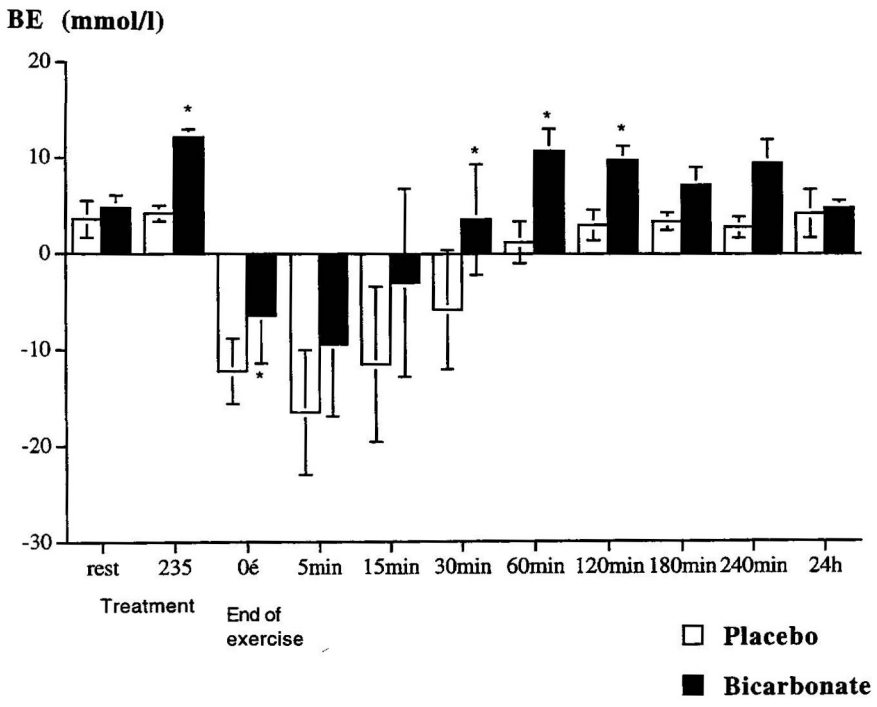

Fig. 5b
Base excess before and after administration of sodium bicarbonate or water, and during repeated intervals post-exercise. *=significant difference between treatments ($p < 0.05$).

Plasma lactate, hypoxanthine, xanthine and uric acid concentrations

Peak PLa concentrations were recorded within 5 minutes of recovery (Fig. 2a). The mean peak PLa concentration was 25.0±7.5 mmol/l after administration of placebo and 27.8±7.8 mmol/l after treatment with bicarbonate. The mean resting concentrations of hypoxanthine (bicarbonate: 1.2±1.0 µmol/l, placebo: 1.0±0.8 µmol/l) and xanthine (bicarbonate: 0.2±0.2 µmol/l, placebo: 0.1±0.2 µmol/l) were low. Hypoxanthine concentrations were significantly increased immediately post-exercise (2.6±0.8 µmol/l) and at 15 minutes of recovery (2.8±2.0 µmol/l) after bicarbonate loading, and at 5 and 15 minutes post-exercise (2.5±2.6 and 2.8±2.5 µmol/l, respectively) after administration of placebo. Xanthine concentrations were significantly increased at 5 and 15 minutes of recovery after bicarbonate loading (1.6±0.9 and 2.4±2.3 µmol/l, respectively) and at 15 minutes of recovery (2.9±3.4 µmol/l) after administration of placebo. Peak uric acid concentrations occurred between 5 and 30 minutes of recovery (Fig.2b). The mean peak uric acid concentration was 126.7±83.9 µmol/l after bicarbonate loading and 133.0±108.8 µmol/l after administration of placebo. No differences were seen in any of these metabolites between treatments.

Muscle metabolic response (Fig. 3a,b)

The concentrations of muscle ATP, CP and glycogen were significantly decreased immediately post-exercise in comparison with resting concentrations

both after placebo and after bicarbonate administration. The muscle ADP and lactate concentrations were significantly increased after both treatments. IMP was significantly increased after administration of placebo but not after bicarbonate treatment, although four of the five horses had higher IMP concentrations after treatment with bicarbonate. No significant change in the concentration of AMP was observed at any time, but four of the five horses had higher AMP concentrations immediately post-exercise after both treatments, compared with the resting value. At 15 minutes of recovery the muscle ATP, ADP and AMP concentrations were not significantly different from the resting values. IMP was still elevated and CP still decreased after bicarbonate treatment. After administration of placebo four of the five horses had higher IMP concentrations immediately post-exercise than at rest, but this difference was not significant. The muscle lactate concentration was still significantly increased and the glycogen concentration was still decreased at 15 minutes of recovery.

Total blood and red cell volumes

No significant differences were seen in the mean total blood volume (placebo: 131.7±12.7 ml/kg b.w., bicarbonate: 133.8±13.7 ml/kg b.w.) or mean red cell volume (placebo: 75.7±9.5 ml/kg b.w., bicarbonate: 74.6±10.9 ml/kg b.w) between the two treatments

Study V

Heart rate

The heart rate increased with speed and the mean peak heart rate was 212±8 beats/min.

Duration of exercise

The duration of exercise varied between horses from 180-300 seconds.

Oxygen uptake during exercise

Oxygen uptake increased with speed and the highest mean oxygen uptake was 64.4±6.5 l/min.

Stride length and stride frequency

The stride length and stride frequency increased with speed and at the last speed step the mean stride length was 4.8±0.4 m and the mean stride frequency was 122±4.6 strides/min.

Muscle metabolic response

The muscle glycogen, CP and ATP concentrations were significantly lower, and the muscle lactate, ADP, AMP and IMP concentrations significantly higher after termination of exercise. The stride frequency close to the onset of fatigue

in each horse was negatively correlated with the increase in muscle ADP post-exercise (r=-0.85).

Duration of exercise and peak plasma lactate, hypoxanthine, xanthine and uric acid concentrations in relation to muscle metabolic response (studies I, II and IV)

When the values from 16 horses (one horse excluded due to the misunderstanding at the last speed step in study IV) in studies I, II and IV were combined, a metabolic response similar to that in the separate studies was observed immediately post-exercise, with significant decreases in muscle ATP, CP and glycogen concentrations and increases in muscle IMP and lactate and in plasma lactate and uric acid (Figs. 3 a,b). Peak post-exercise hypoxanthine and xanthine concentrations were significantly increased in relation to resting concentrations when values from 16 horses were combined. A significant increase in muscle ADP and AMP was also found immediately post-exercise, when the values for 16 horses were combined (Fig. 3 a). In the separate studies a significant increase in AMP immediately post-exercise was only found in test 1a in study I and in the baseline situation in study II (Fig. 3 a). A significantly increased ADP concentration was only found in study IV. After 15 minutes of recovery (n=16) the muscle ATP, CP and glycogen concentrations were still significantly decreased and the muscle AMP, IMP, CP and lactate concentrations significantly increased compared with the resting values.

The peak increases in hypoxanthine and xanthine were positively correlated with the increase in IMP (r=0.63 and r=0.74 respectively) and the increase in peak plasma lactate (r=0.77 and r=0.90 respectively). The peak increase in xanthine was also positively correlated with the increase in peak uric acid (r=0.52).

	ΔATP	ΔADP	ΔAMP	ΔIMP	ΔLa	Δpeak plasma uric acid	Δpeak plasma lactate
Duration of exercise	ns	ns	0.69*	0.71*	0.60*	0.55*	0.62*
Δpeak plasma uric acid	-0.64*	ns	0.63*	0.64*	0.51*	-	0.59*
Δpeak plasma lactate	-0.50*	ns	ns	0.74*	0.57*	0.59*	-

Table 1
Interrelationships (n=16 horses) between duration of exercise, changes in peak uric acid and peak lactate in plasma, and changes in muscle ATP, ADP, AMP, IMP and lactate immediately post-exercise * $p<0.05$. ns=non-significant.

Discussion

Metabolic responses to exercise

Markers for depletion of adenine nucleotides

One of the aims of the present investigation was to develop a standardised maximal exercise test protocol for studies of the muscle anaerobic metabolic response to exercise and to find markers in plasma for the adenine nucleotide degradation that occurs in muscle during intensive exercise. These markers would make it possible to determine the muscle metabolic changes that take place during maximal exercise without using the muscle biopsy technique, which is somewhat impractical. The results of this investigation show that the standardised incremental maximal exercise test caused an anaerobic metabolic response, observed as decreases in ATP and CP concentrations in muscle, and increases in muscle IMP and lactate and plasma lactate and uric acid concentrations post-exercise (studies I, II, IV and V). This is in agreement with the findings in several earlier studies, which have also shown the importance of anaerobic metabolism for energy production in horses during intensive treadmill or track exercise (Lindholm and Saltin 1974; Snow *et al.* 1985; Harris *et al.* 1987; Valberg 1987; Valberg *et al.* 1989; Harris *et al.* 1991; Essén-Gustavsson *et al.* 1995; Ronéus and Essén-Gustavsson 1997). In the present investigation, plasma hypoxanthine and xanthine concentrations were also found to be increased during recovery. IMP can either be reaminated to AMP via the purine nucleotide cycle or further degraded to uric acid through formation of hypoxanthine and xanthine (Lowenstein 1972). Increased concentrations of hypoxanthine, xanthine and uric acid in plasma suggests an energy deficiency resulting from a degradation of ATP. The increases in ADP, AMP and IMP found in the present investigation further indicates that the exercise performed during the standardised incremental maximal exercise test was in fact maximal. It is well documented in rats that large increases in IMP only occur under conditions of maximal contraction (Meyer and Terjung 1980). The fact that the muscle concentrations of ATP, CP and glycogen were still decreased and those of AMP, IMP and lactate were still increased at 15 minutes of recovery also shows that it takes a considerable time for muscle to recover after exercise at maximal intensities. This is in agreement with findings in a study on Thoroughbreds performing four maximal gallops, each gallop covering a distance of 620 m, where practically no resynthesis of muscle ATP after 30 minutes of recovery was found (Snow *et al.* 1985).

The correlation found between an increase in peak plasma uric acid and decrease in muscle ATP and increase in AMP and IMP in the present investigation shows that uric acid may be used as a marker for the adenine nucleotide depletion that occurs in muscle during intensive exercise (Table 1). This finding is supported by results from an earlier study in which Thoroughbreds performed intensive exercise on an inclined treadmill (5°) for

2 minutes (Harris *et al.* 1991). In that study increases in plasma hypoxanthine, ammonia and uric acid showed significant correlations to a decrease in muscle ATP post-exercise. In the present study, no correlation was found between plasma hypoxanthine during recovery and depletion of muscle ATP, which might be due to the fact that the plasma hypoxanthine concentration only increased slightly during recovery. The reason for the finding of such a correlation in the study by Harris et al. (1991) may be that their horses performed exercise at a higher intensity and therefore showed more marked increases in hypoxanthine concentrations. Correlations were found in the present study, however, between the peak change in hypoxanthine and xanthine and the increase in muscle IMP, which suggests that these metabolites can mirror the adenine nucleotide degradation. In a study on Standardbred trotters and Finnhorses that raced 4 x 1,000 m at increasing speeds, no correlation was found between uric acid and the concentrations of ATP immediately post-exercise (Räsänen *et al.* 1993). These differences between studies are probably not related to differences in sampling procedures, as the muscle samples were collected immediately after exercise and blood samples were drawn at frequent intervals during recovery in all studies. One reason for these divergent results could be that the latter study did not show such marked changes in uric acid as the other two studies. In the study by Räsänen et al. the exercise test was performed on a track under less standardised conditions. The distances covered by the horses in the present study (nearly 4,000 m) and the study by Räsänen et al were, however, similar. The fact that the exercise test in the present study was performed on an inclined treadmill and not on the flat is probably the reason for the higher uric acid concentrations in our horses.

The increase in peak plasma lactate was shown to correlate with the increase in IMP and the increase in plasma hypoxanthine, xanthine and peak uric acid, indicating that anaerobic glycolysis has an impact on adenine nucleotide degradation. This has been suggested in a previous study on horses, where the onset of adenine nucleotide degradation was found to be coupled to a lactate threshold value in muscle of 40 mmol/kg d.w (Harris *et al.* 1991). This relationship is probably partly due to an increased availability of AMP in connection with a reduced pH, which will stimulate AMP deaminase to form IMP (Newsholme and Leech 1983; Dudley and Terjung 1985b; Sahlin 1986).

Metabolic response and relation to performance

In the present investigation the duration of exercise showed a relationship both to the changes in muscle IMP, AMP and lactate post-exercise and to the changes in peak plasma uric acid and peak plasma lactate during recovery. This illustrates the importance of anaerobic metabolism, including adenine nucleotide degradation, during intensive exercise. It has been suggested that the depletion of ATP may be partly responsible for the onset of fatigue both in horses and in humans (Snow *et al.* 1985; Sahlin 1986; Harris *et al.* 1991). Previous studies have shown a relationship between the accumulation of adenine nucleotide degradation products (ADP, AMP and uric acid

concentrations) post-race and the performance of the horse (Räsänen 1995; Essén-Gustavsson *et al.* 1997). Horses with better performance tend to have lower accumulation of ADP and AMP (Essén-Gustavsson *et al.* 1997). It has also been found that superior Standardbred trotters have a lower production of lactate during exercise at submaximal intensities in comparison with other Standardbred trotters (Kryzwanek 1973; Persson and Ullberg 1974; Wilson *et al.* 1983; Bayly *et al.* 1987; Evans *et al.* 1993; Ronéus *et al.* 1994). These findings indicate that a well developed aerobic capacity is beneficial for racing performance, as it prevents the accumulation of lactate and the breakdown of adenine nucleotides early in the race.

An optimal balance between the aerobic and anaerobic capacities seems to be important for the performance during a race. During the finish of a race the demand for energy is extremely high and a well developed anaerobic capacity is necessary for a successful result. In the present study, the horses that showed the best performance (longest duration of exercise) were those with the largest accumulations of plasma uric acid and plasma lactate post-exercise. This result is obviously related to the intensity and duration of exercise, as the degree of lactate and uric acid accumulation in plasma and the time it takes to reach peak concentrations of these metabolites have been shown to be related to these factors (Harris *et al.* 1987; Harris *et al.* 1991; Räsänen *et al.* 1996). It may be speculated, however, that a high capacity for adenine nucleotide breakdown and anaerobic glycolysis is beneficial for the ability to increase speed at the end of the race. This is supported by the fact that faster horses reach higher blood lactate levels than slower horses (Saibene *et al.* 1985; Ronéus and Essén-Gustavsson 1997; Bayly *et al.* 1987; Harkins *et al.* 1993; Räsänen *et al.* 1995). If the aerobic capacity increases at the expense of the anaerobic capacity, it is reasonable to assume that the horse may lose its ability to increase speed at the finish of a race. The ability to endure these high lactate concentrations is probably coupled to a well developed buffering capacity. This is indicated by the finding of a negative correlation between running speed and minimum pH and minimum bicarbonate concentration in the blood after a race (Harkins *et al.* 1993). A seven-week training period has been shown to increase the intramuscular buffering capacity in Thoroughbred horses (McCutcheon *et al.* 1987). Training has also been shown to diminish the fall in pH during exercise in Thoroughbreds performing a 3 x 600 m race (Snow and MacKenzie 1977). A suggested reason for this is an increased capacity for lactate removal by oxidative muscle fibres and by the liver.

Although all horses in the present investigation showed obvious signs of fatigue at the end of the standardised incremental maximal exercise test, individual variations were observed in the extent of ATP depletion and plasma lactate and uric acid accumulation during recovery. Some of the horses were unable to continue the exercise despite the fact that only minor changes in muscle adenine nucleotides and in plasma lactate and uric acid concentrations occurred. These individual variations in adenine nucleotide depletion have been observed previously in Thoroughbreds that have raced over distances of 1,400 to 3,800 m (Sewell *et al.* 1992). The reason for these differences in metabolic responses between horses could be related to factors such as training

status, fibre type composition and the oxidative capacity of the muscle (Lindholm *et al.* 1974; Valberg *et al.* 1985; Valberg *et al.* 1987). The acitvity of key regulatory enzymes such as myokinase and AMP-deaminase might also vary between horses. Studies in rats have shown that highly oxidative fast-twitch fibres have a higher potential to maintain their ATP content than low-oxidative fast-twitch fibres (Dudley and Terjung 1985a,b). Higher ammonia concentrations have also been found after intesive exercise in humans with a higher proportion of type II fibres (Dudley *et al.* 1983). After racing, the percentage of type II fibres in the muscle of horses have also been shown to be positively correlated with muscle ammonia and to the allantoin concentration in plasma and negatively correlated with ATP concentrations in muscle (Essén-Gustavsson and Valberg 1987; Räsänen *et al.* 1993). This indicates that AMP-deamination is of importance for energy release in type II fibres in connection with intensive exercise. After racing, muscle ATP concentrations have also been reported to be lower in pools of type IIB than in type I and IIA fibres (Valberg and Essén Gustavsson 1987). These results suggest that the muscle fibre composition and the oxidative capacity has an influence on the degree of adenine nucleotide degradation. The fibre composition and oxidative capacity in muscle of the different horses were not investigated in the present study.

The fibre recruitment pattern is, however, another factor to take into consideration when discussing metabolic response and onset of fatigue. A previous study on horses has shown that after a race the concentration of muscle ATP is lowered but may vary between different single fibres (Essén-Gustavsson *et al.* 1997). Some fibres are completely depleted of ATP, whereas others show only minor depletion. This is in agreement with a conclusion from another study that the decrease in muscle ATP after exercise may vary between fibres (Harris *et al.* 1997). The importance of these differences in ATP depletion between single fibres is unclear. It may only be speculated that the onset of fatigue in some horses might be related to depletion of ATP in certain fibres. The number of fibres showing such changes is also probably important for the influence on performance. In the current investigation measurements were made on whole muscle and therefore nothing can be said about the ATP concentrations in single fibres. Further investigations on the metabolic response in single fibres are therefore needed to establish whether this might influence the time to fatigue in some horses.

Methodological considerations

To be able to use peak plasma uric acid as a valid marker for the adenine nucleotide degradation in muscle during maximal exercise, it is important to standardise the exercise conditions so that the horses have a similar work load. Caution should therefore be observed when comparing results between track and treadmill studies, as track tests are less standardised and the metabolic response can be influenced by weather conditions. Exercise on a wet track involves a heavier work load and therefore a greater demand on the anaerobic capacity than exercise on a dry and firm track. The standardised incremental maximal exercise test used in the present study induced an anaerobic metabolic response (Fig. 3 a,b) that was similar between test occasions (study

I). The rapid changes in blood and muscle metabolites that occur during high intensity exercise are one of the difficulties encountered when studying the anaerobic metabolic response. This means that small differences in sampling time and duration of exercise can lead to marked differences in the concentrations recorded, making the results difficult to repeat. Many of the studies in the area of exercise physiology have therefore been conducted with submaximal exercise tests, where the metabolic changes occur more slowly and reach steady state levels. Another factor to take into consideration when studying Standardbred trotters is that these horses can alter their gait and start to gallop when exercising at high speeds. This might influence the fibre recruitment and the muscle metabolic response to exercise.

The fact that some metabolites (muscle ADP, AMP and plasma xanthine and hypoxanthine) showed significant changes with exercise on some test occasions but not on others, is probably due to the small number of horses used in the separate studies. This means that a different metabolic response in one horse could have a great effect on the results obtained. This is supported by the fact that significant increases were found in muscle ADP and AMP (Fig. 3a) and plasma hypoxanthine and xanthine concentrations when the values from all horses were calculated together.

Creatine supplementation

In study II no significant increase in the plasma creatine or muscle total creatine concentration was found after supplementation with creatine monohydrate in a daily dose of 50 g for 6.5 days. This is in agreement with the results of the two previous studies regarding creatine uptake in horses (Essén-Gustavsson *et al.* 1994; Sewell and Harris 1995). The findings regarding the uptake of creatine in horses are in sharp contrast to those in human beings, in whom a daily creatine dose of 20 g for 5 days has been shown to considerably increase both the plasma creatine and TCr concentrations (Harris *et al.* 1992; Greenhaff *et al.* 1994; Balsom *et al.* 1995; Green *et al.* 1996; Casey *et al.* 1996; Hultman *et al.* 1996; Engelhardt *et al.* 1998). The reason for these discrepancies between species could be related to the fact that horses, being herbivores, might have a diminished capacity for intestinal absorption of creatine in comparison with humans, in whom creatine is a natural part of the diet. Other factors that could have an influence on the uptake of creatine from the gut are the dose of creatine used and the mode of administration. In the study by Essén-Gustavsson et al. (1994), a creatine monohydrate dose of 0.1 g/kg b.w. was given twice daily in the feed, whereas in the study by Sewell and Harris (1995) a dose of 0.15 g/kg b.w creatine monohydrate was given three times daily in the water. The daily dose used in the present study was somewhat lower (0.1 g/kg b.w.), but was chosen in accordance with the manufacturer's recommendation for horses, and is the dose commonly used. Factors that are known to enhance the uptake of creatine in humans is a diet rich in carbohydrates as well as exercise during the

supplementation period (Harris *et al.* 1992; Green *et al.* 1996). The horses in the present investigation were all regularly exercised during the supplementation period and as the diet of the horse normally consists mainly of carbohydrates, the conditions ought to have been optimal for uptake from the gastrointestinal system.

The initial total creatine concentration in muscle may also have an impact on the uptake of creatine by muscle from the blood. This has been suggested from studies on humans, in which large individual variations in the initial muscle TCr concentration were seen (Harris *et al.* 1992). The mean resting concentration of muscle TCr in man has been reported to be 125 mmol/kg b.w, with a range of 90-160 mmol/kg b.w (Greenhaff *et al.* 1995). There are indications that individuals with low initial TCr concentrations can increase their muscle TCr concentration to a greater extent than those with higher initial concentrations (Harris *et al.* 1992; Ekblom *et al.* 1996). There are also indications that there may be an upper level of creatine storage of 150-160 mmol/kg b.w. which cannot be exceeded (Harris *et al.* 1992; Greenhaff *et al.* 1994). Large variations in the initial TCr concentration were also found among the horses of the present investigation (mean TCr: 130 mmol/kg b.w; range: 110-160 mmol/kg). Such individual variations were also observed in the other two studies of creatine uptake in horses by Essén-Gustavsson et al. (1994) and Sewell and Harris (1995), in which the mean initial muscle TCr concentrations were 127±15 and 104±4 mmol/kg respectively. The fact that no changes were observed in muscle TCr concentrations after creatine supplementation in these studies might indicate that the horses used had already reached their maximal creatine storage capacity in muscle and therefore did not benefit from creatine supplementation. Further studies on horses with low initial muscle TCr concentrations are therefore needed to establish whether in horses the muscle can take up creatine from the blood. As no significant increase in the muscle TCr or plasma creatinine concentration was observed after supplementation with creatine, no effect on the metabolic response could be expected. This also proved to be the case, as there were no changes in the depletion of muscle ATP, CP or glycogen or increases in the muscle IMP or muscle or plasma lactate or uric acid concentration after treatment with creatine. The standardised incremental maximal exercise test, however, caused an anaerobic response and adenine nucleotide degradation in all horses. If uptake of creatine had in fact occurred in the present study, this is the exercise intensity at which the horse should benefit from creatine supplementation

Supplementation with creatine has been shown to increase the body weight in human beings, possibly as a result of increased water retention (Balsom *et al.* 1993b; Balsom *et al.* 1995; Green *et al.* 1996; Hultman *et al.* 1996; Cooke and Barnes 1997; Dentkowskii *et al.* 1997; Engelhardt *et al.* 1998). There are also indications that creatine supplementation might increase the protein

synthesis and thereby the lean body mass (Williams and Branch 1998, review). Despite the fact that no significant uptake of creatine could be detected in horses, an increase in total blood volume has been observed after supplementation with creatine (Essén-Gustavsson *et al.* 1994). The total blood volume did not increase after supplementation with creatine in the present investigation. The reason for these contradictory results could be that the creatine dose was lower (by half) than that used in the study by Essén-Gustavsson et al. (1994).

Bicarbonate loading

Measurement of total carbon dioxide post-race

Despite the fact that no effects of sodium bicarbonate administration on performance have been documented, bicarbonate loading before races may still be practised. Pre-race measurement of plasma TCO_2 in horses has previously been proved to be a reliable indicator of the administration of sodium bicarbonate, and a pre-race value exceeding the threshold value of 37 mmol/l is regarded as evidence that a horse has received an alkalising agent (Lloyd and Rose 1992; Auer *et al.* 1993; Kallings and Persson 1994; Reilly *et al.* 1996). In study III it was found that administration of 0.6 g/kg b.w of sodium bicarbonate caused an increase in the plasma TCO_2 concentration to above 37 mmol/l both before and 3 hours after a race. This shows that a post-race plasma TCO_2 value exceeding the internationally established threshold of 37 mmol/l is a reliable indicator of pre-race bicarbonate loading. This could have practical consequences in Sweden, where the regular doping controls are usually performed post-race. There is a current suggestion, however, that the international threshold should be lowered from 37 to 36 mmol/l. The present findings indicate that lowering of the threshold value would make it possible to continue having the Swedish regular doping control within 2 hours post-race, as the plasma TCO_2 exceeded 36 mmol/l in all cases after bicarbonate loading but in no case after administration of placebo.

Effects on acid- base balance and metabolic response to exercise

Administration of sodium bicarbonate caused marked metabolic alkalosis (studies III and IV), in accordance with previous findings in both horses and humans (Kelso *et al.* 1987; Lawrence *et al.* 1987; Horswill *et al.* 1988; Greenhaff *et al.* 1990; Lawrence *et al.* 1990; Harkins and Kamerling 1992; Lambert *et al.* 1993; Lloyd *et al.* 1993; Lloyd and Rose 1995). Despite the alterations in the blood acid-base status after bicarbonate administration, no effect on the muscle metabolic response to intensive exercise on track (study III) or on the treadmill (study IV) was observed. These findings are in agreement with the results from another study performed on Thoroughbreds racing a distance of 1,600 m on a track, where no significant differences in muscle metabolic responses (muscle CP, ATP, lactate and pH) were found after bicarbonate administration (Kelso *et al.* 1987). There are indications, however,

that bicarbonate loading can influence muscle metabolic responses, as a reduced decline in muscle ATP and a diminished increase in muscle IMP have been seen in horses exercising on a treadmill (2 min at 12 m/s on an inclined treadmill) after administration of bicarbonate in comparison with placebo (Greenhaff *et al.* 1991b). These horses also showed lower accumulation of ammonia in plasma during recovery, indicating diminished adenine nucleotide depletion (Harris *et al.* 1991). The lack of effect on the metabolism in the former study by Kelso et al. (1987) could be related to the fact that a lower dosage (0.4 g/kg b.w) and a shorter period between administration and exercise (1 hour) were used than in the study by Greenhaff et al. (1991b). This is not a likely explanation for the lack of effect on the metabolism in the present study, as the same dosage and the same interval between administration and exercise were used as in the study by Greenhaff et al. (1991b). This interval has been shown to be adequate, as maximal changes in acid-base balance do not occur until al least 3 hours after bicarbonate administration with this dose (Greenhaff *et al.* 1990). A more likely explanation for the lack of effect on the metabolism after bicarbonate loading in the present investigation is that the work load might have varied between the studies. This is supported by the fact that the changes in ATP, IMP and lactate in muscle were greater in the study by Greenhaff et al. (1991b). These differences are probably due to the fact that their exercise test was performed at the same high speed up to fatigue, whereas in the present study an incremental test was used. Another factor that might have influenced the results is that the former study was performed on Thoroughbreds, which are known to have a greater proportion of type II fibres than Standardbreds (Valberg 1987). These fibres are more glycolytic and have been shown to exhibit greater depletions of ATP and accumulations of lactate after exercise than type I fibres (Valberg and Essén-Gustavsson 1987). Studies on rats have also shown a greater accumulation of IMP in type II than in type I fibres (Meyer *et al.* 1980). This indicates a higher capacity for adenine nucleotide degradation in these fibres. In addition to factors such as motivation to run, differences in fibre-recruitment patterns might have influenced the results, as discussed earlier in this thesis.

Significantly higher plasma and blood lactate concentrations have been found after bicarbonate administration in horses, both before and after exercise (Lawrence *et al.* 1987; Greenhaff *et al.* 1991a; Harkins and Kamerling 1992; Lloyd *et al.* 1993; Kallings and Persson 1994). The absence of significant changes in plasma lactate in the present study could be due to the small number of horses used, in that deviating values in one individual could have had a great effect on the result. This is illustrated by the fact that all horses except one had higher concentrations of plasma lactate at the end of exercise. Another explanation could be that the measurements in the present study were made on plasma instead of blood. This might have affected the results, as lactate can be transported, by passive diffusion or by active transportation, into

red blood cells when the lactate production is high (Pösö *et al.* 1995; Väihkönen *et al.* 1998). As the active transport mechanism can vary between individuals, this could be a factor to take into consideration. In some studies, however, no significant increase in plasma or blood lactate has been found after bicarbonate administration (Kelso *et al.* 1987; Lawrence *et al.* 1990). In those studies, lower dosages of bicarbonate and/or shorter periods between administration and exercise were used than in the studies that have shown altered plasma lactate concentrations after bicarbonate treatment. This is probably one explanation for the divergent results in these studies.

The results regarding the effect of sodium bicarbonate are conflicting. The effectiveness of this treatment, however, seems to be dependent on the intensity and duration of exercise. Studies on humans have shown that sodium bicarbonate administration can be beneficial for performance during high-intensity exercise, or when exercise is performed in short repeated bouts (Costill *et al.* 1984; Lindeman and Fahey 1991; Harkins and Lawrence 1994). In horses no significant influence on the running time on track or on a treadmill have been observed after bicarbonate administration, although some studies have indicated a tendency towards improved performance after bicarbonate treatment in comparison with placebo (Kelso *et al.* 1987; Lawrence *et al.* 1987; Lawrence *et al.* 1990; Greenhaff *et al.* 1991a; Harkins and Kamerling 1992). In the present investigation no significant effect was found in the running time or placement over a distance of 2,000 m on a track (study III), or in the duration of exercise on a treadmill (study IV). Two of the horses in study IV, however, continued to run for 45 seconds longer when treated with bicarbonate as compared to placebo, which was more than the expected variation of 10-15 seconds which had been seen earlier (study I) with this test protocol. On the other hand one of the horses ran for 25 seconds longer when given placebo. This, together with the fact that the two horses that continued to run for a longer duration when given bicarbonate were the horses with the lowest plasma lactate concentrations, suggests that bicarbonate has no influence on performance. If bicarbonate does have an effect on performance, it would have been expected to be found in horses with high plasma lactate concentrations. It is also important to remember that the horse's motivation to run may also have differed from the test occasions and the fact that one person decided when the horses could not continue to exercise also may have influenced the results. These factors together are probably the reason for the increase in running time observed in some horses rather than an actual effect of bicarbonate.

Locomotion pattern and its relation to metabolic response

In the present study differences were seen in the stride length and stride frequency close to fatigue. These variations might be partly attributable to individual differences in fibre type composition, metabolic profiles in the muscle fibres and recruitment pattern. A previous study has shown a negative

relationship between stance time and percentage of type IIB fibres in young Standardbred trotters performing maximal exercise on track (Ronéus *et al.* 1995). That study also showed a negative relationship between plasma lactate and both stride length and stance time. This indicates that young horses with a low proportion of type IIB fibres perform maximal exercise with a low lactate production and long stride length and stance time. In older horses, stride length at the anaerobic threshold has been shown to be positively related to the percentage of type I and IIA as well as the red cell volume, which is a marker for oxygen transport capacity (Persson *et al.* 1991). These findings indicate that stride length is important for aerobic energy expenditure.

Little is known about the possible influence of fatigue on the locomotion pattern during exercise, but the stride frequency has been found to decrease in Thoroughbreds showing signs of fatigue (Leach and Springings 1979, 1980). The relationship observed between the accumulation of ADP post-exercise and the stride frequency close to fatigue in the present study indicates that the anaerobic metabolism can influence the locomotion of the horse. It is therefore reasonable to assume that the ability to maintain low concentrations of ADP in muscle is beneficial for performance. A high proportion of type IIA fibres is thought to have a beneficial effect on performance since horses with a high proportion of these fibres are able to increase their stride length more and thereby minimise their energy cost. Of note is that a higher type IIA/IIB ratio is often seen in racehorses with better racing records (Essén-Gustavsson and Lindholm 1985).

Different fibre type composition, metabolic profile and recruitment pattern may therefore all be factors that could explain the development of fatigue and why a variation was seen among horses in the present study. Another important factor that can influence the onset of fatigue, although it is hard to control, is the motivation of the horse itself to run.

General conclusions

The standardised incremental maximal treadmill exercise test makes it possible to make qualitative and quantitative studies of the anaerobic metabolic response in skeletal muscle both regarding the glycogen breakdown and lactate production and the adenine nucleotide degradation. During intensive treadmill exercise to fatigue, the degradation of adenine nucleotides is of considerable importance for the duration of exercise. The depletion of adenine nucleotides in muscle was also shown to be reflected by the change in peak plasma uric acid during recovery.

There seems to be a relationship between the stride frequency close to fatigue and the accumulation of ADP in muscle. This suggests that an ability to maintain low concentrations of ADP in muscle is beneficial for an increase in speed at high exercise intensities.

The muscle metabolic response during intensive exercise was not influenced by the administration of creatine or bicarbonate, indicating that these substances have no effect on the anaerobic metabolic response with the dosage used in the present investigation. Administration of sodium bicarbonate does not seem to influence the speed or placement during a simulated race on a track.

The TCO_2 concentration in plasma 3 hours post-race proved to be a reliable marker for detecting pre-race administration of sodium bicarbonate. This study indicates that a reduction of the internationally established threshold value for plasma TCO_2 from 37 to 36 mmol/l would make it possible to continue having the ordinary Swedish doping control at 2 hours post-race.

Future clinical and practical aspects:

Some aims of our planned future research are:

To study the muscle metabolic responses in horses with impaired performance (red cell hypervolaemic horses), using the standardised incremental maximal exercise test, and to determine whether these horses have a decreased anaerobic metabolic capacity.

To introduce a track test under as standardised conditions as possible, yielding similar anaerobic metabolic responses, and to determine whether the plasma uric acid concentration can be used as a marker for adenine nucleotide degradation in muscle in horses performing maximal exercise on the track.

To examine the metabolic changes that occur in single fibres after performance of the standardised incremental maximal exercise test, and to determine wheter horses with impaired performance show similar changes in single fibres to those found in horses with normal performance.

To measure the activities of myokinase and AMP-deaminase in different horses and to determine whether training can influence the activities of these enzymes and thereby the adenine nucleotide degradation.

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