ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 6

Muscle Metabolic Response to Track Exercise in Standardbred Trotters

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SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Akademisk avhandling som för vinnande av veterinärmedicine doktorsexamen kommer att offentligen försvaras i Ettans föreläsningssal. Klinikcentrum, Ultuna, fredagen den 17 januari 1997, kl 09.15.

Abstract

This study was undertaken to investigate if measurement of plasma lactate concentration and certain muscle characteristics following a track test can provide important information about a horse's performance capacity.

In Standardbred trotters, marked individual differences are evident in plasma lactate concentration and muscle metabolic responses to maximal and submaximal work performed on a race-course. A negative correlation was found between plasma lactate concentration to a submaximal test, and the horse's maximal trotting speed over 1600 m, and a more marked anaerobic glycolysis and ATP breakdown during maximal exercise, than had slower horses.

Inverse correlations were found between stance time and both plasma lactate and percentage of type IIB fibres, indicating that locomotion pattern may be partly dependent on both fibre type composition and metabolic profile in muscle.

Neither fibre type composition, enzyme activities, nor plasma lactate or ammonia concentration after a race showed any correlation with performance potential, expressed as individual performance index (IPI).

The results of this study also show that adenine nucleotide breakdown in muscle is of great importance for energy release during racing and that ATP and IMP concentrations can vary markedly among individual fibres after a race. Thus, metabolite determinations in whole muscle must be evaluated with caution as they are no more than mean values for metabolic response in different fibres during racing.

Tactical driving is important in racing; running against time in a track test or an exercise test on a treadmill is therefore more standardized than racing proper.

It is evident that measurement of plasma lactate and certain muscle characteristics after a standardized track test in 2-year-old Standardbred trotters can provide important information about a horse's performance capacity. This type of information can be useful for trainers when subjecting a young horse to more individual training. However, further research is required before specific, detailed recommendations can be made regarding optimal training methods.

Keywords: horse, plasma lactate, muscle, muscle characteristics, track test.

Distribution:
Swedish University of Agricultural Sciences
Department of Medicine and Surgery
P.O.Box 7018, S-750 07 UPPSALA, Sweden

Uppsala 1996
ISSN 1401-6257
ISBN 91-576-5233-3

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Doctoral thesis
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Acta Universitatis Agriculturae Sueciae
Veterinaria 6

ISSN 1401-6257
ISBN 91-576-5233-3

Tryck: SLU Repro, Uppsala 1996

To the young Standardbred trotter
with poor performance capacity

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Contents

Appendix

Papers I-V

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

I. Plasma lactate response to submaximal and maximal exercise tests with training, and its relation to performance and muscle characteristics in Standardbred trotters. 1994. Ronéus, N., Essén-Gustavsson, B., Lindholm, A. and Eriksson, Y. *Equine.Vet. J.* 26, 117-121.

II. Muscle characteristics and metabolic response to a 1600 m track test in 2-year-old Standardbred trotters. 1996. Ronéus, N., Essén-Gustavsson, B. Accepted for publ. in *Am. J. Vet. Res.*

III. Lactate response to maximal exercise on the track: relation to muscle characteristics and kinematic variables. 1995. Ronéus, N., Essén-Gustavsson, B., Johnston, C., Drevemo, S. and Persson, S.G.B. *Equine. Vet. J.* Suppl. 18, 191-195.

IV. Muscle characteristics and plasma lactate response in Standardbred trotters after racing: relation to performance. 1996. Ronéus, N., Essén-Gustavsson, B., Lindholm, A. and Persson, S.G.B. Submitted for publication.

V. Metabolic response in skeletal muscle fibres of Standardbred trotters after racing. 1996. Essén-Gustavsson, B., Ronéus, N. and Pösö, R. Accepted for publication in *Comparative Biochemistry and Physiology.*

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Abbreviations

ADP	Adenosine diphosphate, mmol/kg
AMP	Adenosine monophosphate, mmol/kg
ATP	Adenosine triphosphate, mmol/kg
CP	Creatine phosphate, mmol/kg
CS	Citrate synthase, mmol/kg/min
G-6-P	Glucose-6-phosphate, mmol/kg
GLY	Glycogen, mmol/kg
HAD	3-OH-acyl CoA dehydrogenase, mmol/kg/min
HK	Hexokinase, mmol/kg/min
IMP	Inosine monophosphate, mmol/kg
IPI	Individual performance index, %
LDH	Lactate dehydrogenase, mmol/kg/min
PLA	Plasma lactate, mmol/l
SL	Stride length, m
ST	Stance time, ms

Introduction

The sporting horse is an impressive animal, endowed with both grace and stamina, an animal with unique physical capacity. It has a high heart to body weight ratio, abundant cardiac output, and substantial scope for expansion of the oxygen carrying capacity made possible by splenic erythrocyte release during exercise (for references, see Hodgson and Rose, 1994). The muscle power in horses is probably a result of the need for horses in the wild to produce sudden bursts of highly intense physical effort when attempting to escape from predators. Their anatomy and instincts are adapted to "flight rather than fight".

Excellence in sporting performance requires a complex interaction of physiologic mechanisms such as cardiocirculatory oxygen transport capacity, muscle characteristics, ability to recruit muscle fibres, locomotion pattern, motivation and adaptation to training. All of the above mentioned factors are of course important for performance capacity but each factor could also be a limitation. Standardbred trotters have been carefully selected and bred for speed and performance over many generations, and there is really not any big difference in the speed capabilities of the majority of horses. The successful racehorse, however, must perform fast trotting over at least 2000 m as well as performing intermittent increments of speeds. This requires additional attributes of stamina and "the will to win". The genetic inheritance of the right combination of these factors is the primary requisite for an outstanding racehorse, and no horses will be successful lacking these traits.

At rest, equine blood lactate concentrations are normally <1 mmol/l. This is the consequence of there being only minimal production of lactate within the metabolically active tissues since all energy needs are satisfied by the aerobic processes. The accumulation of blood lactate in response to exertion is generally regarded as an indicator of fitness and standard of training, as it reflects the dependence on anaerobic metabolic processes. Several major adaptations occur in connection with physical training that influence lactate metabolism, including improved circulatory function and metabolic efficiency of skeletal muscle. An identifiable effect of this enhanced metabolic capacity is the finding that blood lactate concentrations decrease, following a prescribed standardized spell of exercise, compared with levels before training (Persson and Ullberg, 1974; Jones and Campbell, 1982; Persson, 1983; Harris *et al.*, 1991; Evans *et al.*, 1993). The differences in metabolic response to exercise are probably related to individual genetic factors associated with potential for aerobic and anaerobic energy production, locomotion pattern and motivation.

Muscle characteristics

In the Standardbred trotter muscle mass constitutes approximately 42-45 % of total body weight (Gunn, 1987; Karlström *et al.*, 1994).

Sampling of skeletal muscle by the needle biopsy technique was first introduced into equine exercise physiology by Lindholm and Piehl (1974). Depending on myosin ATPase activity at pH 9.4, muscle fibre types are classified as Type I (slow twitch) and Type II (fast twitch fibres) fibres (Engel, 1962). Type II fibres can be subdivided into Type IIA and Type IIB, according to preincubation at different pH values before ATPase staining (Brooke and Kaiser, 1970).

Histochemical analysis of skeletal muscle samples has shown that the equine gluteus medius consists of a mixture of slow-contracting Type I fibres and fast-contracting Type II fibres having different metabolic properties (Lindholm and Piehl, 1974; Essén *et al.*, 1980; Valberg and Essén-Gustavsson, 1987). In the horse Type I fibres have a low and Type II fibres a high glycolytic capacity, whereas oxidative capacity is demonstrably higher in Type I and Type IIA fibres, than in Type IIB fibres (Valberg and Essén-Gustavsson, 1987). The largest proportion of type II fibres is found superficially, with an increasing proportion of type I fibres in the deeper parts (Kline *et al.*, 1987; Karlström *et al.*,1994).

Enzymes can be studied in mitochondria and sarcoplasm. A marker for the oxidative pathway in the Krebs cycle is citrate synthase (CS), while β-oxidation is represented by 3-OH-acyl CoA dehydrogenase (HAD). The stage where glucose becomes involved in the metabolic breakdown of carbohydrate (glycolysis) is regulated by the activity of enzyme hexokinase (HK), while the anaerobic chain and lactate formation, can be studied by lactate dehydrogenase (LDH) activity determination.

How muscle characteristics are related to performance

All muscle fibres at rest contain glycogen and studies on the glycogen depletion pattern during muscle work have shown that the fibres are recruited in order, Type I through IIA to IIB as the horse accelerates (Hodgson *et al.*, 1983; Valberg, 1986; White *et al.*, 1987). Oxidative capacity increases in Type IIB fibres with ageing and training (Essén *et al.*, 1980; Ronéus *et al.*, 1992). A longitudinal study of Standardbred trotters using muscle biopsies obtained from the foal stage up to 4-5 years of age showed the significance of a highly developed oxidative capacity, especially of Type IIB fibres, for racing performance (Ronéus *et al.*, 1992)

The importance of muscle characteristics for performance has been demonstrated in several studies. Snow and Guy (1980) studied six limb muscles in different breeds of horse and found relationships between the type of performance the breed had been selected for and the fibre type

composition of the middle gluteal muscle. Quarter horses, which run short races over 400 m, have the largest proportion (90-95 %) of Type II fibres. Thoroughbreds racing over 1000 up to 3000 m, have approximately 85-90 % Type II fibres, while Standardbreds, which have been developed for harness racing, i.e. trotting or pacing from 1600 up to 2600 m, have approximately 75-85 % Type II fibres (Essén *et al.*,1980; Snow and Guy, 1980; Essén-Gustavsson and Lindholm, 1985). Endurance breeds such as the Arab, which has been bred for stamina rather than speed, and the Spanish Andalusian horse, are known to have approximately 70 % Type II fibres (Lopez-Rivero *et al.*, 1991). Among Standardbred trotters, those that do well have a higher Type IIA/IIB ratio than horses with poorer performance (Essén-Gustavsson and Lindholm, 1985; Ronéus *et al.*, 1992).

Although the Type I/II fibre ratio is genetically predetermined, training can alter the relative proportions, especially a transition of Type IIB to Type IIA fibres, depending on the training regimen (Essén-Gustavsson and Lindholm, 1985; Hodgson *et al.*, 1986; Snow and Vogel, 1987; Ronéus *et al.*,1992). Muscle characteristics of horses differ between individuals with age, sex, and state of training (Ronéus *et al.*,1991,1992).

It is known that horses with differences in muscle fibre composition have different metabolic responses to standardized submaximal or near-maximal exercise (Valberg *et al.*, 1985). In earlier studies well trained horses were found to have a high muscle oxidative capacity, demonstrated by high CS and HAD activities and relied to a great extent on aerobic energy release, in comparison with untrained horses (Thornton *et al.*, 1983; Bayly *et al.*, 1987). Blood lactate response to a given exercise can differ markedly between individuals and this may be related to muscle fibre properties (Valberg *et al.*, 1987). A close correlation exists between lactate accumulation and the proportion of type IIB fibres in the muscle. As the intensity of muscle work increases, more low-oxidative type IIB fibres are recruited and the energy production becomes more dependent on anaerobic metabolism, but leads to the formation of lactate. Horses with a large proportion of type IIB fibres are susceptible to greater lactate production, as these fibres have the highest glycolytic and lowest oxidative capacities (Valberg *et al.*, 1985; Ronéus *et al.*, 1987; Valberg, 1987). When adult horses perform submaximal work of equal intensity, those with a greater proportion of type IIB fibres produce more lactate (Valberg *et al.*, 1985).

Muscle metabolic response to exercise

Skeletal muscle is composed of fibres that run parallel to the long axis of the muscle. At the head of each myosin filament is an adenosine triphosphate (ATP) molecule that during contraction becomes hydrolysed and releases energy. This reaction is expressed as $ATP + H_2O \rightarrow ADP + Pi$

+ H^+ + energy. The energy is expended when the cross-bridges change their orientation in relation to the axis of the myosin core, thus pulling the actin filaments along the myosin filaments. This process is repeated millions of times during muscular contraction.

All metabolic processes are designed to produce ATP, which is the ultimate substrate consumed by muscle. As the ATP is degraded, it must constantly be replenished and the rate of replacement must match the rate of consumption if muscle contraction is to continue. This is accomplished by a variety of ATP-producing reactions that are available in the muscle. An extremely rapid mechanism comes into play when creatine phosphate (CP), which is present in muscle, transfers its phosphoryl group to adenosine diphosphate (ADP) in order to replenish ATP. This reaction is catalysed by the enzyme creatine phosphokinase. The store of CP is expended within a few seconds. If muscle work continues, glycolytic metabolic reactions involving glycogen and glucose must occur quickly in order to supply additional ATP.

If enough oxygen is delivered via the cardio-respiratory system to the working muscle, ATP is produced by aerobic pathways within the mitochondria. Although slower, aerobic processes are more efficient, producing 38 moles of ATP per molecule of glucose via the Krebs cycle and oxidative phophorylation. During aerobic work, glucose is broken down by the process of glycolysis to produce energy.

Lipids are stored within the muscle as well as in extramuscular depots such as adipose tissue, where triglycerides undergo hydrolysis to glycerol and free fatty acids. During prolonged low-intensity muscle work, both fat and glycogen serve as major energy sources (Essén-Gustavsson *et al.*,1984).

Should the oxygen supply be inadequate or if the metabolic demand is high, anaerobic processes become more important. Several studies have shown that the horse has a remarkable capacity for glycolysis and anaerobic metabolism during high-intensity effort as evidenced by the high lactate levels in muscle and blood (Lindholm and Saltin, 1974; Persson and Ullberg, 1974; Valberg *et al.*, 1985; Essén-Gustavsson and Valberg, 1986; Harris *et al.*,1991; Lovell and Rose, 1991; Evans *et al.*,1993; Räsänen *et al.*, 1995). Performance capacity during brief but intense effort such as racing is not limited by the availability of glycogen but, rather by insufficient energy production accompaned by an increase in muscle lactate concentration and a decrease in adenosine triphosphate (ATP) (Snow *et al.*, 1985; Valberg *et al.*, 1985; Essén-Gustavsson and Valberg, 1986; Valberg, 1986; Lovell and Rose, 1991; Räsänen *et al.*, 1995).

The great advantage of anaerobic pathways is that they serve as an immediate rapid source means of energy: 1 mole of glycogen or glucose produces 3 or 2 moles of ATP by glycolysis, but also increase muscle and plasma lactate concentrations, which cause a decrease in muscle and blood pH, from around 7.0 to 7.1 at rest to 6.4 or less during fatigue (Harris *et al.*, 1989). Reduced pH is known to reduce the respiratory capacity of muscle (Gollnick *et al.*, 1990) and to have a direct effect on the contractile apparatus (McCutcheon *et al.*, 1991, 1992).

There are few reports on the effects of training on the activity of enzymes directly associated with purine nucleotide metabolism. The greater the capacity for speed an animal has, the higher is the enzyme activity of creatine kinase and AMP deaminase (Snow and Harris, 1986; Cutmore *et al.*, 1986). The increase in AMP deaminase activity may be responsible for ensuring a rapid stimulation of glycolysis during intense work (Cutmore *et al.*, 1986).

In a few horses ATP concentration has been analysed post-race in pools of either Type I, IIA or IIB fibres and was found to vary both between horses and in fibre pools. The lowest ATP concentrations were measured after intense effort in Type II and especially in type IIB fibre pools (Valberg and Essén-Gustavsson, 1987). That study also indicated that the oxidative and glycolytic capacities of the fibres influence the metabolic response, as the lowest CS and highest LDH activities were found in fibre pools with lowest ATP concentration.

That the anaerobic capacity of muscles is of importance for the racehorse is evidenced by the high lactate and ammonia values and low ATP and CP concentrations following intense exercise and racing (Essén-Gustavsson and Valberg, 1987; Räsänen *et al.*, 1993).

Low ATP and CP levels in muscle, concomitant with an increase in inosine monophosphate (IMP) and ammonia levels, show the importance of CP and adenine nucleotide breakdown for the rephosphorylation of ADP to ATP. It has been suggested that IMP forms in response to a sudden rise in the concentration of ADP and consequently the concentration of AMP. Moreover there is a critical pH below which there may be a substantial reduction in the kinetics of ADP rephosphorylation provided by CP, resulting in an increase in ADP, which is the trigger for adenine nucleotide degradation during intense exercise (Sewell and Harris, 1992). The balance between formation and consumption of ATP can no longer be maintained when AMP is deaminated to IMP, with concomitant release of ammonia to the circulation.

Fatigue is the result of a complex chain of events, with central (psychological/neurologic) as well as peripheral elements (muscular). Decreased pH, a diminished nucleotide pool, increased temperature, and altered electrolyte gradients are all likely to have deleterious effects on a

number of metabolic processes in muscle, resulting in fatigue (for ref, see Hodges and Rose, 1994).

Locomotion pattern

The locomotor muscles in the horse, which are located proximally on the skeleton, create a pendulum-like effect that reduces the amount of energy needed to swing the limb. Farley and Taylor (1991) suggested that animals change their gait at different speeds in order to minimize energy consumption. Elite pacers appear to have a greater range of limb motion and longer stride than less successful performers (Wilson *et al.*,1988). In a previous study the reproducibility of certain gait characteristics was investigated in Standardbred trotters at a speed of 12.0 m/s. In the long-term study it was found that stride length and duration of swing and stride extended with training (Drevemo *et al.*, 1980). A previous study on older horses showed that the locomotion pattern depends on muscle characteristics (Persson *et al.*, 1991). In that study, stride length during treadmill trotting at the anaerobic threshold was dependent on aerobic power and consequently correlated negatively with the proportion of type IIB fibres and LDH activity. Several studies involving mature horses have shown that a relationship often prevails between the proportion of type IIB fibres and LDH activity (Essén *et al.*, 1980;Valberg *et al.*, 1985; Valberg, 1987). While training of horses leads to increased aerobic capacity, regardless of training regime, little is known about the effects of training and individual variation on muscle characteristics and locomotion pattern of the young Standardbred trotter.

Performance testing

Studies on equine exercise physiology from the end of the 19th century (Zuntz and Hagemann, 1898) up to the mid-1930s (Proctor *et al.*,1934) were focused on energy metabolism, especially with regard to the draught horse.

Standardized protocols for the investigation of physical capacity in race horses, using a high-speed treadmill were intruduced by Persson (1967). In trotters, heart rate and blood lactate responses to submaximal exercise, and also total red cell volume and haemoglobin were correlated with performance capacity (Persson, 1967, 1969; Persson and Ullberg, 1974). In experimental studies, telemetric measurements of breaths per minute, tidal volume, and oxygen uptake have been made (Hörnicke *et al.*, 1983, 1987) as well as collection of arterial blood for measurement of blood gases (Bayly *et a.*,1983; Thornton *et al.*, 1983; Persson *et al.*, 1987). These studies have shown the importance of oxidative capacity for stamina and

performance capacity. However, most of the latter measurements require sophisticated equipment and are restricted to experimental studies.

Work testing provides a means for functional evaluation of a range of physical systems under standardized exercise conditions. A number of studies have been published where cardiorespiratory and metabolic responses in athletic horses by means of work tests were undertaken either on the track or on a treadmill. Most studies have used experimental horses of varying background, age and athletic ability.

Several studies have investigated the correlation between blood lactate concentration and speed during exercise tests on the track. These work tests were performed either at submaximal or at high speeds, in Standardbreds (Åsheim *et al.*, 1970; Krzywanek, 1973; Lindholm and Saltin, 1974; Persson and Ullberg, 1974; Wilson *et al.*,1983; Valette *et. al.*, 1993) and in Thoroughbreds (Snow and MacKenzie, 1977; Kubo *et al.*, 1984; Bayly *et al.*, 1987; Ronéus *et al.*,1987; Foreman *et al.*, 1990; Erikson *et al.*,1991; Lindner *et al.*, 1992; Harkins *et al.*,1993; Evans *et al.*, 1993). These studies have indicated that horses with superior ability, or those in better condition, had lower blood lactate values in response to a given submaximal work load.

Indicators of performance capacity

Common indicators of performance have included: prize money won, best race time, prize money per start, class of race, treadmill run time and "time for rating". Individual performance index (IPI) is calculated annually from the individual horse's racing performance (% placing 1, 2 or 3, earnings, average earning per start, and best racing record), converted to and expressed as standardized deviation from the average record in the same sex and age group (Arnason *et al.*, 1982).

A recent study in Standardbred trotters, raised and trained in Finland, showed that blood lactate, and uric acid concentrations were correlated to racing time and individual performance index (IPI). However, no correlation was found between plasma lactate concentration and IPI. The conclusion was that the accumulation of lactate in the blood was greater, and loss of purine nucleotides was less in the superior horses (Räsänen *et al.*, 1995).

Track testing

Under laboratory conditions, equine fitness can be determined by subjecting horses to exercise tests on a high-speed treadmill (Bayly *et al.*,1983; Persson, 1983). These tests do not accurately reproduce exercise conditions "in the field" where weather and track conditions, driver and sulky must be

considered. Both submaximal and maximal track testing have a number of advantages over treadmill testing. For instance the horse is examined whilst performing the activity for which it is bred and trained. The effect of gear is not overlooked. The time required for habituation is short and the test does not require expensive equipment. It should be possible to perform such tests regularly, as part of a horse's training regimen, in order to monitor performance and response to training.

Several work tests have been described in field conditions for horses in order to estimate precisely their level of fitness or the influence of certain training programmes (Åsheim *et al.*, 1970; Persson and Ullberg, 1974; Wilson *et al.*, 1983; Dubreucq *et al.*, 1995). These tests involve the relationships between speed, heart rate and blood lactate concentration.

Aims of the study

The several objectives of these studies were:

- to investigate plasma lactate concentration in 2-year-old Standardbred trotters undergoing training and to study their metabolic response to submaximal and high speed exercise on the track and their muscle characteristics. A specific aim was to ascertain whether performance capacity is correlated to muscle characteristics and muscle metabolic response to exercise.

- to study if relationships occur between muscle characteristics and kinematic variables in young Standardbred trotters during high speed exercise on the track;

- to investigate muscle characteristics, plasma lactate and ammonia responses after a race, in relation to individual performance index (IPI);

- to investigate and describe muscle characteristics of racehorses and their relationship to muscle metabolic responses after racing. A specific aim was to analyse ATP and its breakdown products in muscle and especially within individual type I and type II fibres.

- to develop a simple practical field test which can give information about the individual horse's metabolic potential.

Materials and methods

For a more detailed account of the Materials and Methods used, see papers **I** to **V**.

Horses

Study I

Seven 2-year-old Standardbred trotters, Horses A-G (3 colts, 4 fillies) with an age range of ±1 month, were all bred, raised and trained at the same camp in Sweden. Three of the horses could not participate during the whole study, which lasted from 24 to 40 months of age. Horse G was lame from the age of 29 to 39 months and Horses C and F were sold when 3 years old.

Study II

Thirteen 2-year-old Standardbred trotters (10 colts and 3 fillies) were raised and trained at the same training facility and were clinically normal and free of lameness during the work test.

Study III

Fourteen 2-year-old Standardbred trotters, bred, raised and trained at the same training facility (7 colts and 7 fillies) were used in this study. The horses were clinically normal and free of lameness during the work test.

Study IV

Twentyfive 3-5-year-old racing Standardbred trotters, trained at the same camp, were examined in this study (6 fillies, 12 colts and 7 geldings). Nine of the horses were 3 years old, 10 were 4 years old and 6 were 5 years old when they participated in this study. Ten of these horses also performed a submaximal exercise test.

Study V

Eighteen horses (4 mares, 1 gelding and 13 stallions) with a mean age of 4 years (range 3- 6), trained at the same camp, were studied after racing. Five horses participated in racing over distance of 2,640 m, 11 horses over 2,140-2,180 m and 2 horses over distances of 1,640 m. Eleven of these horses were placed among the 6 best, 3 were placed first, two second and

one third. Four of the horses broke into a gallop either at the beginning and/or at the finish of the race.

Training programme

Studies I-III

The regular training programme started when the horses were aproximately 15 months old. During the first months, the training consisted of slow trotting and walking. At about 18 months the horses trotted at slow speed over a distance of 6,000-7,000m interspersed with short bursts of speed over a distance of 200-300 m, 5 days a week. In addition, the horses trotted near their maximal speed once a week on a high quality track and from the age of 22 months twice a week. Two heats covering 1,000 m were performed. They were all fed a similar diet.

Work tests

The work tests (studies I-III) were performed under good weather conditions and on the same track as was used when the horses were being trained (Seglinge, Almunge).
The horses raced (studies IV-V) on three different occasions, on the same track (Solvalla, Stockholm), over distances of 1,640-2,640 m.
The same professional trainer handled the horses during all the tests (I-V).
The same person took all the muscle biopsies and blood samples.

Study I

Two types of work test were performed on the track, one at submaximal speed and one as fast as possible.
Submaximal test: This test consisted of four 1000 m runs and the speed was increased after each run from 1.50, 1.45, 1.40 to 1.35 min/km corresponding to 9.1, 9.5, 10.0, and 10.5 m/sec. The driver kept the speed as constant as possible with speed checkpoints every 100 m, The exact speed was recorded by stopwatch. The time between each 1000 m run was approximately 2.5 min. After each run, a blood sample was drawn from the jugular vein within 30 sec. This test was performed when the horses were 24, 26, 29 and 40 months old.
High speed test: The horses trotted as fast as they could over a distance of 1,600 m, with an older horse as a pacer during the test. The speed was recorded and immediately after the finish a blood sample was drawn and a muscle biopsy was obtained. The high-speed test was performed one week after the submaximal test, at an age of 24 and 29 months.

Study II

Exercise track test over 1,600 m:
An exercise test over 1,600 m was performed when the horses were approximately 28 months old. The horses trotted as fast as they could over a distance of 1,600 m, with an older horse as a "pacer" during the test. The speed was recorded, a blood sample from the jugular vein and a muscle biopsy (gluteus medius) were obtained from each horse within one minute after the test. Subcutaneous anesthesia was carried out about 10 min before the test on a site approximately 15 cm from *tuber coxae* in line with the root of the tail so that muscle biopsy could be obtained as soon as possible after the test.

Study III

A work test was performed when the horses were approximately 26 months old. The horses trotted close to their maximum speed while maintaining a regular gait over a distence of 1,000 m. Concurrent with this run the horses were filmed along a straight part of the track. Their speed was recorded, blood samples were collected less than one minute after the finish and a muscle biopsy was obtained approximately 3 h after exercise.

Kinematic parameters: Five consecutive strides were filmed at 250 frames/sec left laterally with a stationary panning camera (Locam 164-5 DC. Red Lake Laboratories, Santa Clara, California, USA). The horses were filmed while trotting along a straight side of track (Drevemo *et al.*, 1993). The developed films were converted to video image in the Trackeye (Innovativ Vision AB, Linköping, Sweden) analysis system (Drevemo and Johnston, 1994). From the video images, first contact (when the left fore hoof was visibly in contact with the track surface) was determined. Stance time was determined by taking the difference in time between toe off and first contact. Stride duration was the difference in time between two consecutive first contacts, while stride length was the distance travelled over the track during one stride duration. Velocity was quantified by the quotient stride length/stride duration. The mean ± s.d. for each kinematic variable were derived from five consecutive strides.

Studies IV-V

Racing: The horses raced (on three different occasions) on the same track, over distances of 1,640-2,640 m. Blood samples were collected from the jugular vein 5 min after the finish of the race. Gluteus medius muscle biopsies were obtained from each horse within 10 min after racing.

Submaximal exercise track test: (Study IV). Ten of the horses also accomplished a submaximal track test consisting of five incremental 1,000 m work spells at approximately 9.1, 9.5, 10.0, 10.5, 11.1 m/sec. After each run a blood sample was drawn from the jugular vein within 30 sec. The intervals were interspersed with 2-minute walking spells.

Muscle biopsies

Muscle biopsy specimens were taken from the m. gluteus medius (Lindholm and Piehl, 1974). The same person took all the biopsies. One sample for biochemical analysis was immediately frozen in liquid nitrogen, while another sample for histochemical analyses was rolled in talcum powder before being frozen. All samples were stored at -80°C until analysed.

Histochemical analyses: Transverse serial sections (10μm) of the muscle biopsy specimen were cut in a cryostat at -20°C. These sections were stained for myosin ATPase after both acid (pH 4.3 and pH 4.6) and alkaline (pH 10.3) preincubation (Brooke and Kaiser, 1970). Muscle fibres were identified as Type I, Type IIA or Type IIB. Muscle composition was determined by typing at least 200 fibres.
In study I, a computerized image analysis system (BIO-RAD, Scan-Beam, Hadsun, Denmark) was used to calculate fibre type distribution (%) and mean fibre area (μm) for each fibre type.

Biochemical analysis: Freeze-dried muscle samples were dissected free of fat, connective tissue and blood using a dissection microscope and were then weighed. The activities of citrate synthase (CS) as a marker for oxidative capacity, 3-OH-acyl-CoA dehydrogenase (HAD) as a marker for β-oxidation, (lactate dehydrogenase) LDH as a marker for glycolytic capacity, and hexokinase (HK) as a marker for capacity of phosphorylation of glucose, were analysed after the sample had been homogenized in a phosphate buffer (pH 7.3). All enzyme activities were analysed at +25°C using fluorimetric technique (Essén *et al.*, 1980; Essén-Gustavsson *et al.*, 1983). The concentrations of glycogen, lactate, ATP, ADP, AMP, IMP, CP and G-6-P were measured according to fluorimetric techniques of Lowry and Passoneau (1973). In study V, single fibres were dissected free from the muscle samples. The fibre varied in length some millimetres. Type I and type II fibres could be identified after two small pieces from each fibre were cut off and stained for myofibrillar ATPase activity after acid (pH 4.3) and alkaline (pH 10.3) preincubation. The fibres were then weighed using a fish-pole balance (Lowery and Passoneau, 1970). The fibres were then extracted in perchloric acid and neutralized with potassium hydroxide. ATP

and IMP were analysed with the high-performance liquid chromatography (HPLC) technique.

Blood samples

All blood samples were collected in sodium heparin vacutainer tubs (Becton-Dickson). They were kept on ice until centrifugation, which was done within 6 hours. Plasma lactate was analysed by means of a lactate analyser (Analox system, GM7, London, England). Plasma ammonia concentration was determined using a fluorimetric technique (Kun and Kearney, 1974) and uric acid using the HPLC-technique.

Individual Performance Index (IPI)

Each year the Swedish Trotting Association (STC) publishes performance traits (best time notation per km using volt start and autostart, total numbers of starts with and without placings and accumulated life-time earnings) in the Annual Statistics for Swedish Trotting (*Årsstatistik för svensk travsport*). All of these performance traits are also given for each start and are summarized for the year in question. IPI weighs together the deviation of converted racing performance records of the individual horses from the contemporary averages. These measuements were made on the basis of their own racing performance by the following indices, % placing 1 to 3, earnings, average earnings per start, and best racing record, respectively, expressed as deviations from the average records belonging to the same sex and age groups in standard deviation (SD) units. The racing performance records exhibit skewed distribution on the orginal scale and were transformed as described below in order to obtain approximate normal distributions (Arnason *et. a.l.*,1982).

1. $\sqrt{}$Percentage of races placed 1-3.
2. $4\sqrt{}$earnings.
3. Log 10[$\sqrt{}$earnings/(no. of starts) + 1].
4. Log 10 record (best racing time as time/km - 1 min), to the nearest 1/10 s.

The IPI was computed for each individual horse as 100 + 10 (average standardized deviation for the four racing variables (with the sign for best racing time reversed)). The annual IPI for each age and sex group has therefore a mean of 100 and 10 index units correspond to one standard deviation in yearling performance within groups.

Statistical analysis

Data were analysed by one-factor ANOVA repeated measures and simple linear regression. Tests of differences between sexes (study II) were done with Student's *t*-test for unpaired data. Calculations were carried out by using a computer and the StatView SE program. The results are presented as mean values and standard deviations (SD). Significance was accepted when $p<0.05$.

Results

Submaximal exercise track test

Studies I and IV

The plasma lactate response after the submaximal track test at 24, 26 and 29 months of age did not differ markedly within horse, but large individual differences between horses were seen (Fig. 1). All the horses that performed a submaximal test at 40 months of age showed a lower lactate response than in the earlier tests. The mean lactate response in the final interval of the submaximal test decreased from 14.5 to 7.2 mmol/l during the training period of 16 months ($p<0.001$).

The horse's speed when it performed the maximal test over 1,600 m was negatively correlated with the lactate values after each run during the submaximal test at 29 months of age (Fig. 2).

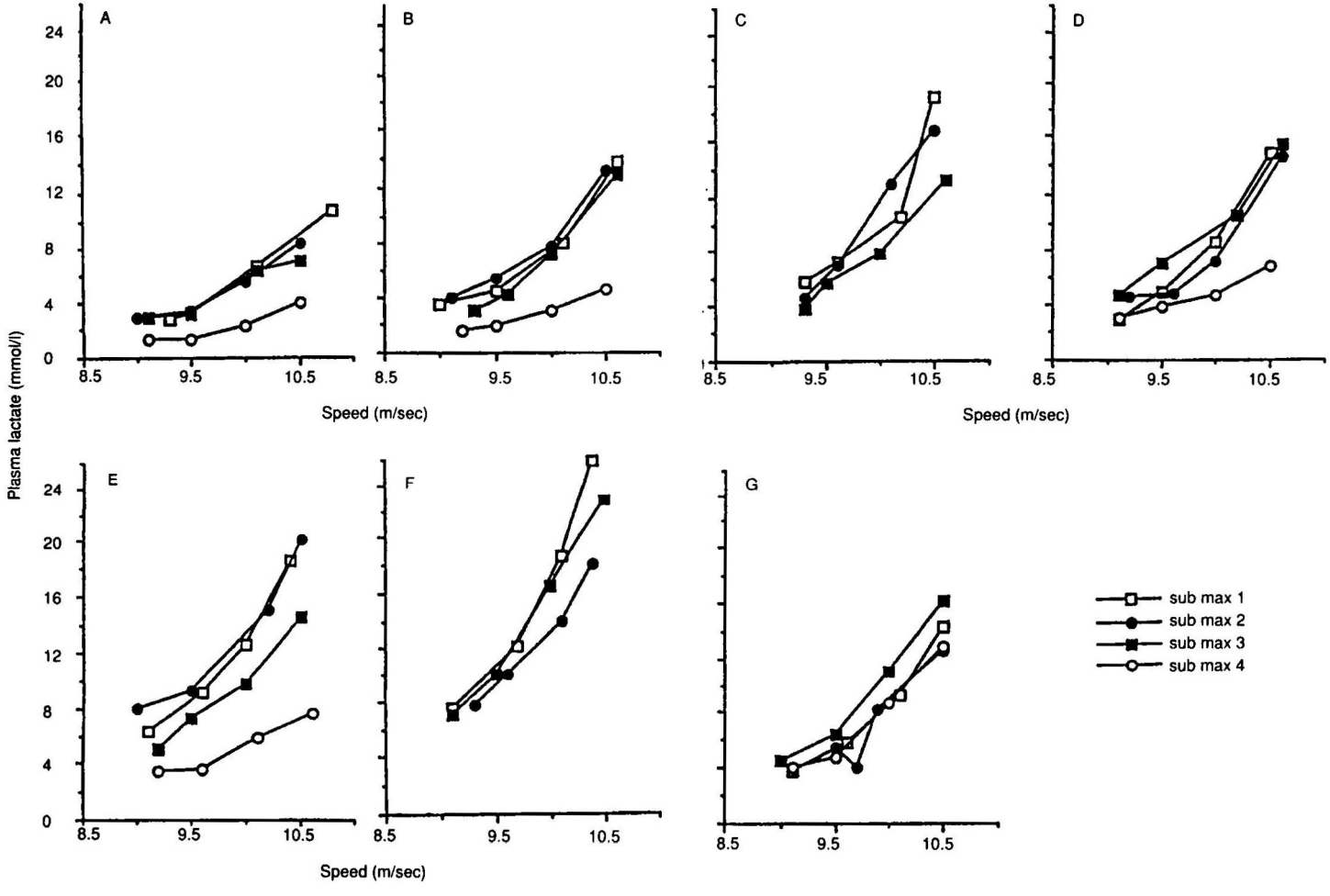

Fig 1: Plasma lactate concentrations in 7 horses (A–G) at 24 (submax 1), 26 (submax 2), 29 (submax 3) and 40 months (submax 4) of age. Each submax test consisted of 4 x 1,000 m runs at the trot, with increasing speed at each run.

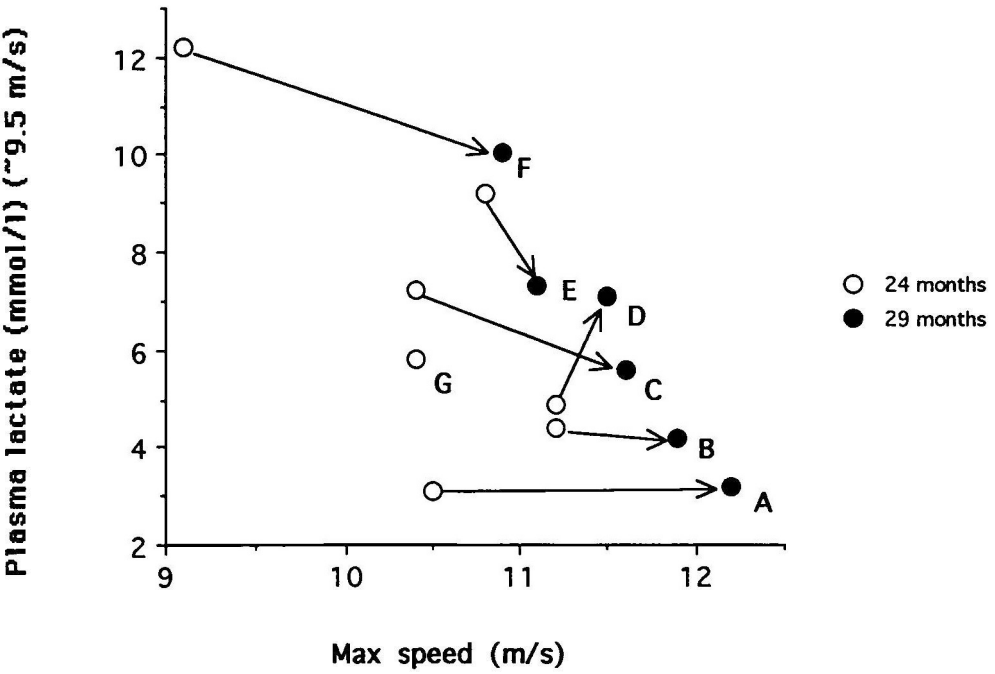

Fig. 2: Plasma lactate concentrations after submaximal speed (1,000 m, 9.5 m/s) in relation to maximal speed (after 1,600 m) in 7 horses (A-G) at 24 and 29 months of age.

Muscle biopsy: Individual differences in fibre composition were seen between horses. The relative distribution in area of Type I fibres increased, from 11.5% to 15% ($p<0.05$) during the period of 5 months training, while the mean percentage of Type IIB fibres decreased, from 44% to 37% ($p<0.05$). The relative distribution in area of Type IIB fibres decreased, from 56% to 49% ($p<0.05$). The mean CS activity increased during this period from 40 to 47

μmol/g/min ($p<0.01$).

The plasma lactate response after the submaximal test, in racing Standardbred trotters, also showed individual differences (study IV). Lactate concentration ranged from 2.7 to 10.8 mmol/l (mean 5,1) after the fourth submaximal interval.

High-speed exercise track test

Study I

Plasma lactate concentrations ranged from 12.8 to 27.3 mmol/l after the first maximal test at 24 months and from 24.2 to 33.6 mmol/l after the second test at 29 months old. The relationship between maximal speed over

a distance of 1,600 m and plasma lactate concentrations, when the horses were 24 and 29 months old, showed individual differences. There was no correlation between lactate values after the high-speed test and horse's speed at 24 or 29 months of age (Fig. 3).

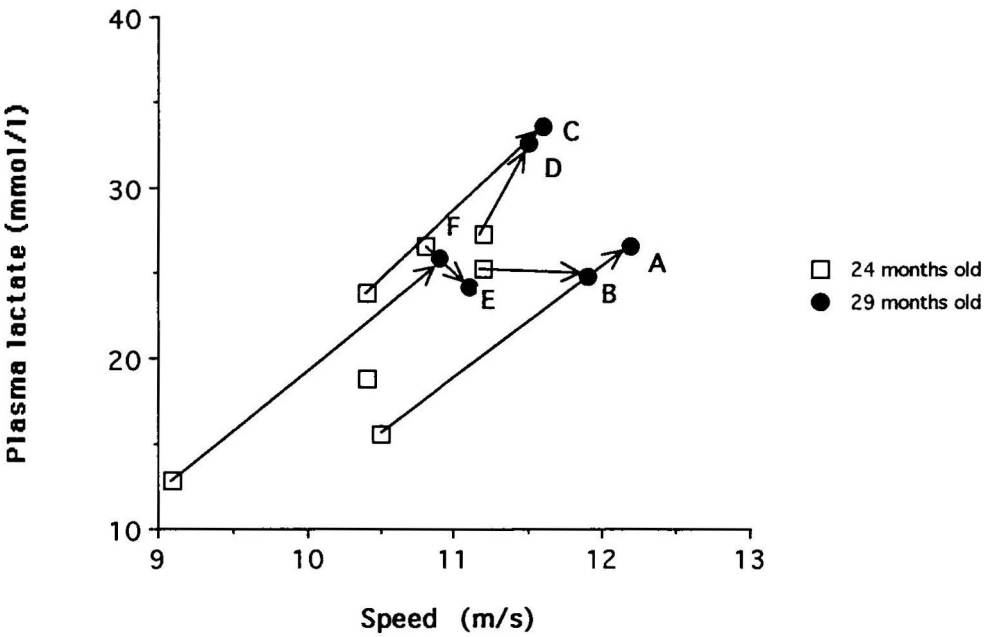

Fig. 3: Plasma lactate concentration after maximal work (1,600 m) in relation to speed (A-F) at 24 and 29 months of age.

Study II

Both plasma lactate (r=0.69) and muscle G-6-P (r=0.72) concentrations showed a positive correlation to speed over 1,600 m (Fig. 4)(Fig. 5). ATP concentration showed a negative correlation to speed (r=-0.61)(Fig. 6), plasma lactate (r=-0.79), muscle lactate (r=-0.66) and G-6-P (r=0.57) concentration. G-6-P concentration showed a positive correlation to muscle lactate (r=0.73) and plasma lactate (r=0.74) concentrations. Neither the percentages of Type I, IIA, IIB fibres, nor the enzyme activities of HK, HAD, LDH and CS correlated with the horses speed while trotting over 1,600 m.

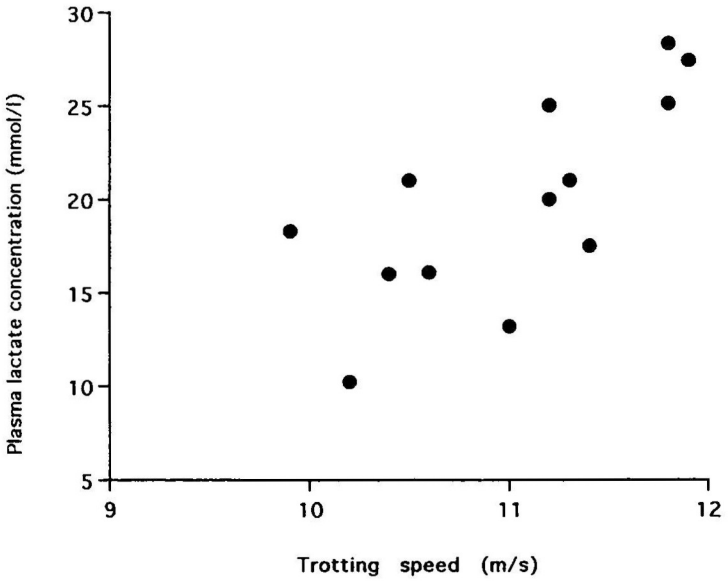

Fig. 4: Plasma lactate concentration (mmol/l) vs trotting speed (m/s) in 2 year old Standardbreds (r=0.69).

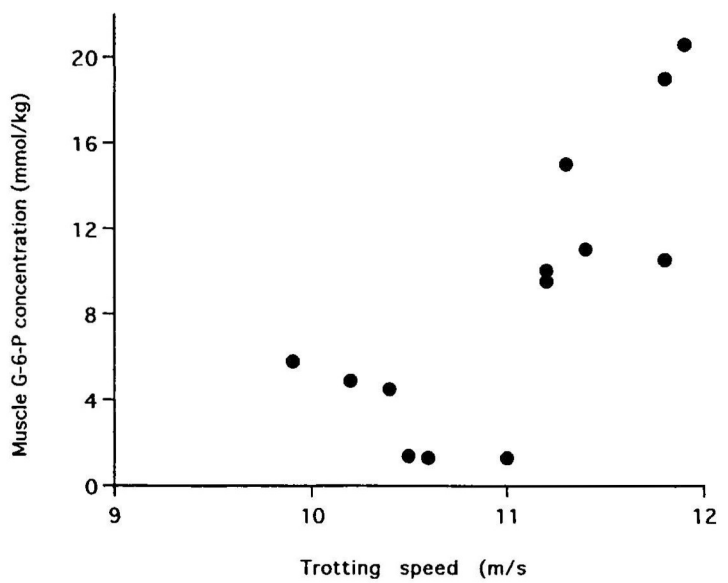

Fig. 5: Muscle glucose-6-phosphate (G-6-P) vs trotting speed (m/s, r=0.72).

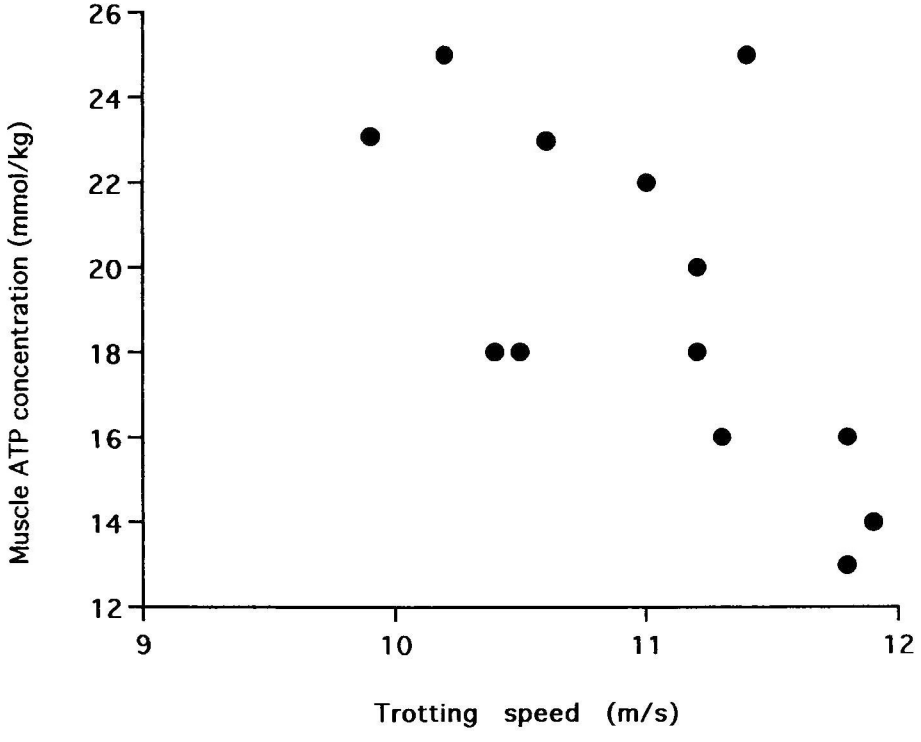

Fig. 6: Muscle ATP concentration (mmol/kg) vs speed (m/s, r=-0.61).

Study III

Large individual variations were found in muscle characteristic, plasma lactate response and locomotion pattern to high-speed exercise on the track. The percentage of type IIB fibres ranged between 44% and 58%, SL ranged between 4.98 m and 5.98 m and ST ranged between 119 ms. and 146 ms. A significant positive correlation was found between PLA after the work test and the percentage of type IIB fibres. PLA showed significant negative correlations to both ST (Fig. 7) and SL. A significant negative correlation was found between the percentage of type IIB fibres and ST. The enzyme activities of CS, HAD, and LDH in muscle did not correlate with either PLA or the kinematic variables.

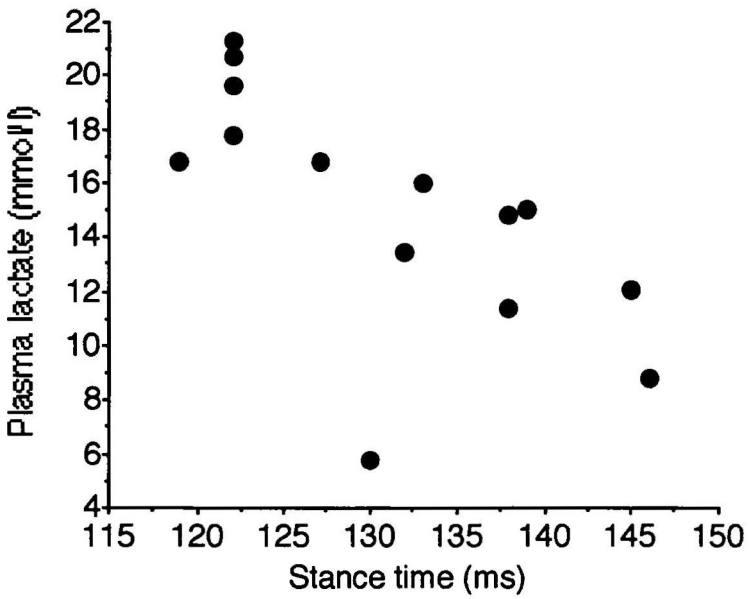

Fig. 7: Plasma lactate (mmol/l) concentrations after trotting for 1.000 m, (~11.5 m/s) in relation to stance time (ms) in fourteen 2-year-old Standardbred trotters.

After racing

Study IV

The mean fibre type distribution for the 25 horses was 19% type I, 46% type IIA, and 35% IIB fibres. The mean activity was 59 mmol/kg/min for CS, HAD 37 mmol/kg/min, LDH 1685 mmol/kg/min, and HK 2.5 mmol/kg/min.

The mean concentration after racing was 445 mmol/kg/min for glycogen, plasma lactate 31 mmol/l and ammonia 141 mmol/l. Fibre type composition (Type I, IIA, IIB), enzyme activities (CS, HAD, LDH, HK), plasma lactate and ammonia concentrations after racing and submaximal test showed no correlations to IPI.

Study V

The mean concentration for the 18 horses was 18.3 mmol/kg/min for ATP, ADP 2.66 mmol/kg/min, AMP 0.52 mmol/kg/min and IMP 7.17 mmol/kg/min. The ATP and IMP concentrations within individual fibres from different horses are shown in Fig. 8. Some fibres showed low ATP concentrations and some of these had high IMP concentrations, whereas some fibres showed low IMP concentrations. High ATP concentrations were also seen in some fibres which had low to moderate IMP concentrations. The percentage of type IIB fibres showed a negative correlation to CS activity and positive to LDH. The ATP concentration in whole muscle was negatively correlated both to plasma lactate concentration and to muscle lactate and IMP concentrations. The lactate concentration correlated positively with G-6-P and IMP concentrations.

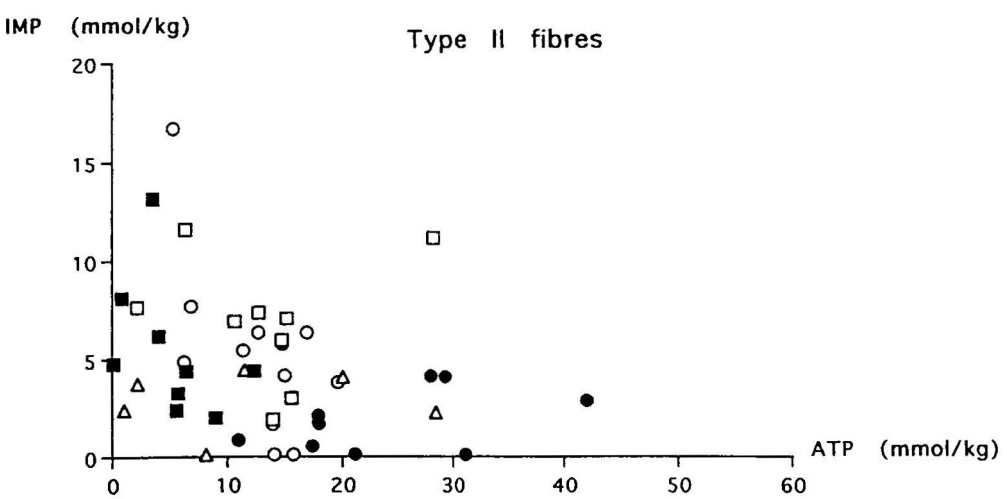

Fig. 8: ATP and IMP concentrations (mmol/kg) within individual fibres from five different horses after racing.

General discussion

The studies presented in this thesis revealed marked individual differences in plasma lactate and muscle metabolic responses to submaximal and high speed-work in young and mature racehorses. The 2-year-old Standardbreds were bred, raised and trained at the same training facility and the adult racing horses were also trained by the same trainer. Thus, since all horses in these studies were trained at the same establishment and fed the same diet, any effect of environmental or nutritional factors is probably of little importance regarding the results.

Muscle metabolic response to submaximal work:
Several earlier studies demonstrated correlations between blood lactate concentration and speed during exercise tests (Krzywanek, 1973; Lindholm and Saltin, 1974; Persson and Ullberg, 1974; Wilson *et al.*, 1983; Bayly *et al.*,1987; Evans, 1993). Most of those reports on submaximal exercise indicated that horses having a superior performance capacity have a low blood lactate concentration in response to a submaximal work load. The negative correlation evident between plasma lactate concentration after a submaximal test and the horse's maximal trotting speed over 1600 m at the age of 29 months in study **I** corroborates those previous reports. However, this correlation was not seen in the early phase of the training period, at the age of 24 months, which suggests that other factors, such as "trotting technique" may be of importance for a young Standardbred's performance capacity (Study **I**).

The horses that had lower plasma lactate values after the submaximal test at 29 months, and trotted 1600 m at high speed, were also those with a lower proportion of Type IIB fibres and a higher CS activity in the skeletal muscle (Study **I**). After 40 months of age the lactate production in the final submaximal heat was significantly less (7.2 mmol/l) than at 29 months (14.5 mmol/l), showing that an important response to training could be associated eith an increase in aerobic capacity. A submaximal work test with analyses of lactate concentration in plasma can be a very useful means to monitor improvements in a young horse's form.

Previous studies have shown a close correlation between lactate accumulation and proportion of Type IIB fibres in the muscle (Valberg *et al.*,1985; Valberg, 1987). The results of this study also corroborate previous observations that Type IIA/IIB fibre ratio, Type I/II fibre ratio and CS activity all increase with training (Lindholm *et al.*, 1983; Henkel, 1983; Ronéus *et al.*, 1992).

It is concluded that measurement of plasma lactate concentration after a submaximal track test can be an important tool with which to assess

performance capacity and to monitor training development of individual 2-year-old horses. The submaximal test performed in study **IV** showed that plasma lactate concentrations differed between horses, but no significant correlation was seen between plasma lactate response and IPI or muscle characteristics. This is most probably due to the fact that this group of mature racing horses were too alike to show any marked differences in metabolic response to a submaximal exercise test.

Muscle metabolic response to maximal work:
The results of study **II** show, as does study **I,** that in 2-year-old horses, there are considerable individual differences in muscle metabolic and plasma lactate responses to high speed-work over a distance of 1,600 m.

The concentrations of ATP, G-6-P and plasma lactate correlated with trotting speed over 1,600 m on a track. Our findings corroborate those of Kubo *et al.* (1984) who reported a significant positive correlation between gallop speed over a distance of 1,600 m and blood lactate concentrations in six 2-year-old Thoroughbreds 3 to 5 min after completion of the exercise.

In study **I**, no correlation was found between plasma lactate and time taken to trot 1,600 m, most probably because these horses were too alike to show any marked differences in metabolic response to a maximal exercise test. However, the study did show that plasma lactate concentrations were higher when the horses were trotted at a faster speed (Fig. 3).

Study **II** showed that horses with the best performance capacity over a distance of 1,600 m had better capacity for anaerobic glycolysis and ATP breakdown in muscles, than horses that trotted at a slower speed.

Variations in enzyme activities were seen between horses, but no correlations were found between oxidative or glycolytic enzymes and speed.

In a recent study on Standardbreds raised and trained in Finland, blood and plasma lactate and purine concentrations were measured after races over 2,100 m (Räsänen *et al.*, 1995). The results showed that accumulation of lactate in the blood was greater, and loss of purine nucleotides was less in the superior performing horses. Although the horses had been selected to reflect a wide range of racing abilities, there was no correlation between plasma lactate concentration and performance, expressed as IPI. Those results agree with study **IV**, where racing performance, expressed as IPI with a variation between 100 and 116 in racehorses, was not associated either with muscle characteristics or with plasma lactate or ammonia concentrations after racing. A study by Krzywanek (1974) also found a lack of relationship between racing and post-race blood lactate concentration in 156 trotters. That anaerobic metabolism is of great importance for energy production during racing was shown by the high lactate and ammonia concentrations in blood, and low ATP and high lactate and IMP levels in muscle determined after racing (study **V**).

Racing is often characterized by a high speed at the start, in order to achieve an advantageous position, maximal speed during the race to improve one's position, and a burst of speed at the finish in an all-out effort to win. Both aerobic and anaerobic pathways are therefore of importance for energy release during the race. Muscle fibre recruitment patterns may vary according to tactics, which are an important factor for winning.

That plasma lactate concentration is correlated with performance capacity, expressed as speed in study **II**, may be related to the more standardized work carried out during exercise track testing over 1,600 m. Fibres are selectively recruited in a specific pattern that varies according to gait, speed, and duration of exertion (Lindholm *et al.*, 1974; Snow *et al.*, 1982; Hodgson *et al.*, 1983; Essén-Gustavsson *et al.*, 1984; Valberg, 1986; Gottlieb *et al.*, 1989). Fibre type recruitment probably follows a more distinct order during a standardized work test than in a race, and this will influence plasma lactate concentrations.

Several studies have demonstrated a relationship between the proportion of Type II fibres, especially Type IIB fibres, and lactate accumulation and adenine nucleotide degradation in muscle during intense work (Valberg *et al.*, 1985; Essén-Gustavsson and Valberg, 1986; Lovell and Rose, 1991; Räsänen *et al.*, 1995). Previous studies have also indicated a relationship between running time and muscle ATP content (Snow *et al.*,1985; Gollnick *et al.*, 1990). Sewell and Harris (1992) suggested that IMP formation occurs as a result of an increase in the concentration of ADP and consequently the concentration of AMP in muscle. Moreover, when Type IIB fibres are recruited, the result will be a gradual decrease in muscle pH due to lactate accumulation and breakdown of ATP (Lindholm and Saltin, 1974; Valberg and Essén-Gustavsson, 1987). It is well known that these metabolic changes in fibres could be associated with fatigue. Horses possessing a capacity for superior athletic performance are able to offset these deleterious effects, at least to some degree, as they possess local intracellular and circulating buffer systems (Harris *et al.*, 1990). The high buffering capacity of muscle in horses is thought to be related to the high concentation of carnosine in muscle fibres. Carnosine contributes approximately 30% of the non-bicarbonate buffering, with the greatest concentrations of this dipeptide being found in type IIB fibres (Sewell *et al.*, 1992). The main buffer is the blood bicarbonate system, and obviously, any improvement in circulation through the muscles and lungs in response to training must be beneficial. Other important factors causing fatigue during intense exertion are that, during exercise, about 80% of the energy produced is liberated in the form of heat, which results in the diversion of blood flow away from working muscle to skin in order to dissipate heat (Rowell, 1983). Equine sweat is hypertonic, and therefore electrolyte losses

accompany fluid losses. These changes in fluid and electrolyte balance are directly related to reductions in performance capacity (Carlsson, 1987).

That anaerobic metabolism is of great importance for energy production during racing was demonstrated in study **V** by the high lactate and ammonia levels in blood and low ATP and high lactate and IMP levels in muscle, measured after racing. A previous study showed that poor capillary density, which is due to a limited oxygen supply to and lactate removal from fibres (Essén-Gustavsson and Valberg, 1986; Karlström *el al.*, 1991).

A study of pooled single fibres dissected from biopsy samples collected after racing found the greatest ATP depletion in type IIB fibres, but little change in Type I (Valberg and Essén-Gustavsson, 1987).

The results of study **V**, like those in studies **I** and **II**, with high lactate, ammonia and uric acid concentrations in plasma and low ATP and high G-6-P and IMP concentrations in muscle, show the importance of anaerobic glycolysis and purine nucleotide degradation for energy production during racing and high intensity work.

The new interesting finding in study **V** was that adenine nucleotide loss varied markedly among individual fibres, with ATP levels as low as 1-5 mmol/kg in some fibres and as high as 40-58 mmol/kg in others. Some individual fibres had high IMP levels, concomitant with low ATP levels, while other fibres showed only a slight increase in IMP concentration, possibly a result of the fibres having been recruited at different stages during the race.

In an earlier study, on Thoroughbreds, low ATP levels were also found in some fibres after racing but were said to be due to an artefact (Foster *et al.*, 1986). IMP levels were not measured in that study. The fact that fibres in our study were found to contain high IMP concomitant with low ATP concentration indicates that nucleotides had actually been degraded to a great extent. This indicates large variations in ATP levels between different fibres, which is why metabolite analyses of whole muscle must be interpreted with caution.

The low ATP concentrations found in some fibres indicate that these had been recruited during racing and that rephosphorylation of ADP and AMP could not match the demand for ATP. ADP and AMP may therefore accumulate in these fibres if the capacity of the myokinase (the key enzymes in the degradation of AMP during exercise are myokinase and AMP-deaminase) and AMP-deaminase reactions to stabilize the ADP/ATP ratio is insufficient.

It has previously been suggested that muscle fatige is related to increased AMP and ADP concentrations (Sahlin, 1986). An interesting finding in study **V** was therefore that AMP and ADP concentrations after racing appeared to be correlated to the placing of the horses in the races. The fact

that AMP and ADP concentrations were increased in the horses that were not among the first indicates that the maximal rate for ATP regeneration in the fibres could not be maintained, which is why the horse could not maintain its speed.

As tactics are so important in a race, speed - and thus the recruitment of fibres - may vary among the competing horses and throughout the race, compared with a more standardized work on a track or treadmill.

Muscle characteristics and kinematic variables:
In Study **III**, significant negative correlations were found between stance time and both plasma lactate concentration and proportion of type IIB fibres, indicating that the locomotion pattern may be dependent to some degree on both muscle fibre composition and metabolic profile.

The results indicated that young Standardbred trotters with proportionatly fewer Type IIB fibres perform high-speed trotting with less lactate production, longer stride and longer stance time over a distance of 1,000 m. The most efficient locomotion pattern for horses having a high percentage of type IIB fibres seems to be a short stance time, stance being the phase of the stride when the horse's limbs are in contact with the ground and subsequently the phase in which the horse can propels its body forward. Consequently, the power developed by the muscles should be greatest during this phase. The young horses in the present study were observed during maximal effort over a distance of 1,000 m on the track, when there is a high demand for anaerobic energy release. Type IIB fibres would therefore also have been recruited and stride length may have reached its maximum.

A previous study in older horses showed that locomotion pattern is dependent on muscle characteristics (Persson *et al.*,1991). In that study, stride length during treadmill trotting at the anaerobic threshold was negatively correlated to the proportion of Type IIB fibres and LDH activity. Stance time and stride length are significant determinants of aerobic energy consumption during submaximal trotting.

The Standardbred horse must be able to trot at high speed whilst pulling a sulky and the success of a horse's performance depends greatly on its ability to move optimally. Balancing and gearing the horse correctly are essential to allow it to attain and maintain maximal speed. No animal can possibly do its best if its legs collide or if the harness and gear are cumbersome.

Track testing:
The simplest track test is the assessment of exertion capacity by timing the horse over the competition distance. A fast track time and rapid recovery show clearly if the horse is fit to race.

Skeletal muscles undergo rapid changes in metabolic characteristics as training proceeds (Ronéus *et al.*, 1991, 1992). The oxidative capacity of muscles can be improved by training (Lindholm and Piehl, 1974; Essén-Gustavsson *et al.*, 1980; Lindholm *et al.*, 1983; Hodgson *et al.*, 1986; Ronéus *et al.*, 1987; Gottelieb *et al.*, 1989; Lovell and Rose, 1991; Ronéus *et al.*, 1991; Lopez-Rivero *et al.*, 1991).

If the training load is too light to produce lactate, the muscle fibres recruited will not be those that will be required for competitive running, so little adaptation or improvement can be expected. On the other hand, if the load is too heavy, fatigue will soon develop and the metabolic processes will be impaired, with an increased risk of injury.

There seems to be a difference between running against time and running against competitors, since tactics are important in racing and speeds can therefore vary markedly during a race. A track test or an exercise test on a treadmill is therefore more standardized than a race situation, which could thus explain the differences between studies found in lactate response to performance. As indicated by the results of studies **I** and **IV** differences in performance capacity are probably greater among younger horses than in a selected group of adult racing horses. Therefore, the advantage of a performance track test is maybe greater for the younger horses. Track testing is certainly more easily performed than treadmill testing, and has the advantage of being undertaken under conditions similar to those in which the horse has to perform. However, muscle metabolic response and plasma lactate level determined on the track will be influenced by several factors such as track surface, weather conditions, ambient temperature and the driver's skill.

Sporting horses that perform poorly have always posed a challenge for owners, trainers and veterinarians. One of the most important decisions is whether the horse has suffered a reduction in performance capacity or merely lacks ability. It seems that submaximal exercise testing of horses should be able to discriminate between animals having poor vs. superior metabolic capacity.

Stamina can be improved as a result of the functional responses to progressivly increasing levels of repeated workloads specific to the exercise, thus allowing the individual to perform more efficiently. Submaximal testing can be a useful way for trainers to assess and monitor the fitness of their horses, in order to place the individual horse at its optimal training level.

This study has established that measurement of plasma lactate and certain muscle characteristics after a track test, as indicators of metabolic properties, can provide important information about a young horse's performance capacity. However, further research is still required before specific recommendations can be made concerning optimal training methods

or selection with respect to performance potential in horses undergoing training.

Summary of findings

In the studies compiled in the present thesis, the main observations were as follows:

- In 2-year-old Standardbred trotters, marked individual differences were evident in plasma lactate concentrations and muscle metabolic responses to high-speed and submaximal exercise on the track. There was a negative correlation between plasma lactate concentration after a submaximal test and the horse's speed over 1,600 m. Horses with the best performance capacity had a more marked anaerobic glycolysis and ATP breakdown than horses that trotted at a slower speed, over a distance of 1,600 m.

- Correlations between stance time and both plasma lactate concentration and proportion of type IIB fibres suggest that locomotion pattern is partly dependent on both fibre composition and metabolic profile.

- Neither fibre type composition, enzyme activities and plasma lactate and ammonia concentrations after a race nor submaximal testing showed any correlation to IPI.

- Adenine nucleotide degradation in muscle is of great importance for energy release during racing, but ATP and IMP concentrations can vary among individual fibres. Therefore, metabolite analyses based on whole muscle must be evaluated with caution, as they only give mean values for metabolic responses.

Acknowledgements

Sincere thanks are expressed to:

- Docent Birgitta Essén-Gustavsson, my tutor and scientific advisor, for inspirating enthusiasm and her invaluable help with the writing of this thesis. Under her guidance I enjoyed excellent conditions for scientific work.

- Professors Sune Persson and Arne Lindholm , my associated advisors, for constructive criticism and discussions and for valuable help with writing of this thesis.

- Yvette Eriksson, Kristina Karlström, Karin Thulin and Marie Ederoth, Department of Medicine and Surgery, for valuable support throughout the years and for their never failing patience with my many questions concerning the computer work.

- My co-authers Professor Stig Drevemo, Dr Chris Johnston and Yvette Eriksson for their excellent contributions.

- Professional trainers Torbjön Jansson and Göran Lång, for all invaluable help during the tests.

- Kerstin and Hans-Gunnar Tillander who willingly put their fine horses at our disposal.

- Max Brandt for linguistic revision of the manuscripts.

- Librarian Johnny Carlsson, for always being most helpful and providing expert library service when needed.

- My wife Merike, VMD, PhD, for her linguistic knowledge and her scientific know-how, and for being my wife. Our children Carl and Gustav for their patience during the years of my preoccupation with this study.

This work was supported by the Swedish Racing board (ATG) and AGRIA insurance company.

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ISSN 1401-6257
ISBN 91-576-5233-3

REPRO PRINT AB
Stockholm 1996