

maximal speeds decreases. The speed at which lactate begins to accumulate in the blood ( $V_{OBLA}$ ) has been shown to provide an accurate assessment of endurance capacity (Hagberg, 1984). This represents a physiological threshold, reflecting a shift in muscle metabolism leading to increasing lactate production in spite of sufficient molecular oxygen at the cellular level (Karlsson and Jacobs, 1982), and has been shown to occur at blood lactate levels of 4 mmol/l ( $V_{LA4}$ ) (Heck *et al.*, 1985). With the exception of  $VO_2$ max, which has proven difficult to measure, changes comparable to the above have been found in various training studies involving horses (Engelhardt, 1977; Milne *et al.*, 1977; Bayly *et al.*, 1983; Thornton *et al.*, 1983).

Training effects on muscle metabolism in man include pronounced increases in the activity of oxidative enzymes, an increase in the capillary density in the muscle and an increase in the ratio of type IIA to type IIB fibers. In contrast, sprint training results in an increase in fiber size for both type I and type II fibers and an increase in enzymes of anaerobic metabolism. The adaptation of muscles to training has been extensively reviewed (Saltin and Gollnick, 1983; Snow, 1983). However, changes associated with sprint training in horses have not been reported previously.

There is little information on the effect of detraining in horses. Guy and Snow (1977) studied the effect of training and detraining on muscle composition in 6 horses of different breeds. Thornton *et al.* (1983) reported on training and detraining changes in respiratory and cardiovascular parameters in 5 Standardbred trotters.

The purpose of the present study was to examine the changes in muscle composition and metabolism in Thoroughbred racehorses resulting from a training program of repeated bouts of high intensity, short duration exercise and a detraining period of equal length.

### *Materials and Methods*

Five Thoroughbred racehorses were trained by high intensity, short duration interval sprints for 8 weeks, followed by 8 weeks detraining. The horses were trained 5 days per week, being rested on Wednesday and Sunday. At the start of exercise on each training day the horses were walked and trotted at speeds up to 250 m/min for 1200 metres. Following this warm-up period the horses performed increasing numbers of 600 m gallops at speeds between 820 and 860 m/min with 1 minute rest periods between each gallop. The training schedule is outlined in Table 1. By the conclusion of the training program each horse was performing four 600-m gallops, with 1 minute rest periods, five days per week.

Before the training program began, after 2, 4, 6 and 8 weeks training, and after 8 weeks detraining, each horse was submitted to a 5-step, interval-type, standardized exercise test. The horse was ridden around a semicircular track over 600 metres 5 times at increasing speeds, starting at a slow trot and finishing with a fast gallop (Table 1). The same rider was used in all experiments. For each step the horse and rider commenced approximately 50 m before the measured 600 m so that the desired speed was achieved for the full distance. The elapsed time for each 600-m step was recorded. Before the test and at 3 minutes following each step, a venous blood sample (with F1 EDTA as anticoagulant) was taken for lactate determination. Muscle biopsy samples from the left middle gluteal muscle, 10 cm caudodorsal to the tuber coxae, were taken prior to the commencement of the test and between 5 minutes and 10 minutes following

# Skeletal Muscle Adaptation in Racehorses Following High Intensity Interval Training

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## Summary

*Five Thoroughbred racehorses were trained with an increasing number of short gallops (600 metres at 820–860 m/min) 5 days per week for 8 weeks, followed by 8 weeks detraining. Before, at 2-week intervals during training and after 8 weeks detraining, the horses were subjected to a standardized exercise test of 5, 600 m periods of exercise at increasing speeds of 240, 300, 400, 600 and 800 m/min, with a rest of 3 minutes between each exercise. Blood lactate was determined after each period of exercise and muscle biopsies were obtained from the gluteus medius before and after each test to determine muscle fiber types and glycogen content throughout the training program.*

*As a result of training, the speed producing a plasma lactate concentration of 4 mmol/l ( $V_{LA4}$ ) increased significantly from a mean of 540 m/min to 668 m/min. Following detraining  $V_{LA4}$  declined to 598 m/min. There was a significant increase in the number of highly oxidative type II fibers during training. There was no significant change in glycogen content of the gluteus muscles following training.*

*Thoroughbred racehorses appear to adapt to intensive sprint training by increasing the oxidative capacity of their skeletal muscles, and this adaptation is maintained at least in part for 8 weeks of detraining.*

*Index terms:* Lactate; muscle fiber types; glycogen.

## Introduction

Thoroughbred racehorses are required to perform high intensity exercise for short periods of time, in the great majority of cases lasting between 1 and 2 minutes. Routine methods of training these horses involve a high proportion of low intensity, long duration exercise, which as the animal gradually becomes 'fitter' is supplemented with varying amounts of high intensity exercise, which in many cases is performed only twice weekly.

Studies on the effect of training on skeletal muscle metabolism in horses have assessed the effect of endurance training either alone, or in combination with short periods of high intensity exercise. Endurance training in humans results in increased cardiac output, lower heart rates at given levels of activity and an increase in the maximum oxygen uptake ( $\dot{V}O_{2max}$ ) (Åstrand and Rodahl, 1977). Lactate production at given sub-

### Materials and Methods

Eleven Thoroughbred horses, four mares and seven stallions, under the care of four different trainers at Täby racetrack, Stockholm, Sweden, were observed from March 1985, when these horses were 22 months of age, to October 1985.

*Training Programs.* The horses were put in training in October 1984 when they were approximately 17 months of age. During the first months, the training was very slow with walking and trotting over 1700 m and at the end of 1984 horses were cantered for short distances (Table 1). From January the canters became longer (1400–1600 m), and in May 1985, when the horses were approximately 24 months of age, they started to do fast work. This consisted initially of 400 m gallops once a week and increased progressively to 1000 m. Many horses only had 4–7 fast gallop sessions before they entered their first race. The slow type of training at a trot and canter was performed about 4–6 times per week the year around.

*Muscle biopsies.* Gluteus medius muscle biopsies (Lindholm and Piehl, 1974) were obtained at rest from all horses in March, June and October. Muscle biopsies were taken by the same person each time at a point on a straight line between the highest point of the tuber coxae and the head of the tail. A thumb was placed on the highest point of the tuber coxae and with the hand, in a relaxed position, the site of the biopsy was established as the point where the little finger fell upon this line. The depth that the biopsy needle was inserted, was 6 cm. All three biopsies from each horse were obtained within a 1-cm radius measured as the distance from previous biopsy scars. Two samples were obtained, the piece for histochemical analyses was rolled in talcum powder before being frozen in liquid nitrogen, while a second piece for biochemical analyses was frozen immediately in liquid nitrogen. Both samples were stored at  $-80^{\circ}\text{C}$  until analyzed.

*Exercise Tolerance Test.* An exercise test was performed on 8 of the horses in June and October. Three horses were unable to participate in exercise tolerance testing due to lameness or other health problems. The exercise test consisted of two 1000-m gallops with 3–4 min rest in between. All horses carried a telemetric heart rate monitor (Hippocard) with a digital display strapped on the jockey's wrist. The first gallop was performed at a speed which maintained a heart rate (HR) of approximately 170 beats/min, and the second gallop at a heart rate of approximately 200 beats/min. Samples for

TABLE 1 Training program during the first year of training

	1984		1985			
	October–December		Muscle biopsy ↓ January–March	Exercise tolerance test muscle biopsy ↓ April–June	Exercise tolerance test muscle biopsy ↓ July–October	
Slow work 4–6 times/week	walk/trot canters	1700 m 2 × 400 m	trot canters	1700 m 1500 m	trot/canters 2000 m	canters 2500 m
Fast work once a week	—	—	fast gallops 400 m 600 m 800 m 1000 m			

blood lactate (La) analyses were collected within one minute after both heats from a jugular vein catheter. The total blood volume was determined after the second heat of each exercise test by the dye-dilution method (Persson, 1967).

**Biochemical analysis.** For biochemical analyses, freeze-dried muscle samples were dissected free of connective tissue and blood under a dissection microscope and weighed. The activity of citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD) (Essén *et al.*, 1980) and lactate dehydrogenase (LDH) (Essén-Gustavsson *et al.*, 1983) was analyzed after the samples had been homogenized in a phosphate buffer.

**Histochemical analysis.** Transverse serial sections (10  $\mu\text{m}$ ) of muscle were cut in a cryostat microtome at  $-20^{\circ}\text{C}$ . These sections were assayed for myosin adenosine triphosphate after both acid (pH 4.3 and 4.6) and alkaline (pH 10.3) buffer preincubation (Brooke and Kaiser, 1970). Muscle fibers were identified as type I, IIA, and IIB and muscle fiber composition was determined by typing at least 200 fibers from photomicrographs of each section.

**Statistical analysis.** The results from the biochemical and histochemical analyses were compared using analysis of variance and Student's *t* test on paired observations. The relationship between muscle characteristics and time in training was tested using a two-factor ANOVA design with horse and time as factors. The GLM procedure in the SAS package was also utilized. For the entire group of Thoroughbred horses in this study, the relation between time (June and October) and speed was studied using a linear model, where speed was modeled as a function of horse, time in training, HR and the logarithm of the lactate contents (log La). That is, the effect of training was studied after the effects of horse, log La and HR had been eliminated.

## Results

**Fiber composition (Table 2).** Large individual differences in fiber composition were seen between the horses. The mean percentage of type I fibers increased significantly ( $P < 0.05$ ) from 7% in March to 11% in October. In addition, the mean percentage of type IIA fibers increased significantly ( $P < 0.01$ ) from a mean of 32% in March to a mean of 43% in October and a corresponding decrease occurred in the mean percentage of type IIB fibers from 60 to 46% ( $P < 0.001$ ).

**Muscle enzyme activities.** The mean CS activity increased 44% from 27.5  $\mu\text{mol/g/min}$  to 39.5  $\mu\text{mol/g/min}$  ( $P < 0.01$ ) and the mean HAD increased 67% from 15.8  $\mu\text{mol/g/min}$  to 26.4  $\mu\text{mol/g/min}$  ( $P < 0.001$ ) from March to October. The mean

TABLE 2. Mean fiber types and enzyme activities ( $\pm$  standard deviations) from 11 Thoroughbred horses during the 2-year-old training and racing season

1985	Fiber type (%)			Enzyme activity $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$		
	I	II A	II B	CS	HAD	LDH
March	7.0 $\pm$ 4.0	32.4 $\pm$ 9.3	60.5 $\pm$ 9.1	27.5 $\pm$ 3.2	14.0 $\pm$ 4.3	1492 $\pm$ 322
June	8.6 $\pm$ 5.3	37.5 $\pm$ 9.1	53.8 $\pm$ 11.3	32.0 $\pm$ 8.7	20.9 $\pm$ 2.9	1146 $\pm$ 115
October	10.9 $\pm$ 1.8*	42.7 $\pm$ 7.6*	46.4 $\pm$ 7.6*	39.5 $\pm$ 8.2*	26.5 $\pm$ 3.1*	1020 $\pm$ 91*

\* = significantly different from March value

CS = citrate synthase; HAD = 3-OH-acyl CoA dehydrogenase; LDH = lactic dehydrogenase

LDH activity decreased 46% from March to October from 1492  $\mu\text{mol/g/min}$  to 1021  $\mu\text{mol/g/min}$  ( $P < 0.01$ ).

*Exercise tolerance test.* The mean values for HR, speed and blood lactate are shown in Table 3 and statistical analysis of the two exercise tests showed no significant differences ( $P < 0.10$ ). With similar HR in June and October, 5 out of 8 horses had increased speed with unchanged lactate response. During the exercise tolerance test HR and La were significantly correlated to speed ( $P < 0.001$ ) (Fig. 1).

*Total blood volume.* Total blood volume showed a significant mean increase of 14% ( $P < 0.05$ ) (Table 3) during four months.

### Discussion

The low percentage of type I fibers found in the middle gluteal muscle at the age of 2.5 years agree with earlier studies on Thoroughbreds in Sweden (Lindholm *et al.* 1983) and in England (Snow and Guy 1980). Previous studies on both Standardbreds and Thoroughbreds show changes in muscle fiber composition with training, in particular an increase in the type IIA/IIB ratio (Lindholm *et al.* 1983; Guy and Snow 1977; Essén *et al.*, 1980; Henckel, 1983; Raudsepp *et al.*, 1985). In this study, an increase in the percentage of type I fibers was seen with 7 months of training which is similar to observations on trotters by Henckel (1983). Large individual differences in the percentage of type IIA and IIB fibers were found between the horses. However, during the period of time the horses were studied the percentage of these fiber types changed significantly. Not only did the type IIA/IIB fiber ratio increase during the first year of training but an increase occurred in CS and HAD activities and a decrease in LDH activity. An earlier study on Standardbreds between the ages of 6–18 months indicates that changes in muscle characteristics may not only be due to training but also to growth itself (Essén-Gustavsson *et al.*, 1983). The changes in muscle fiber composition found in this study are, however, probably mainly due to a training effect, since horses reach about 80% of their adult weight within 1.5 years of age (Evans *et al.*, 1977) and the horses in this study were between 2 and 2.5 years of age.

The horses were continuously trained from approximately October 1984 (range, August to December) when they were about 17 months of age. During the first 7 months their training consisted predominantly of slow work (Table 1) since rain and snow during the fall and winter months in Sweden result in variable track conditions. In April each year the winter straw racing surface is removed and the condition of both a dirt

TABLE 3. The mean heart rate, speed and blood lactate at the first and second heat (H1 and H2) and total blood volume ( $\pm$  standard deviations).

	Heart rate beats $\text{min}^{-1}$		Speed $\text{m sec}^{-1}$		Blood lactate $\text{mmol l}^{-1}$		Total blood volume $\text{ml/kg}$
	H1	H2	H1	H2	H1	H2	
June	167 $\pm$ 11.5	209 $\pm$ 7.8	8.9 $\pm$ 1.2	11.1 $\pm$ 0.8	1.9 $\pm$ 0.9	5.8 $\pm$ 2.2	109 $\pm$ 6.7
October	177 $\pm$ 10.8	209 $\pm$ 14.0	8.9 $\pm$ 1.2	11.5 $\pm$ 1.0	2.1 $\pm$ 0.7	5.7 $\pm$ 1.2	125 $\pm$ 15.7*

\* = significantly different from June value

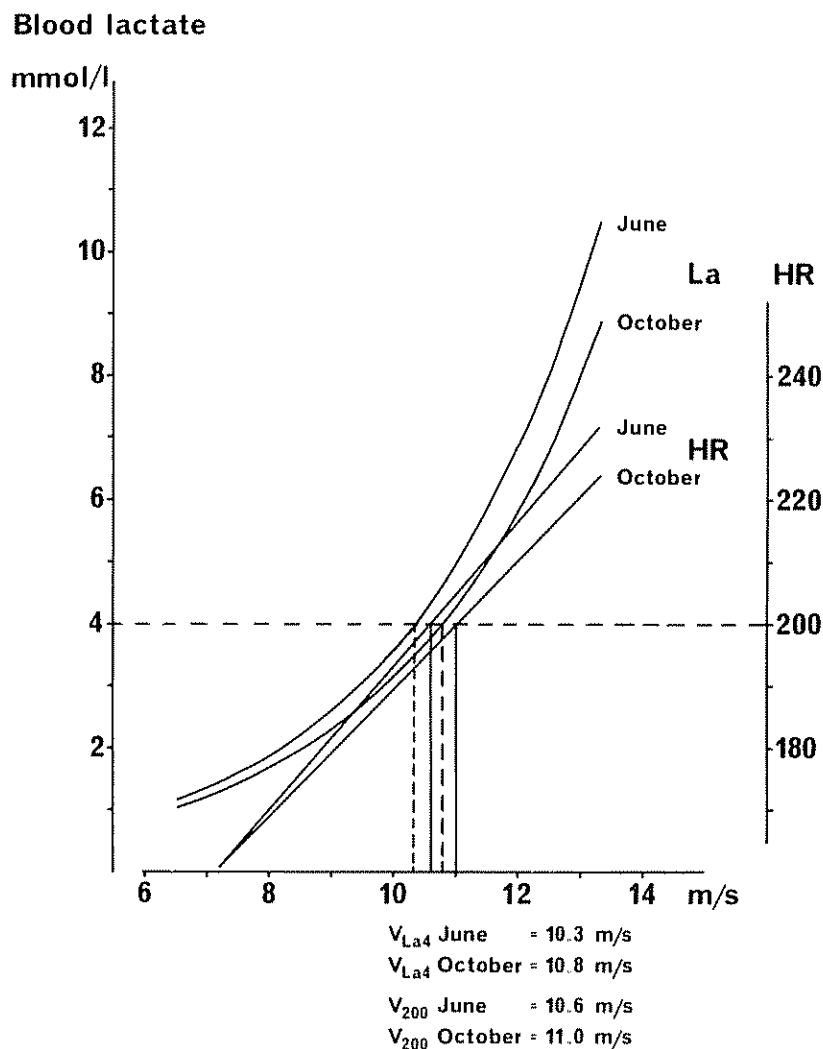


FIGURE 1. Correlation between speed (m/s) and heart-rate (HR) during the exercise tolerance test and between speed and blood lactate (La) measured immediately after the exercise test.

$V_{200}$  = speed producing a heart rate of 200  $\text{min}^{-1}$ ;  $V_{La4}$  = speed producing blood lactate of 4 mmol/l.

track and a sawdust track are usually good enough for the horses to start fast-gallop training in May 1985 (range, April–August). Minor differences in training programs or shorter breaks in training due to injuries or respiratory infections were not taken into account in evaluating the results. Most horses only did one 400–1000 m fast-gallop per week for 4–7 weeks before the first race. The race debut varied from June 1985 to February 1986. The training program for the horses in this study did not markedly differ between the 4 trainers and thus contained to a greater part slow type of work than

intense work-like fast gallops. It is well known that with slow type of training an increase in muscle oxidative capacity usually occurs in both man (Saltin and Gollnick, 1983), rats (Holloszy, 1967) and horses (Hodgson, 1985). The magnitude of the increase however differs between studies and is likely related to duration and intensity of training (Dudley *et al.*, 1982).

The biopsy data showed that muscle had adapted to training through changes in fiber-type composition and through increased oxidative capacity; however, individual differences existed between horses. The tests performed in June and October for blood volume analyses showed that the oxygen transport capacity increased during this period.  $V_{200}$  and  $V_{L_{a4}}$  are variables shown to be useful for evaluating exercise tolerance and fitness in Standardbred horses (Persson, 1983). It has been shown that blood lactate accumulation is exponentially related to both exercise intensity expressed as velocity (V) and heart rate (HR) when performing stepwise incremental treadmill work under standardized conditions in the laboratory (Persson and Ullberg 1974, Persson 1983). The work tests in June and October indicated that it was possible to get information about  $V_{200}$  and  $V_{L_{a4}}$  also for a group of Thoroughbred horses under practical conditions. Heart rate and blood lactate were significantly correlated to speed and  $V_{L_{a4}}$  was related to  $V_{200}$  (Fig. 1). It is important, however, to let the horse run several heats in order to determine  $V_{L_{a4}}$  for the individual horse. In this study each horse only performed two heats due to the fact that all horses had private owners and the trainers did not want these young horses to go more heats. When muscle oxidative capacity increases it has been shown that work intensity, at which lactate in the blood starts to accumulate is increased (Sjödín *et al.*, 1981). The  $V_{L_{a4}}$  is also shown in Standardbreds to increase after training (Thornton *et al.*, 1983). Thus,  $V_{L_{a4}}$  may be one parameter to use for evaluating energy release through mainly oxidative processes during a training period. In the field however, it may be complicated to determine  $V_{L_{a4}}$  as several heats are needed in order to extrapolate for  $V_{L_{a4}}$ . Heart rate monitoring during gallops is an easy method to use in the field. That only five of the eight horses had increased their  $V_{200}$  during the training period from June to October is likely related to different individual training responses. Psychological factors, stress, track conditions and injuries could also have influenced the work test.

In conclusion, this study showed that both a heart rate monitor and blood samples taken after work can be used to define parameters like  $V_{200}$  and  $V_{L_{a4}}$  which may be useful in evaluating fitness and state of training even under field conditions in Thoroughbred horses.

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# Cardio-Respiratory and Muscle Metabolic Responses to Draught Work on a Treadmill in Standardbred Horses

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## Summary

*Cardio-respiratory and muscle metabolic responses to standardized draught work on a treadmill were studied at a slow trotting speed (4.8 m/sec) until the horses could no longer lift weights from the ground. Ten horses performed incremental draught work in which weights were added every two minutes to a rope which ran over a pulley and was connected horizontally to the harness. Heart rates increased linearly to a mean of 209 bpm and blood lactates exponentially to a mean of 10.5 mmol/l with increasing weight loads. Lactate levels increased significantly and glycogen and creatine phosphate levels decreased in both m. gluteus and semitendinosus, and adenosine triphosphate (ATP) levels decreased in m. semitendinosus only. The ATP level in the semitendinosus muscle at the end of exercise was significantly negatively correlated to the percentage of type II fibers. In conclusion, for slow trotting of short duration, glycolysis with lactate accumulation and phosphagen breakdown seem to be important pathways for energy supply in muscles that are involved during exercise with incremental draught loading.*

*Lactate; adenosine triphosphate; creatine phosphate.*

## Introduction

Draught work is used as a method of training Standardbred racehorses in the Nordic countries. Many trainers are of the opinion that the strength of horses can be increased by use of draught work in their training program. Draught work is also often used after horses have had injuries or surgery when conventional training at higher speeds has to be avoided due to stress on joints, ligaments and tendons. The specific metabolic response induced by pulling against increasing resistance and the training effects gained from this are poorly understood. It was therefore of interest to study cardio-respiratory and muscle metabolic responses using a model in which draught work was performed under standardized, measurable and reproducible conditions as close as possible to the way it is performed on the track.