# 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 ($\dot{V}O_2$max) (Åstrand and Rodahl, 1977). Lactate production at given sub-

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

TABLE 1.

A) Interval training program

| Week | No. of Days | No. of Gallops |
|---|---|---|
| 1 | 3 | 1 × 600 m |
| 2 | 5 | 1 × 600 m |
| 3, 4 | 5 | 2 × 600 m |
| 5, 6 | 5 | 3 × 600 m |
| 7, 8 | 5 | 4 × 600 m |

B) Standard Exercise Test

| Step | Speed ($m \cdot min^{-1}$) | |
|---|---|---|
| 1 | 240 | trot |
| 2 | 300 | trot |
| 3 | 400 | canter |
| 4 | 600 | canter |
| 5 | 800 | gallop |

the final gallop at a depth of 6–8 cm, using the technique described by Lindholm and Piehl (1974).

Blood samples for lactate determination were packed in ice and stored at 0°C until analyzed. After centrifugation plasma lactate concentrations were determined enzymatically (Boehringer Mannheim Product No. 149993) within 4 hours of collection.

The muscle sample was divided into two parts. One portion for histochemical analysis was mounted on aluminum foil with OCT embedding medium (Ames Tissue Tek) and then frozen in isopentane cooled in liquid nitrogen, while that for biochemical analysis was frozen directly in isopentane cooled in liquid nitrogen. All samples were stored at −90°C until analyzed.

*Histochemical analyses.* From the samples for histochemical analysis, transverse serial sections (10 μm) were cut on a cryostat at −20°C and mounted on cover slips. Sections for fiber typing were stained for myofibrillar ATPase after alkaline (pH 10.3) buffer preincubation according to the technique of Brooke and Kaiser (1970), while those for estimation of muscle fiber oxidative capacity were stained for reduced nicotinamide dinucleotide tetrazolium reductase (NADH-TR) according to the technique of Novikoff *et al.* (1961).

Photomicrographs were taken of each section and the fibers identified as type I or type II according to their characteristic staining patterns (Brooke and Kaiser 1970). Oxidative capacity was classified as low or high, according to the intensity of the NADH-TR staining as shown in Fig. 1. A minimum of 200 fibers were counted in each sample and all fiber classifications were carried out by the same person.

*Biochemical analyses.* Muscle tissue was freeze-dried and dissected free of blood, fat and connective tissue. A portion of the dissected muscle (0.7 to 1.2 mg) was weighed and the glycogen hydrolyzed to glucose by boiling in 2 ml 1 M hydrochloric acid for 2 hours. The glucose content of the extract was measured enzymatically (Instrumentation Laboratory, Product No. 35162).

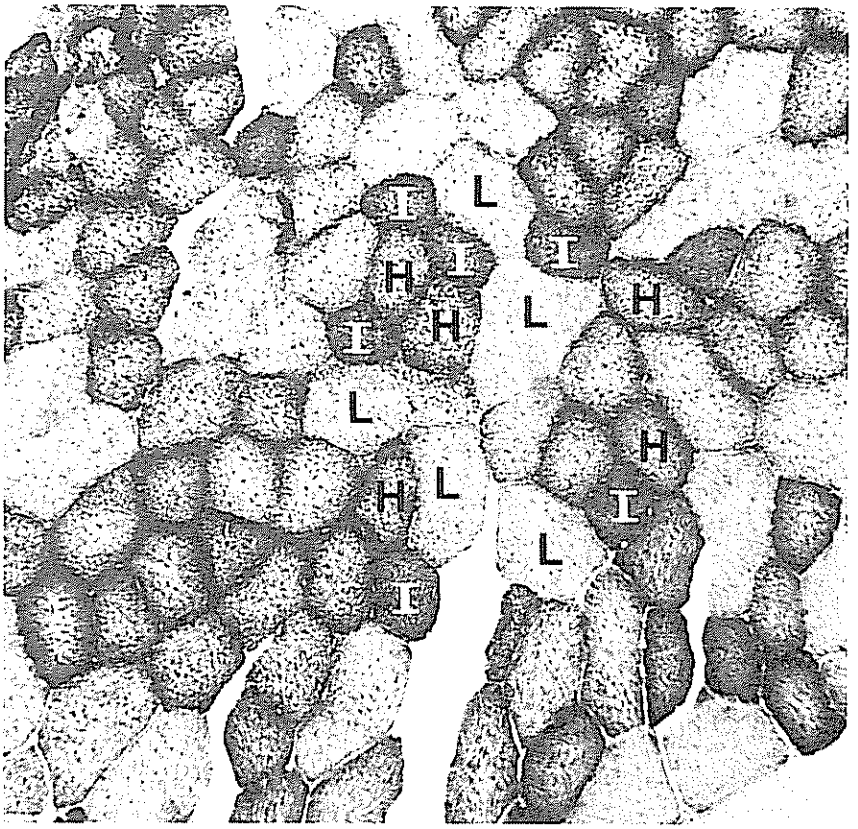


FIGURE 1 Transverse section from the middle gluteal muscle stained for NADH-TR. Type I fibers are shown (I) as are type II fibers of Low (L) and High (H) staining intensity.

*Statistical analyses.* Linear regression analysis of logarithmic transformations of lactate concentrations against speed enabled calculation of the speed at which plasma lactate concentration was 4 mmol/l ($V_{LA\cdot 4}$). Comparison of values from each horse at progressive times throughout the training and detraining periods was performed using a Student's *t* test of paired observations. Results are expressed as means ± SD.

## *Results*

Details of changes in muscle fiber type during the experimental period are shown in Table 2. There was no change in the proportion of type I fibers throughout the training period. Before training commenced there was an approximately equal proportion of type II high oxidative and type II low oxidative fibers. Following 2 weeks of training there was a significant increase ($P < 0.05$) in type II high oxidative fibers which persisted for the 8-week training period. After 6 weeks of training there was a significant ($P < 0.01$) reduction in low oxidative type II fibers which persisted through 8 weeks of detraining.

TABLE 2. Changes in metabolic parameters during the training and detraining program

| Week | Type I (%) | Type II High Oxidative (%) | Type II Low Oxidative (%) | Glycogen (mmol · kg$^{-1}$) | $V_{LA4}$ (m · min$^{-1}$) |
|---|---|---|---|---|---|
| 0 | 12.4 ± 5.0 | 42.8 ± 4.6 | 44.8 ± 3.3 | 491 ± 51 | 540 ± 85 |
| 2 | 9.7 ± 2.4 | 48.7 ± 1.9* | 41.5 ± 1.3 | 545 ± 56 | 571 ± 116 |
| 4 | — | — | — | 494 ± 75 | 600 ± 63 |
| 6 | 11.6 ± 2.9 | 49.6 ± 3.2** | 38.8 ± 2.3* | 428 ± 44* | 628 ± 74* |
| 8 | 11.7 ± 1.3 | 50 ± 4.2* | 38.2 ± 3.2* | 509 ± 56 | 668 ± 76** |
| −8 | 12.7 ± 3.5 | 46.7 ± 2.5 | 40.7 ± 3.0* | — | 598 ± 34* |

$V_{LA4}$ = speed at which plasma lactate concentration was 4 mmol · L$^{-1}$.
*$P < 0.05$
**$P < 0.01$ significant difference from week 0 values.

Muscle glycogen concentration during training showed some variations, but only differed significantly from pretraining levels after 6 weeks of training when there was a significant decrease ($P < 0.05$).

The pretraining value of $V_{LA4}$ was 540 ± 85 m/min. This increased throughout the training period and was significantly greater than pretraining after 6 ($P < 0.05$) and 8 ($P < 0.01$) weeks. Following 8 weeks of detraining $V_{LA4}$ had decreased from 668 ± 76 m/min to 598 ± 34 m/min but was still significantly higher than the pretraining value.

## *Discussion*

The proportions of the type I fibers in the middle gluteal muscle of horses in the present study were similar to those reported by Snow and Guy (1981) and Hodgson *et al.* (1986) for Thoroughbreds. While it is generally believed there is no conversion between type I and type II fibers as a result of training (Lindholm and Piehl, 1974; Taylor and Brassard, 1981), Guy and Snow (1977) reported a significant decrease in the percentage of type I fibers in the deltoid muscle following training. The present study showed no significant change in the proportion of type I fibers throughout the training period.

In horses, training programs consisting of a mixture of aerobic and anaerobic workloads have increased the proportion of type II fibers exhibiting high oxidative capacity (Lindholm and Piehl, 1974; Essén *et al.*, 1980; Hodgson *et al.*, 1986), as has endurance training in humans (Andersen and Henriksson 1978).

In the present study, the repeated bouts of high intensity, short duration activity, resulted in a significant increase in the ratio of type II high oxidative fibers to type II low oxidative fibers and a significant increase in $V_{LA4}$. Thus the aerobic capacity of the horses in this study increased in a similar fashion to that of horses undergoing more routine training programs.

Interestingly, the greatest increase in the ratio of type II high oxidative to type II low oxidative fibers in any two week period, occurred between weeks 0 and 2 when the workload was comparatively small (Table 2). Previous studies have not been sufficiently detailed for a comparison to be made, but it is possible that previously seden-

tary horses exposed to the immediate demands of high intensity exercise may undergo rapid adaptation of skeletal muscle. In humans subjected to training programs at high levels of submaximal exercise (80–90% $\dot{V}O_2$max) there is increased capillarisation of muscle fibers, an enhancement of their mitochrondrial enzyme activities and an accompanying increase in $\dot{V}O_2$max. These adaptations may occur by 2–3 weeks of training (Andersen and Henriksson, 1977, Saltin and Rowell, 1980).

Following 8 weeks of detraining, despite a decrease, the ratio of type II high oxidative to type II low oxidative fibers was still significantly higher than before training. Other detraining studies have not analyzed fiber type changes however the study by Guy and Snow (1977) supports the proposition that skeletal muscle adaptation to 10 weeks of training is reduced following 5 and 10 weeks of detraining.

Increased muscle glycogen levels have been reported following training in both humans and horses. In humans, a 250% increase in muscle glycogen content results from a 5 month training program (Gollnick *et al.*, 1973) whereas trained 4-year and older Standardbred horses have only a 25% greater muscular glycogen content than untrained 2-year-olds (Lindholm and Piehl, 1974). Apart from the decrease after 6 weeks there was no significant change in muscle glycogen content during the 8 weeks of training in the present study.

All horses in the present training program were mature horses (4 years and over) and had previous race training experience. It may be that horses exposed to recurrent conditioning maintain high resting levels of glycogen even following extended periods of relative inactivity. Alternatively, 8 weeks may not be a sufficient period to demonstrate increased glycogen storage. This does not appear likely, however, as other measures of muscular adaptation showed rapid and sustained responses to the training stimulus.

The low glycogen after 6 weeks of training was probably due to still partially depleted glycogen stores at the time of sampling, as the sample was collected on the day following three 600 m gallops. It has been shown that it can take up to 48 hours for muscle glycogen to return to pre-exercise levels following strenuous activity (Lindholm and Piehl, 1974; Snow *et al.*, 1982).

For many years $\dot{V}O_2$max has been regarded in humans as the best measure of cardiovascular fitness and endurance capacity (Shephard *et al.*, 1968), but recently a better measure has proven to be $V_{LA4}$ (Heck *et al.*, 1985). Maximum oxygen uptake has been difficult to measure in horses, but it has been shown that the onset of blood lactate accumulation occurs at $V_{LA4}$ in horses as it does in humans (Wilson *et al.*, 1983).

It would appear from the results that the increase in oxidative capacity in the muscle fibers was associated with the significantly increased $V_{LA4}$ between week 0 and week 8 of training. Following 8 weeks detraining $V_{LA4}$ decreased but was still significantly greater than the pretraining level. Many studies in horses have shown a decrease in lactate production during a standard exercise test following training programs (Krzywanek, 1973; Anderson, 1975; Milne *et al.*, 1977; Snow and Mackenzie, 1977; Thornton *et al.*, 1983) and one, the maintenance of this change with detraining (Thornton *et al.*, 1983). The calculation of $V_{LA4}$ in future tests may prove to be useful in the assessment of performance capacity as it is in humans, particularly in events lasting from 30 seconds to 6 minutes for which it has been stated that lactate production is the true limiting factor of performance (Mader *et al.*, 1978).

In horses performing high intensity exercise the glycolytic process reaches a peak of production in about 30 seconds, and energy production is predominantly anaerobic for

the first 40 to 50 sec (McMiken 1983). It may be expected, therefore, that intermittent exercise of 40 to 45 seconds duration at speeds well in excess of $V_{LA4}$ would stimulate the anaerobic metabolic pathways to a greater extent than the aerobic pathways. From the present study it would appear that this is not the case, although increases in anaerobic capacity are not as readily quantified and it may be that the anaerobic capacity of these horses increased in conjunction with the increase in aerobic capacity. In humans, training programs which lead to increased aerobic capacity have shown no increases in measured anaerobic markers.

It appears, therefore, that to increase anaerobic capacity, if this is possible in the horse, sprints of shorter duration than 40 to 45 s are necessary. Human sprinters train for events lasting 10 to 20 s, and incorporate significant weight lifting or other strength type training in their programs. Training horses to gallop at near maximal speeds for such short periods repeatedly would be difficult without a controlled environment such as a treadmill. Thoroughbred racehorses show a greater genetic adaptation than humans to the demands of short bursts of high speed exercise, having both higher resting levels of muscle glycogen (Guy and Snow, 1977) and higher levels of anaerobic enzyme activity relative to oxidative enzymes (Hodgson *et al.*, 1986) and it appears likely, therefore, that even where training programs are heavily weighted towards maximal activity, the metabolic response will be an increase in oxidative capacity rather than a marked change in glycolytic function.

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# A Field Study of Circulatory Response and Muscle Characteristics in Young Thoroughbreds

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## Summary

*Eleven Thoroughbred horses were examined in March, June and October during their first year of training. Muscle biopsies were taken on each occasion and analyzed for fiber type composition and enzyme activities. Work tests (2 × 1,000 m gallops) were performed in June and October to evaluate heart rate (HR) and blood lactate (La) at each of two speeds and blood volume was determined. A telemetric heart rate monitor was used so that HR could be maintained at approximately 170 bpm in the first gallop and 200 bpm in the second. Muscle oxidative capacity increased significantly from March to October by 40–70% and the mean type IIA/IIB fiber ratio increased by 72%, although there was a large variation among the horses. During the work tests HR and La were significantly correlated to speed. Blood volume increased significantly from a mean of 109 to 125 ml/kg between June and October. It can be concluded that the work tests and variables measured in this field study may be useful in assessing the state of training of Thoroughbred racehorses*

*Index terms: Horse; blood lactate; heart rate; blood volume; muscle fiber types, muscle enzyme activities; training.*

## *Introduction*

Most young racehorses today are trained in a similar fashion. However, large individual differences in muscle fiber characteristics occur between horses and at a similar workload the metabolic response to exercise differs markedly between individuals (Valberg *et al.*, 1985). Great demands are placed on young racehorses and consequently many horses begin training at high speeds at a very early age and if these horses are not properly conditioned this results in an increased risk of injury. Therefore it is important to develop practical field methods for assessing and following an individual horses state of training. The present study was undertaken to examine the adaptations of the middle gluteal muscle in two-year-old Thoroughbred horses to a conventional training program over a period of seven months and to evaluate the state of training during this period with an exercise tolerance test on the racetrack.