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Changes in Skeletal Muscle Composition in Response to Interval and High Intensity Training

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ABSTRACT Seven Thoroughbred horses underwent 12 weeks training on an inclined treadmill. During weeks 1–6, the exercise was essentially aerobic, but during weeks 7–12 the horses underwent increasing amounts of high intensity interval training, at speeds equivalent to 100% of maximum heart rate. Before and every 3 weeks during training, muscle biopsies were collected from the m. gluteus medius for fibre typing and measurements of enzyme activities (CS, HAD, LDH). Metabolic response to exercise was assessed at 0, 6 and 12 weeks of training, by a standardised exercise test: 2 min warm up at 4 m s^{-1}, followed by acceleration over 30 s to a speed of 11.0 m s^{-1} for 60 s. Prior to the test and immediately after, muscle samples were collected for metabolite analyses (ATP, ADP, AMP, PCr, creatine, NAD, citrate, lactate (LA) and pH). Muscle temperature was also measured. After 12 weeks, there was a 35% decrease ($p<0.05$) in the percentage of type I fibres from a mean 25.3±3.3 pre-training. The percentage of type IIa fibres increased from 45.2±2.7 to 55.8±2.0, from pre- to post-training ($p<0.01$). Activities of CS and HAD increased ($p<0.01$) by approximately 140–160%, respectively from pre-training to 6 weeks training and, thereafter, showed little change. LDH showed no change during the first 6 weeks of training, but by 9 weeks increased to 111% ($p<0.05$) of pre-training values and to 123% ($p<0.02$) by 12 weeks. No change was found due to training, in post-exercise ATP, ADP, AMP, PCr, creatine, NAD and citrate. After 12 weeks training, muscle LA and muscle temperature decreased from post-exercise, pre-training values ($p<0.01$), whereas muscle pH increased.

Key words Horses; metabolites; enzymes; training; fibre types; interval training.

INTRODUCTION

Repetitive loading and overloading is the underlying philosophy behind all training programmes for athletes preparing for competitive events. While the specific aspects of training for different forms of athletic competition have been widely investigated in human exercise physiology, there is still a lack of information about the effects of different training regimens in the horse. Thoroughbred horses race over relatively short distances at high exercise intensities and it would be presumed that training for such races should involve repetitive loading of the anaerobic producing systems.

Where studies evaluating muscular adaptations to training in Thoroughbred horses have been investigated, these have involved horses in commercial training[3,20,21] with few investigations of experimental training programmes.[24,25,28] In the majority of these studies, there has not been a major focus on high intensity exercise and the main training adaptations in muscle have been an increase in aerobic enzyme activities.

In this study, the premise that a training programme in Thoroughbred horses which included a large component of high intensity exercise would result in adaptations in skeletal muscle which should enhance anaerobic capacity was investigated.

MATERIALS AND METHODS

Seven Thoroughbred horses aged between 4 and 7 years, underwent a 12 week training

programme on a treadmill (Beltalong, Euroa, Australia). The horses had been at rest for a minimum of 3 months prior to the commencement of training but all horses had been previously acclimatized to treadmill exercise. The 12 week training programme was divided into 4 phases, with horses exercising for 6 days per week.

Phase 1. Low intensity, long distance training

This phase, which involved the first 3 weeks of training, involved horses trotting on the treadmill inclined at 6° at a speed of 4 m s^{-1}. Daily exercise sessions were divided into two bouts, morning and afternoon, and commenced with a daily distance of 4.8 km in Week 1, increasing to between 5.6 and 8.4 km in Week 3. The weekly distances for each horse were, 28.8, 34.0 and 41.6 km, respectively for Weeks 1, 2 and 3. Following the initial training period, all further training was performed on the treadmill at a decreased incline of 3°, so that the training speeds were closer to those that would be experienced by horses on the racetrack.

Phase 2 Combination of low intensity exercise and moderate intensity interval training

This phase, from Weeks 4–6, involved daily trotting exercise at 4 m s^{-1} over a distance of 1 200 m, followed by 800–1 000 m exercise intervals at a speed which was calculated from previous exercise tests to produce 90% of maximum heart rate (HRmax). The speeds at 90% HRmax ranged from 6.4 to 9.7 m s^{-1}. Heart rate vs. speed regression equations were calculated for each horse so that individual speeds for exercise could be determined, using weekly incremental exercise tests.[9] Interval exercise was given 5 days per week, with the number of intervals being initially 3 by 1 000 m, with 1 min rest periods between the exercise bouts. In Week 6, this exercise increased to two by 1 000 m and two by 800 m intervals, separated by 1 min rest periods. The total distance worked by each horse in Weeks 4–6, ranged from 20–24 km per week.

Phase 3 Continuation of moderate intensity intervals and commencement of high intensity training

Weeks 7–9 continued the aerobic interval training, with horses exercising for 3 intervals of 800–1 000 m at 90% HRmax, with 1 min recovery periods between the exercise bouts. The speeds at 90% HRmax ranged from 7.7 to 10.4 m s^{-1}. This training was given on 4–5 days per week. On the remaining days, the horses were given 3 intervals of exercise over 600 m at 100% HRmax, with a recovery period that was three times as long as the exercise duration. The total distances worked by each horse were 12.2, 25.2 and 19.8 km, respectively for Weeks 7, 8 and 9.

Phase 4 High intensity training

On 3 days per week, horses exercised at their HRmax over a distance of 600 m. The speeds at HRmax ranged from 9.0 to 12.0 m s^{-1}. Three bouts of exercise were given over this distance, separated by a recovery period that was three times as long as the exercise duration. On the other days, the horses exercised over distances of 3–4 km at 6–7 m s^{-1}. The total distances worked by each horse were 18.3, 19.8 and 16.2 km, respectively for Weeks 10, 11 and 12.

Prior to exercise commencing, and at 3 weekly intervals throughout training, resting muscle biopsies were collected from the right m. gluteus medius, using the needle biopsy technique.[22] Sample site was kept constant for each biopsy and attempts were made to obtain the muscle samples at a constant depth. The muscle sample was placed on aluminium foil and within 30 seconds of collection, a portion was frozen in liquid nitrogen for enzyme analyses. Remaining muscle was mounted on cork blocks for histochemistry and frozen in iso-pentane, cooled in liquid nitrogen.

For histochemical examination, 10 μm transverse serial sections were cut and mounted onto coverslips. Muscle sections

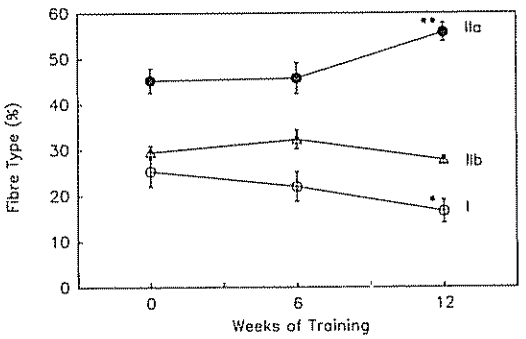

Fig. 1. Percentages (mean ± SEM) of types I, IIa and IIb fibres before and after 6 and 12 weeks of training in 7 Thoroughbred horses. * $p<0.05$; ** $p<0.01$—significant differences from week 0 values.

were stained for myosin ATPase using a method based on the technique of Brooke and Kaiser,[2] following preincubation at acid (pH 4.3, 4.6) and alkaline (pH 10.3) pH values. From these stains, fibres were identified as type I, IIa and IIb and the proportions of the different fibre types determined after counting at least 200 fibres.

After freeze drying, dissection of any blood or connective tissue, and weighing the muscle, activities of three enzymes were measured: citrate synthase (CS) E.C. 4.1.3.7), 3-hydroxyacyl CoA dehydrogenase (HAD) (E.C. 1.1.1.35) and lactate dehydrogenase (LDH) (E.C. 1.1.1.27). All assays were based on the methods of Essén-Gustavsson and Henriksson.[6] Enzyme activities were determined by observation of changes in NADH equivalents *in vitro* at 25°C, using fluorimetry. Enzyme activity was calculated in units of μmol NADH converted min^{-1} mg^{-1} freeze-dried muscle.

Prior to training and following 6 and 12 weeks of training, a standardised exercise test was used to assess the muscle metabolic response to training. For the exercise test, the treadmill inclination was adjusted to 6°. At rest, immediately prior to exercise, a muscle biopsy was collected from the left m. gluteus medius. Muscle temperature was measured following the biopsy using a thermistor (Model 43 TF, Yellow Springs Instruments, OH). The exercise test used was 2 min warm up at 4 m s^{-1} followed by acceleration over 30 s to 11 m s^{-1}, with a further 60 s at this speed. The treadmill was brought to an immediate stop and a muscle biopsy collected from a second prepared site within 10 s. The biopsy was frozen immediately in liquid nitrogen and muscle temperature measured.

Frozen muscle from the pre- and post-exercise biopsies was divided into 3 pieces. Two small pieces, 30–60 mg wet weight, were used for duplicate pH measurements[23] and one large piece for metabolite assays. For muscle metabolite measurements, freeze dried samples of muscle were used. Measurements performed were: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (PCr), creatine, nicotine adenine dinucleotide (NAD), lactate (La) and citrate. These measurements were made using a fluorimetric method.[23]

The data was analysed using a repeated measures analysis of variance, to determine any significant differences from pre-training values. Results are presented as mean ± SEM.

RESULTS

All horses tolerated the training well for the first 9 weeks and there were no indications of any musculoskeletal disorders. During the last 3 weeks of training with high intensity exercise intervals, some of the horses showed decreased appetite but there was no weight loss.

There were no alterations in fibre composition during the first 6 weeks of training, but in muscle biopsies collected after 12 weeks of training, there was a significant decrease ($p<0.05$) in the percentage of type I fibres and an increase ($p<0.01$) in type IIa fibres (Fig. 1).

Significant increases ($p<0.05$) in CS and HAD occurred after 6 weeks training after which the activities of both enzymes plateaued until the end of training. At this time,

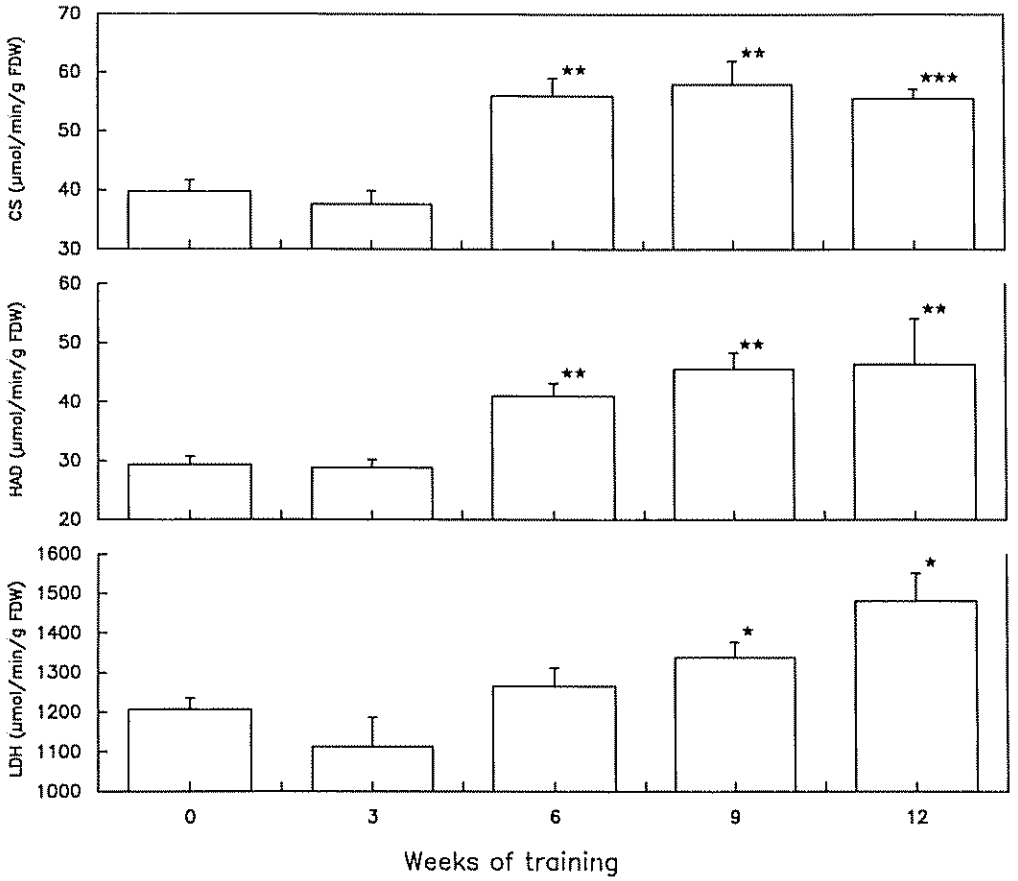

Fig 2 Activities (mean ± SEM) of citrate synthase (CS), 3-hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) in freeze dried muscle from 7 horses before, during and after 12 weeks of training * $p<0.05$; ** $p<0.01$; *** $p<0.001$—significant differences from pre-training (week 0) values

values for CS and HAD were respectively 141 and 161% higher than pre-training values. No changes occurred in LDH until Week 9 when LDH values were 111% above pre-training values ($p<0.05$). Activity of LDH continued to increase from Weeks 9–12, there being an increase over pre-training values of 23% ($p<0.01$) after 12 weeks training. These results are illustrated in Fig. 2.

No changes were found in the muscle concentrations of ATP, ADP, AMP, NAD or citrate throughout training. However, at 12 weeks training, post-exercise muscle LA was significantly lower ($p<0.01$) than pre-training values. Post-exercise muscle pH was higher ($p<0.01$) and muscle temperature lower ($p<0.01$) than the pre-training values. These results are shown in Table 1.

DISCUSSION

The concept of this training study was to provide horses with a gradual increase in the intensity of exercise, which after a period of initial slow trotting training, was based on the percentage of individual horse's HRmax. We wanted to determine if racehorses could tolerate greater amounts of high intensity exercise than are currently given in commer-

Table 1. *Muscle metabolities (mean ± SEM) after exercise at 0, 6 and 12 weeks of training*

Results from 7 horses after a standardised exercise test. All metabolities expressed as μmol g^{-1} D. W.

	Week 0	Week 6	Week 12
ATP	28.0±1.9	26.0±1.5	27.3±1.8
ADP	3.5±0.3	3.0±0.2	4.2±0.3
AMP	0.72±0.08	0.62±0.06	0.58±0.08
PCr	27.6±1.8	26.5±2.7	33.1±2.6
Creatine	94.9±4.8	94.3±4.9	98.8±4.5
NAD	1.64±0.23	1.39±0.12	1.86±0.29
Lactate	158.0±15.3	158.0±11.2	93.4±10.5**
Citrate	2.7±1.2	2.1±0.3	1.7±0.4
Temp (°C)	40.1±0.2	40.0±0.2	39.5±0.1**
pH (units)	6.73±0.03	6.78±0.02	36.89±0.04*

* $p<0.05$; ** $p<0.01$—differences from week 0.

cial training programmes. The reason for selection of exercise intensity as a percentage of HRmax was based on the finding that the speeds at HRmax and $\dot{V}O_2$max are highly correlated.[8] Training horses at the same percentage of HRmax ensures that all horses were exercising at similar exercise intensities. At speeds equivalent to HRmax, horses are exercising at about $\dot{V}O_2$max but well below their maximum speeds. Thus there should be enhancement of both aerobic and anaerobic capacities. Training horses at a set speed or at a speed equivalent to a heart rate of 200 bpm (V_{200}), makes no allowance for individual differences in relative exercise intensities or individual HRmax values.

The large increase in aerobic enzyme markers in muscle as a response to endurance training is well documented in both man[1,10,12,16] and horses.[13,15,18,19] There have been relatively few studies of the effects of high intensity exercise on muscle composition or the metabolic responses to exercise in either humans or horses. Studies in human athletes have investigated changes in enzymes and fibre composition, with some finding no changes in the activities of anaerobic enzyme markers,[4,27] and others finding increases.[10,26] In studies of horses where LDH has been used as a marker of anaerobic enzymes activities, no increases have been reported.[7,20,22,25] However in all the latter studies, the high intensity training component of the training programmes was relatively small. Where more intense training programmes were undertaken,[24,28] anaerobic enzyme markers in muscle were not investigated. The current study found that there was enhancement of the capacities of muscle for beta and end-terminal oxidation after 6 weeks of training involving aerobic exercise over distances which represented 2 to 3 times those performed by Thoroughbred horses undergoing commercial training in Australia or North America.[20] The 10.7% increase in LDH during the final 3 weeks of training, when the programme included a large component of high intensity exercise intervals, was an interesting finding as it represents the first report of an enhancement of LDH activity in response to training in the horse. It is interesting to note that during this period of high intensity training, there was little change in either CS or HAD, suggesting a plateau in activity in response to the decrease in aerobic exercise. It is evident, therefore, that if substantial amounts of high intensity exercise are included in a training programme, enhancement of anaerobic enzyme activity is possible. An additional benefit is the likely increase in muscle buffering capacity, as previously reported[11,24] in horses undergoing training which included some high intensity exercise. The horses in the current study showed no adverse effects on the musculoskeletal system during training, indicating that it is possible for horses to tolerate substantial increases on the currently accepted amounts of exercise. However, training was undertaken on a treadmill, which with a consistent surface and no bends, may not impose the same musculoskeletal stresses as a racetrack. It is not possible to determine whether there would have been any substantial benefits to performance from the increase in anaerobic enzyme activity. However, the standardized exercise test performed at intensities at or above

$\dot{V}O_2$max, resulted in a decrease in muscle LA following the high intensity training. When these findings are translated into possible advantages on the racetrack, a lower muscle LA and higher muscle pH may be found after racing. If a decrease in muscle pH has an effect in limiting performance by enzyme inhibition,[5] then a large component of high intensity training could be advantageous if improved buffering capacity limits the decrease in muscle pH. A possible mechanism for the decrease in muscle LA in the current study could be that lactate became utilised as a substrate in muscle, the increase in LDH being due to an increase in the heart isoenzymes resulting in conversion of lactate to pyruvate. While the possibility exists that the decrease in La was due to enhanced oxygen delivery to muscle, the increase in $\dot{V}O_2$max in these same horses[9] was maximal after 7 weeks training and showed no further change with time. The values for $\dot{V}O_2$max at 0, 7 and 12 weeks of training were respectively, 130 ± 3, 160 ± 11 and 150 ± 5 ml kg^{-1} min^{-1} (Evans and Rose, unpublished data). A possible increase in muscle buffering capacity[11,24] could partially explain the higher post-exercise muscle pH, together with the lower muscle La.

The other metabolic responses to exercise were similar to those reported previously,[23] although there was no change in muscle AMP. While decreases in ATP in muscle have been reported after single bouts of track exercise,[14] the exercise intensity in the current study did not approach maximum intensity. Our aim was to exercise the horses at or above an intensity equivalent to $\dot{V}O_2$max, which was confirmed by concurrent measurements of $\dot{V}O_2$max during training. Because the $\dot{V}O_2$max increased by around 23% with training,[9] the relative exercise intensity after training would have been less than that before training. This could also explain the decrease in muscle temperature after 12 weeks training which would be likely to have a beneficial effect on the horses' abilities to withstand fatigue.

While an increase in percentage of type IIa fibres is a well recognised adaptation to training in horses,[15,22] the decrease in type I fibres was an unexpected finding. Where an increase in the percentage of type IIA fibres has been found in response to training, the increase has usually been at the expense of the type IIb fibres.[15,21] In studies where shorter training periods were investigated[7] or where less intense training regimens were adopted,[20] no changes in the proportions of type I, IIa or IIb fibres were found. While the possibility exists that muscle fibre type transformation from type I to type II has occurred in response to the high intensity training, this seems unlikely. The only previous study in which a change in the proportion of type I fibres occurred during training was in the study of Henckel,[15] where an increase in type I fibres was found after 6 months of moderate intensity training in Standardbred horses. Given the large variation in the proportion of type I fibres that may occur with repeated sampling from the same site,[17] the decrease may simply represent sample variation. Because the proportion of type I fibres increases with depth in the m. gluteus medius[29] it is possible that despite attempts to keep our biopsy technique constant, biopsies at 12 weeks could have been collected from more superficial areas of the muscle.

In conclusion, this study has shown that increases in anaerobic enzyme activity are possible in response to high intensity interval training in Thoroughbred racehorses. Whether this provides any benefit in terms of improved performance and resistance to fatigue awaits further investigation. Possible benefits have to be offset against the possibly greater potential for musculoskeletal injuries.

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