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Effect of Training Intensity and Detraining on Adaptations in Different Skeletal Muscles

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ABSTRACT. This study examined the effects of two different submaximal exercise loads on the nature and magnitude of training adaptations in muscle, for training over a constant distance. Ten Thoroughbred horses underwent 6 weeks of treadmill training followed by 6 weeks of detraining. The horses exercised either at 40% $\dot{V}O_2$max (slow group) or at 80% $\dot{V}O_2$max (fast group), for two 1 500 m exercise bouts, 6 days per week, on a treadmill set at a 6° slope. Prior to training and at two weekly intervals during training and detraining, resting muscle biopsies were taken from m. gluteus medius and m. biceps femoris. Fibre type composition (ST, FTH, FT) was assessed histochemically. Buffering capacity was measured on frozen muscle and freeze dried samples were analysed for citrate synthase (CS), 3 hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH). There were no changes in fibre type proportions in the slow group, but in the fast group, there was an increase in the ratio of FTH/FT fibres in m. gluteus medius. The activities of enzymes in both muscles were largely unaltered by training or detraining. However, muscle buffering capacity increased by 8% during training in the fast group and decreased during detraining. We conclude that a relatively short period of training with a constant exercise load results in few adaptations in skeletal muscle. To achieve an optimal training effect, the degree of exercise load at submaximal intensities may not be the critical factor, but rather, an increasing exercise intensity may be necessary.

Key words. Fibre types; enzymes; buffering; horses; oxidative; submaximal exercise.

INTRODUCTION

Studies in both Standardbred[8,9,12,14,24] and Thoroughbred horses[1,5,15,18–20,27] have described various histochemical and biochemical changes in muscle associated with training. While these studies have provided important descriptive information, little is known about specific effects of factors such as training intensity and duration.

The aim of this study was to investigate the time-course of skeletal muscle adaptations to training of differing intensities, in horses undergoing a 6 week treadmill training programme, and the effect of a subsequent 6 week detraining period on any training induced changes.

MATERIALS AND METHODS

Ten Thoroughbred horses ranging in age from 2 to 9 years, that had rested for 4 months prior to the study, underwent 6 weeks of training, followed by 6 weeks of detraining. Horses exercised over a total distance of 3 000 m per day (1 500 m morning and afternoon), on a treadmill (Beltalong, Euroa, Australia), set at a 6° slope. The horses were divided into two groups: a slow group exercised at 40% of maximum oxygen uptake ($\dot{V}O_2$max), and a fast group at 80% $\dot{V}O_2$max.[16] This was equivalent to speeds of approximately 3–5 m s^{-1} and 7–9 m s^{-1}, respectively. During detraining, the horses were confined to small yards. Immediately

prior to training and at two weekly intervals throughout training and detraining, the horses underwent a standardised exercise test and resting muscle biopsies.

The exercise test, conducted on the treadmill set at a 6° slope, consisted of 3 min warm up at 4 m s^{-1}, followed by 90 s at 6 and 10 m s^{-1} and 60 s at 11 m s^{-1} and 12 m s^{-1}. Not all horses completed the test, which was terminated when the horse could no longer keep pace with the speed of the treadmill. $\dot{V}O_2$max was determined on each occasion throughout training[16] and exercise speed adjusted accordingly, so that the horses continued to exercise at the same relative intensity, despite any changes in $\dot{V}O_2$max. Muscle biopsies were collected bilaterally from m. gluteus medius and m. biceps femoris according to the technique of Bergström,[2] as modified by Lindholm and Piehl.[17] An approximately 1 cm^2 area of skin 17 cm caudodorsal to the more dorsal projection of the tuber coxae (for m. gluteus medius), and at the level of the third trochanter, 5 cm cranial to the caudal muscle border (for m. biceps femoris) was aseptically prepared. M. gluteus medius was biopsied at a depth of 8 cm and m. biceps femoris at a depth of 5 cm. With multiple biopsies being taken from the same site, careful control was necessary to minimise between biopsy variation. In all cases, following skin preparation, it was possible to identify the earlier biopsy site, and this facilitated restriction of the site for needle insertion to a 2 cm^2 area. The muscles were biopsied at a constant depth, as indicated by depth markers on the biopsy needles. Immediately following collection, the muscle sample was placed on aluminium foil and, within 30 seconds of collection, a portion was frozen in liquid nitrogen for biochemical determinations. Samples, mounted on cork blocks for histochemistry, were frozen in isopentane, cooled in liquid nitrogen.

For histochemical examination 10 µm transverse serial sections were cut and mounted onto 1 oz. (22 mm × 22 mm) coverslips. Muscle sections were stained for myosin ATPase using a method based on the original technique of Brooke and Kaiser.[3] Serial sections were stained for NADH-TR.[21] Fibre type composition for m. biceps femoris and m. gluteus medius was determined using a combination of the myosin ATPase (pH 10.3) and NADH-TR stains. Fibres were classified as slow-twitch (ST), fast twitch-high oxidative (FTH) or fast twitch-low oxidative (FT).[17]

Following freeze drying, dissection and weighing of muscle, activities of three enzymes were measured: citrate synthase (CS) (E.C. 4.1.3.7) and 3-hydroxyacyl CoA dehydrogenase (HAD) (E.C. 1.1.1.35), as markers of end-terminal oxidative capacity (TCA cycle) and beta-oxidation, respectively, and lactate dehydrogenase (LDH) (E.C. 1.1.1.27) as an anaerobic marker. All assays were based on the methods of Essén-Gustavsson and Henriksson.[7] Enzyme activities were determined by observation of changes in NADH equivalents *in vitro* (25°C), using fluorimetry. Enzyme activity was calculated in units of µmol NADH converted min^{-1} mg^{-1} freeze-dried muscle. Muscle pH was determined in frozen muscle based on the method described by Sahlin, Harris, Nylind, and Hultman[23] as modified by McCutcheon et al.[19] Buffering capacity (Slykes) was measured as the number of moles of H^+ required per gram of tissue, to produce a change in pH from 6.0–7.0 or 7.0–6.0, at 25°C.

All biochemical measurements were based on muscle samples obtained from the left side, as were most histochemical measurements. In a few cases samples from the right side were used for histochemistry; previous workers have reported no significant differences in histochemical data obtained from contralateral muscles.[8]

RESULTS

Enzyme analyses

Changes in the activities of CS, LDH, and HAD during training and detraining, are presented in Figs. 1 and 2.

M. gluteus medius. There were no significant changes in enzyme activities as a result

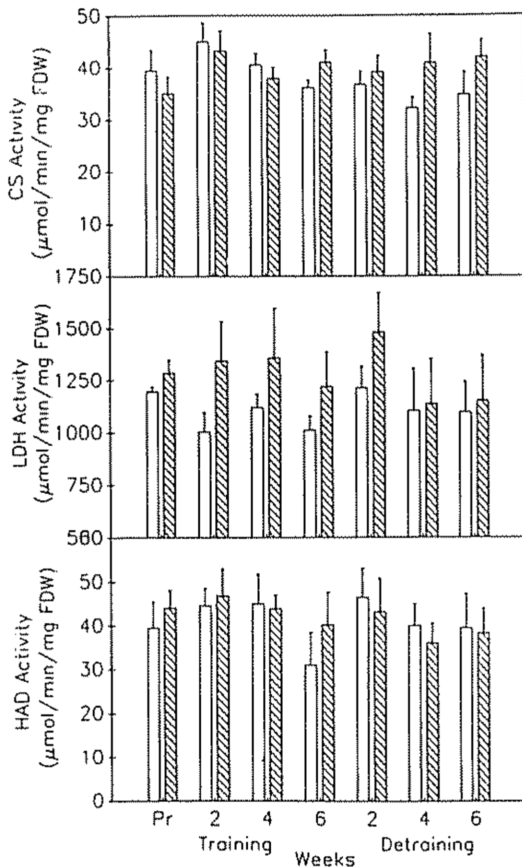

Fig. 1. Changes in the activities of citrate synthase (CS), lactate dehydrogenase (LDH) and 3-hydroxyacyl CoA dehydrogenase (HAD), (mean ± SEM), for m. gluteus medius, throughout training and detraining. □, Slow group; ▨, fast group.

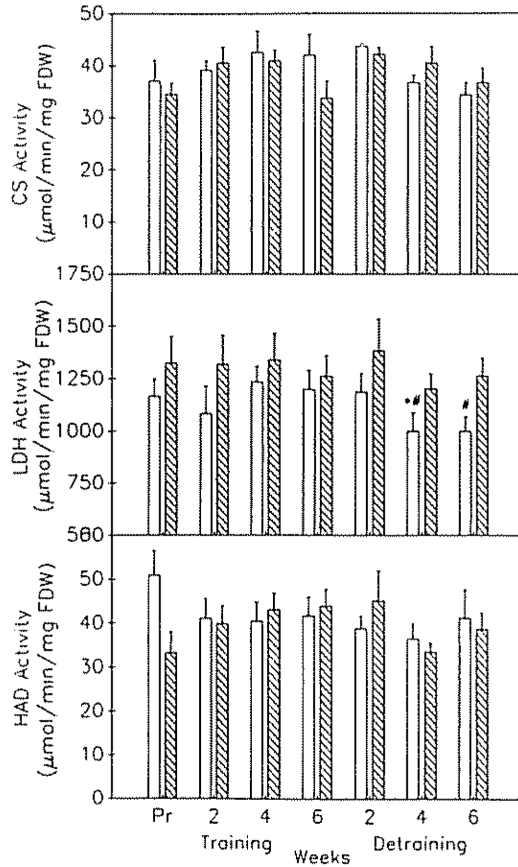

Fig. 2. Changes in the activities of citrate synthase (CS), lactate dehydrogenase (LDH) and 3-hydroxyacyl CoA dehydrogenase (HAD), (mean ± SEM), for m. biceps femoris, throughout training and detraining. □, Slow group; ▨, fast group. * Significantly different ($p<0.05$) from enzyme activity pre-training; # significantly different ($p<0.05$) from enzyme activity at week 6 (end training).

of training or detraining for either training group.

M. biceps femoris. In the fast group there were no significant changes in enzyme activities as a result of training or detraining. In the slow group there was an effect of detraining with LDH values after 4 weeks of detraining being lower ($p<0.05$) than both pretraining, end of training values and values after 2 weeks of detraining (decrease of 16.7%, 20.3%, and 18.7% respectively). After 6 weeks of detraining, enzyme activities were significantly decreased ($p<0.05$) below end of training and 2 weeks detraining values (19.6% and 18%, respectively). A comparison of week 0 (pre-training) values for m. gluteus medius and m. biceps femoris revealed no significant differences in the activities of CS, LDH, or HAD.

Muscle buffering capacity

Mean values (± SEM) for muscle buffering capacity measured in biopsies obtained from the m. gluteus medius after 0, 2, 4, and 6 weeks training, and 2 and 6 weeks detrain-

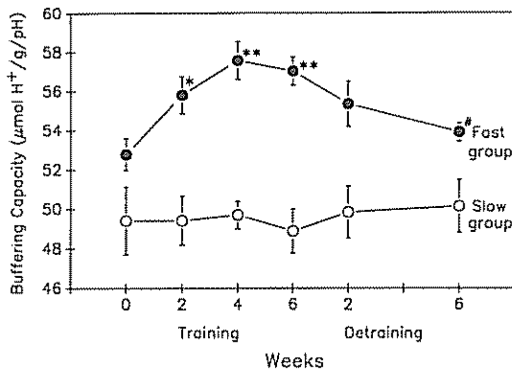

Fig. 3 Muscle buffering capacity, measured in m. gluteus medius biopsies, during training and detraining (mean ± SEM). * $p<0.05$, ** $p<0.01$ = significantly different from buffering capacity pre-training. # $p<0.05$ = significantly different from buffering capacity at week 6 (end training).

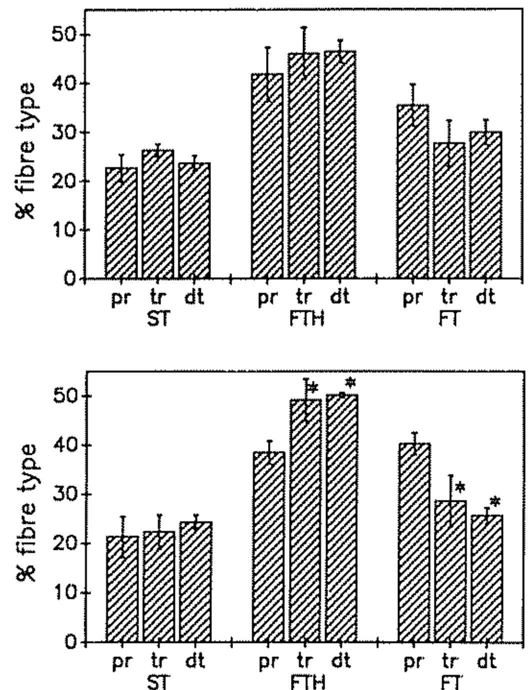

Fig. 4 Proportions of slow twitch (ST), fast twitch–high oxidative (FTH) and fast twitch–low oxidative (FT) fibres for m. gluteus medius, expressed as a percentage of the total fibres counted, pre-training (pr), after 6 weeks of training (tr) and after 6 weeks of detraining (dt) (mean ± SEM). (*a*) Slow group; (*b*) fast group. * Significantly different ($p<0.05$) from pre-training value.

ing, are presented in Fig. 3. In the fast group, buffering capacity increased by 8%, from a mean value of 52.78±0.82 slykes (µmol H^+ g^{-1} pH^{-1}) to 57.02±0.72 slykes (mean ± SEM) following 6 weeks of training. The value after 2 weeks of training was significantly higher ($p<0.05$) than the week 0 (pre-training value) as were the values after 4 weeks and 6 weeks (end) of training ($p<0.001$). In addition, buffering capacity in the fast group after 6 weeks of detraining was significantly lower ($p<0.05$), than the end of training value.

Buffering capacity in the slow group was unaffected by training or detraining. There was no significant difference in week 0 (pre-training) buffering capacity for the two groups.

Fibre type composition

Mean percentage values (± SEM) pre-training, after 6 weeks of training, and after 6 weeks of detraining for ST, FTH, and FT fibres are given in Figs. 4 and 5.

M. gluteus medius. In the fast group there was a 27.8% increase ($p<0.05$) in the proportion of FTH fibres accompanied by 28.9% decrease in the proportion of FT fibres ($p<0.05$) as a result of training. Proportions of FTH and FT fibres were still significantly different ($p<0.05$) to pre-training values, after 6 weeks of detraining (increased by 30.3% and decreased by 36.3%, respectively). In addition, the ratio of FTH to FT fibres in the fast group was significantly higher than the pre-training value ($p<0.05$) in samples obtained after 6 weeks of training (114.8% increase) and following 6 weeks of detraining (107.3% increase). The proportion of ST fibres was not altered by training or detraining. In the slow group the proportions of ST, FTH, and FT fibres, and the FTH/FT ratio were unaffected by training and detraining.

M. biceps femoris. There were no significant changes in fibre type composition in m. biceps femoris in either group as a result of training or detraining.

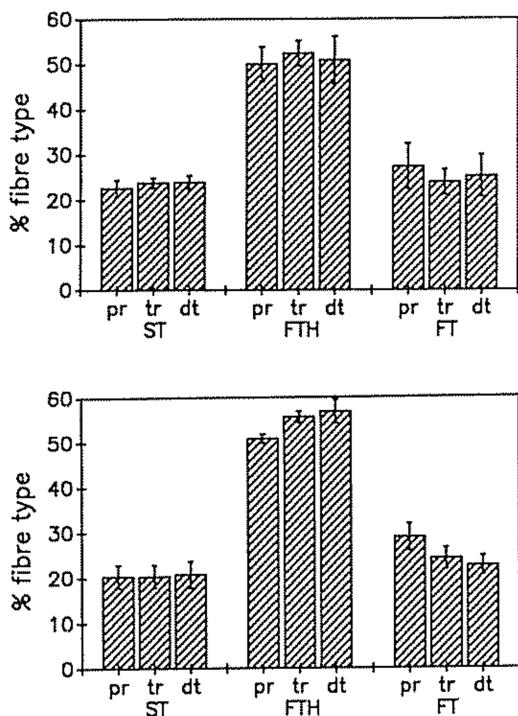

Fig. 5 Proportions of slow twitch (ST), fast twitch–high oxidative (FTH) and fast twitch–low oxidative (FT) fibres for m. biceps femoris, expressed as a percentage of the total fibres counted, pre-training (pr), after 6 weeks of training (tr) and after 6 weeks of detraining (dt) (mean ± SEM) (*a*) Slow group; (*b*) fast group.

Comparison of pretraining fibre composition. Mean pre-training percentages (± SEM) for ST fibres were not significantly different for the two muscles studied: GM-22.0±2.4%, BF-21.5±1.6%. However, prior to training, the mean percentage of FTH fibres (± SEM) in m. biceps femoris (50.4±2.1%) was significantly higher ($p<0.01$) than that in m. gluteus medius (40.1±2.9%) and the mean percentage of FT fibres (± SEM) for m. biceps femoris (28.1±3.0%) was significantly lower ($p<0.05$) than that for m. gluteus medius (37.8±2.4%). This meant that the ratio of FTH to FT fibres (mean ± SEM) for m. biceps femoris (1.99±0.22) was significantly greater ($p<0.005$) than that for m. gluteus medius (1.14±0.15).

DISCUSSION

This study was designed to examine the interaction between training intensity and duration. We compared two submaximal exercise intensities over a constant distance, to determine whether muscle adapts in a similar manner to that in conventional training, where there are progressive increases in exercise intensity. While the m. gluteus medius has commonly been used in evaluating muscular responses to exercise and training, the m. biceps femoris was also sampled in this study. Recent studies have shown that the distribution of fibre types in equine muscle is not homogeneous, there being an increase in the proportion of ST fibres with increasing depth. Van der Hoven et al.[26] reported little variation in muscle fibre composition of m. biceps femoris, compared to m. gluteus medius. In the current study, precisely defined biopsy sites were maintained, and the muscles sampled at constant depths, in order to minimize the methodological error associated with the needle biospy technique. Despite the limitations of fibre classification schemes dependent upon histochemical assessment of oxidative capacity, the classification into ST, FTH and FT fibres has been commonly used as a simple, qualitative means of assessing any changes in oxidative capacity that occur with training. In the current study, the increase in the proportion of FTH fibres and the decrease in FT fibres, with an accompanying increase in the FTH/FT ratio for m. gluteus medius of the fast group indicates an increase in oxidative capacity of the muscle in response to training. However, this was not accompanied by an increased activity of oxidative enzymes (CS, HAD) despite an increase in $\dot{V}O_2$max.[16] In common with results of other studies,[13,20,27] the training-induced increase in the proportion of highly oxidative fibres persisted throughout detraining.

These findings support work reporting a discrepancy between oxidative capacity as assessed by histochemical staining compared to biochemical assessment of whole muscle

oxidative capacity (enzyme assays).[25] Assessment of oxidative capacity from the NADH-TR stain is very subjective and visual assessment of increases in staining intensity may be difficult, particularly in Thoroughbreds, which even before training, tend to possess a majority of highly oxidative fibres.[14] In the current study, enzyme activity determinations were performed using whole muscle homogenates, therefore changes in oxidative capacity of a small number of fibres may be masked. This may also contribute to the discrepancy between results for histochemical and biochemical indicators of oxidative capacity.

While there was a statistically significant increase in the proportion of FTH fibres in m. gluteus medius of the fast group in the current study, this was not the case for m. biceps femoris. As the proportion of FTH fibres in m. biceps femoris was significantly higher than that for m. gluteus medius prior to training, visual appraisal of any increase in staining intensity was more difficult to assess. The failure of training to result in a statistically significant increase in the proportion of FTH fibres for the slow training group is likely to be attributable to the short duration and low intensity of the training stimulus.

The training undertaken by both groups of horses in the current study was predominantly aerobic. A number of previous studies of horses have reported significantly increased levels of oxidative enzymes in response to predominantly aerobic training of 5 weeks to 12 months duration.[1,5,8,11–15,18–20,22] In the current study there were no changes in the activities of CS or HAD. However, all of the previous studies involved training of increasing intensity. In this study for both training groups, training intensity was maintained at the initial level, in terms of percentage of $\dot{V}O_2max$, for the entire 6 week period. It has been demonstrated in rats[6] that if the intensity and/or duration of exercise is increased above an initial level, limited increases in oxidative enzyme activities occur, related to the change in activity, but the enzyme activity plateaus if the training stimulus remains constant. Hodgson et al.[14] in view of results of a 7 week submaximal training programme using Standardbreds, noted that a similar mechanism may occur in horses. In Standardbred horses undergoing a 12 week interval training programme involving walking and trotting exercise with a draught load, at a constant exercise intensity throughout training, a significant increase in CS activity occurred after 2 weeks of training, but with no further increases.[10] While the duration and intensity of training for both groups in the current study may have been insufficient for any initial exercise-induced increase in oxidative enzyme activities, horses in the fast group did demonstrate a trend towards an increase in mean CS activity following 2 weeks of training that was greater than that seen for horses in the slow training group.

Most equine studies have evaluated results of training that involved predominantly aerobic work and have reported no change in the activity of LDH,[13,15,18,20] similar to the findings in the present study.

The pre-training values for buffering capacity in the current study were similar to those reported in Thoroughbreds[19] and in other terrestrial mammals.[4] The significant increase in buffering capacity in association with training in horses in the fast group in the current study is consistent with the results reporting a 60% increase in buffering capacity of m. gluteus medius following 7 weeks of more intense training.[19]

Buffering capacity in m. gluteus medius of horses in the slow training group was unaltered by training. Previous work in humans and horses has indicated that muscle buffering capacity is significantly enhanced by high intensity (predominantly anaerobic) training,[19] but not by endurance work. No previous studies have examined the effect of detraining on muscle buffering capacity. In view of our results, it appears that in the absence of a training stimulus, muscle buffering capacity previously elevated by training, rapidly returns to resting levels.

Although there was a large difference in the intensity of exercise undertaken by the two groups in this study, few adaptations in muscle were found. However, there were sufficient differences in terms of muscle buffering capacity and FTS/FT ratio, to indicate that training intensity was important. At submaximal intensities, exercise duration and intensity may interact, as although the slow group trained at half the intensity of the fast group, the exercise duration was about twice as long.

The relatively small changes in skeletal muscle in the current study, where a constant exercise load was used, contrast with the more substantial changes found in a training study of similar duration,[8] but with an increasing exercise intensity. It appears that a graded increase in exercise load is necessary to produce major adaptations in muscle.

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