

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

A. K. SINHA, S. P. RAY and R. J. ROSE

*Department of Veterinary Clinical Sciences, University of Sydney, NSW 2006, Australia*

**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%  $\dot{V}O_2\text{max}$  (slow group) or at 80%  $\dot{V}O_2\text{max}$  (fast group), for two 1500 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<sup>8,9,12,14,24</sup> and Thoroughbred horses<sup>1,5,15,18–20,27</sup> 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 3000 m per day (1500 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 ( $\dot{V}O_2\text{max}$ ), and a fast group at 80%  $\dot{V}O_2\text{max}$ .<sup>16</sup> This was equivalent to speeds of approximately 3–5 m s<sup>-1</sup> and 7–9 m s<sup>-1</sup>, 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<sup>-1</sup>, followed by 90 s at 6 and 10 m s<sup>-1</sup> and 60 s at 11 m s<sup>-1</sup> and 12 m s<sup>-1</sup>. 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<sup>16</sup> 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,<sup>2</sup> as modified by Lindholm and Piehl.<sup>17</sup> An approximately 1 cm<sup>2</sup> 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<sup>2</sup> 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.<sup>3</sup> Serial sections were stained for NADH-TR.<sup>21</sup> 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).<sup>17</sup>

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.<sup>7</sup> 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<sup>-1</sup> mg<sup>-1</sup> freeze-dried muscle. Muscle pH was determined in frozen muscle based on the method described by Sahlin, Harris, Nylind, and Hultman<sup>23</sup> as modified by McCutcheon et al.<sup>19</sup> Buffering capacity (Slykes) was measured as the number of moles of H<sup>+</sup> 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.<sup>8</sup>

## 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

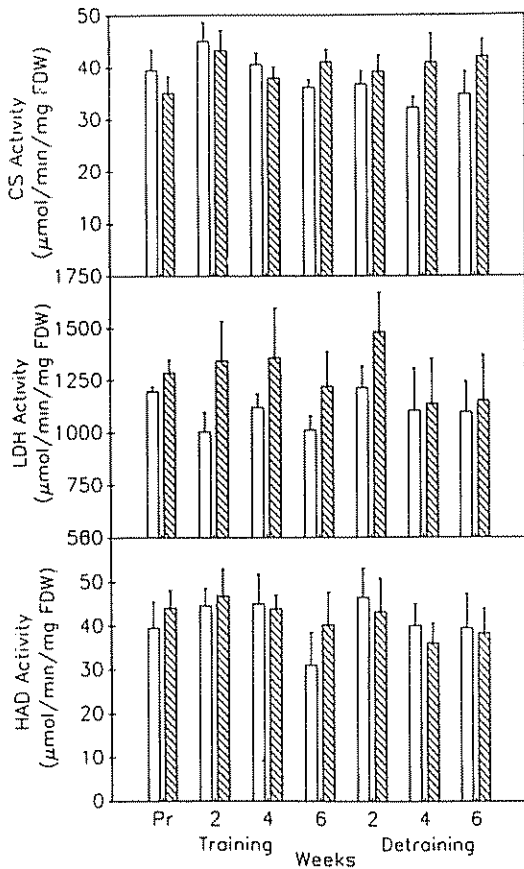


Fig. 1. Changes in the activities of citrate synthase (CS), lactate dehydrogenase (LDH) and 3-hydroxyacyl CoA dehydrogenase (HAD), (mean  $\pm$  SEM), for *m. gluteus medius*, throughout training and detraining.  $\square$ , Slow group;  $\square$  (hatched), fast group.

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 val-

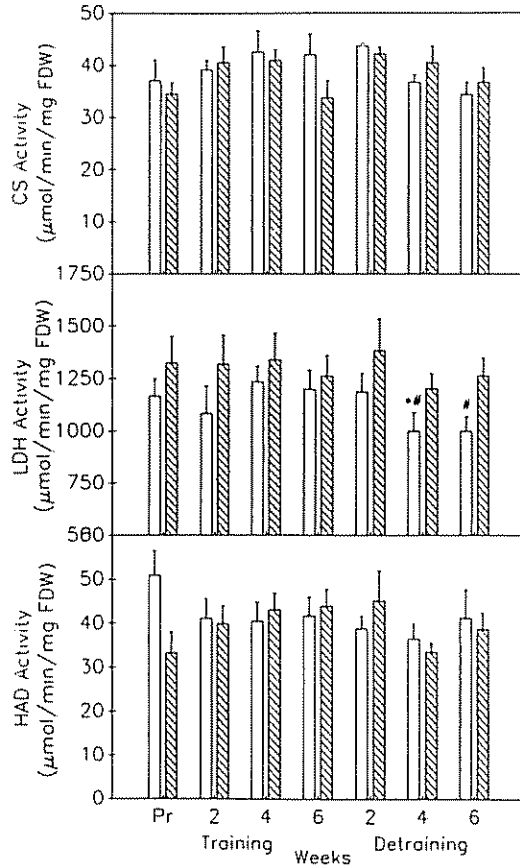


Fig. 2. Changes in the activities of citrate synthase (CS), lactate dehydrogenase (LDH) and 3-hydroxyacyl CoA dehydrogenase (HAD), (mean  $\pm$  SEM), for *m. biceps femoris*, throughout training and detraining.  $\square$ , Slow group;  $\square$  (hatched), fast group. \* Significantly different (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

ues (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 ( $\pm$  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 detraining-

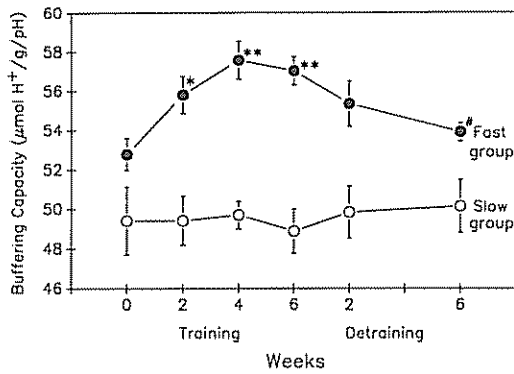


Fig 3 Muscle buffering capacity, measured in m. gluteus medius biopsies, during training and detraining (mean  $\pm$  SEM). \*  $p < 0.05$ , \*\*  $p < 0.01$  = significantly different from buffering capacity pre-training. #  $p < 0.05$  = significantly different from buffering capacity at week 6 (end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

ing, are presented in Fig. 3. In the fast group, buffering capacity increased by 8%, from a mean value of  $52.78 \pm 0.82$  slykes ( $\mu\text{mol H}^+ \text{g}^{-1} \text{pH}^{-1}$ ) to  $57.02 \pm 0.72$  slykes (mean  $\pm$  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 ( $\pm$  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 signifi-

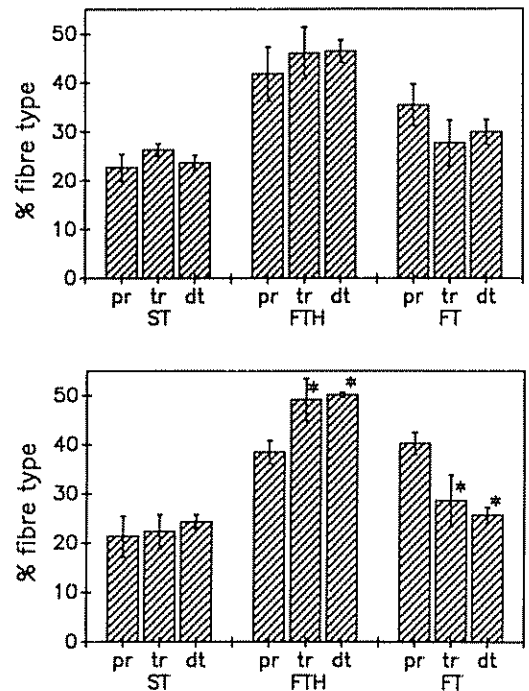


Fig 4 Proportions of slow twitch (ST), fast twitch-high oxidative (FTH) and fast twitch-low oxidative (FT) fibres for m. gluteus medius, expressed as a percentage of the total fibres counted, pre-training (pr), after 6 weeks of training (tr) and after 6 weeks of detraining (dt) (mean  $\pm$  SEM). (a)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

cantly 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.

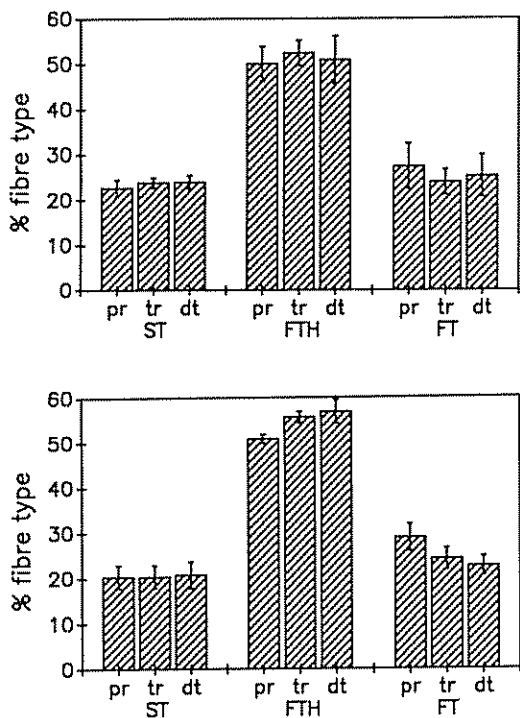


Fig. 5 Proportions of slow twitch (ST), fast twitch-high oxidative (FTH) and fast twitch-low oxidative (FT) fibres for m. biceps femoris, expressed as a percentage of the total fibres counted, pre-training (pr), after 6 weeks of training (tr) and after 6 weeks of detraining (dt) (mean  $\pm$  SEM) (a)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

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

## 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.<sup>26</sup> 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 biopsy 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  $\dot{V}O_2\max$ .<sup>16</sup> In common with results of other studies,<sup>13,20,27</sup> 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).<sup>25</sup> 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.<sup>14</sup> 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.<sup>1, 5, 8, 11–15, 18–20, 22</sup> 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  $\text{VO}_2\text{max}$ , for the entire 6 week period. It has been demonstrated in rats<sup>6</sup> 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.<sup>14</sup> 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.<sup>10</sup> 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,<sup>13, 15, 18, 20</sup> 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<sup>19</sup> and in other terrestrial mammals.<sup>4</sup> 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.<sup>19</sup>

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,<sup>19</sup> 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,<sup>8</sup> 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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#### REFERENCES

1. Bayly, W. M., Foreman, J. H., Reidy, M. A., Matoba, H., Grant, B. D. and Gollnick, P. D. (1983) Histochemical studies of equine skeletal muscle and their relationship to training and fitness. *In Proceedings 8th Equine Nutrition and Physiology Symposium*, pp. 221–226.
2. Bergström, J. (1962) Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest., Suppl.* 68, 7–101.
3. Brooke, M. M. and Kaiser, K. K. (1970) Muscle fiber types: How many and what kind? *Arch. Neurol.* 23, 369–379.
4. Castellini, M. and Somero, G. N. (1981) Buffering capacity of vertebrate muscle: Correlations with potentials for anaerobic function. *J. Comp. Physiol.* 143, 191–198.
5. Cutmore, C. M., Snow, D. H. and Newsholme, E. A. (1985) Activities of key enzymes of aerobic and anaerobic metabolism in middle gluteal muscle from trained and detrained horses. *Equine Vet. J.* 17, 354–356.
6. Dudley, G. A., Abraham, W. M. and Terjung, R. L. (1982) Influence of exercise intensity and duration on biochemical adaptations in skeletal muscle. *J. Appl. Physiol.: Respir. Environ. Exercise Physiol.* 53, 844–850.
7. Essén-Gustavsson, B. and Henriksson, J. (1984) Enzyme levels in pools of microdissected human muscle fibres of identified type. *Acta Physiol. Scand.* 120, 505–515.
8. Essén-Gustavsson, B., McMiken, D., Karlström, K., Lindholm, A., Persson, S. and Thornton, J. (1989) Muscular adaptations of horses during intensive training and detraining. *Equine Vet. J.* 21, 27–33.
9. Fox, G., Henckel, P., Juell, C., Falk-Ronne, J. and Saltin, B. (1987) Skeletal muscle buffering capacity changes in Standardbred horses: effects of growth and training. *In Gillespie, J. R. and Robinson, N. E. (eds): Equine Exercise Physiology 2*. ICEEP Publications, Davis, CA, pp. 341–347.
10. Gottlieb-Vedi, M. (1988) Responses to draught work of varying intensity and duration in Standardbred horses. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.
11. Guy, P. S. and Snow, D. H. (1977) The effect of training and detraining on muscle composition in the horse. *J. Physiol.* 269, 33–51.
12. Henckel, P. (1983) Training and growth induced changes in the middle gluteal muscle of young Standardbred trotters. *Equine Vet. J.* 15, 134–140.
13. Hodgson, D. R. and Rose, R. J. (1987) Effects of a nine-month endurance training program on muscle composition in the horse. *Vet. Rec.* 121, 271–274.
14. Hodgson, D. R., Rose, R. J., DiMauro, J. and Allen, J. R. (1985) Effects of a submaximal treadmill training programme on histochemical properties, enzyme activities and glycogen utilisation of skeletal muscle in the horse. *Equine Vet. J.* 17, 300–305.
15. Hodgson, D. R., Rose, R. J., DiMauro, J. and Allen, J. R. (1986) Effects of training on muscle composition in horses. *Am. J. Vet. Res.* 47, 12–15.
16. Knight, P. K., Sinha, A. K. and Rose, R. J. (1990) Effects of training intensity on maximum oxygen uptake. *In Persson, S. G. B., Lindholm, A. and Jeffcott, L. B. (eds): Equine Exercise Physiology 3*, ICEEP Publications, Davis, CA, pp. 77–82.
17. Lindholm, A. and Piehl, K. (1974) Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of Standardbred horses. *Acta. Vet. Scand.* 15, 287–309.
18. Lindholm, A., Essén-Gustavsson, B., McMiken, D., Persson, S. and Thornton, J. R. (1983) Muscle histochemistry and biochemistry of Thoroughbred horses during growth and training. *In Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds): Equine Exercise Physiology*. Granta Editions, Cambridge, pp. 211–217.
19. McCutcheon, L. J., Kelso, T. B., Bertocci, L. A.,

- Hodgson, D. R., Bayly, W. M. and Gollnick, P. D. (1987). Buffering and aerobic capacity in equine muscle: variation and effect of training. In Gillespie, J. R. and Robinson, N. E. (eds.): *Equine Exercise Physiology 2*. ICEEP Publications, Davis, CA, pp 348-357
- 20 Nimmo, M. A., Snow, D. H. and Munro, C. D. (1982). Effects of nandrolone phenylpropionate in the horse. 3 Skeletal muscle composition in the exercising animal. *Equine Vet J* 14, 229-233
- 21 Novikoff, A. B., Shin, W.-Y. and Drucker, J. (1961). Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. *J. Biophys. Biochem. Cytol.* 9, 47-61.
- 22 Snow, D. H. and Guy, P. S. (1979). The effects of training and detraining on several enzymes in horse skeletal muscle. *Arch. Int. Physiol. Biochem.* 87, 87-93
- 23 Sahlin, K., Harris, R. C., Nylind, B. and Hultman, E. (1976). Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflügers Arch.* 367, 143-149.
- 24 Taylor, A. W. and Brassard, L. (1981). Skeletal muscle fiber distribution and area in trained and stalled Standardbred horses. *Can. J. Anim. Sci.* 61, 601-605
- 25 Valberg, S., Essén-Gustavsson, B. and Skoglund-Walberg, H. (1988). Oxidative capacity of skeletal muscle fibres in racehorses: histochemical versus biochemical analysis. *Equine Vet J.* 20, 291-295
- 26 Van der Hoven, R., Wensing, T., Breukink, H. J., Meijer, A. E. and Kruij, T. A. (1985). Variation of fiber types in the triceps brachii, longissimus dorsi, gluteus medius, and biceps femoris of horses. *Am. J. Vet. Res.* 46, 939-941.
- 27 Wilson, R. G., Thornton, J. R., Inglis, S. and Ainscow, J. (1987). Skeletal muscle adaptation in racehorses following high intensity interval training. In Gillespie, J. R. and Robinson, N. E. (eds.): *Equine Exercise Physiology 2*. ICEEP Publications, Davis, CA, pp 367-375