

## The Use of Morphometry and Enzyme Activity Measurements in Skeletal Muscles for the Assessment of the Working Capacity of Horses

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### Summary

*Enzyme activity measurements, histochemistry and an ultrastructural morphometric analysis were carried out on the semitendinosus muscle of two groups of Halfbred stallions before and after training lasting four weeks. The aim of the study was to find out whether the different methods of analyzing skeletal muscle tissue would allow an objective assessment of the working capacity and of the ability of the horses to be trained. The specific training for 'Novice eventing' consisted of three intervals of three minutes duration with maximal alactic work load twice weekly. There were significant increases in the volume density of mitochondria (VMT) and in the activities of glyceraldehyde 3-phosphate dehydrogenase and malate-dehydrogenase in the muscle samples taken after the training period. A significant negative correlation was found between the increase of VMT in the semitendinosus muscle and the decrease in plasma lactate after a standardized three-minute workload. The morphometry of the mitochondrial compartment of skeletal muscles and the measurement of enzymes of the aerobic pathway were considered to be reasonably sensitive methods to allow some assessment of the aerobic working capacity in horses.*

### Introduction

The evaluation of the performance capacity and the ability of horses to be trained is rather difficult, as changes of blood parameters during and after certain workloads may be the results of both aerobic and anaerobic conditions in the working skeletal muscles. Performances of more than two minutes duration mainly depend on the maximal oxygen uptake capacity ( $VO_{2max}$ ) of the organism which is determined by its overall capacity for oxygen transport and its potential for oxidative phosphorylation in skeletal muscles.  $VO_{2max}$  is generally recognized to be the best global descriptor of aerobic performance. As it is not possible at present to measure  $VO_{2max}$  directly under field conditions in the horse, biochemical parameters of the pathway of energy as well as quantitative morphology of

skeletal muscles have been used to indirectly assess the aerobic working capacity. The aim of this study was to clarify whether biochemical and stereological methods of analyzing skeletal muscle tissue comply with more conventional physiological parameters of the aerobic working capacity, such as plasma lactate after defined workloads.

### Materials and Methods

**Animals:** Six 4- to 5-year-old Halfbred stallions of the 'Bundeshengstestallamt Stadl Paura' in Austria were examined. Before the study, all the animals were kept under identical conditions.

**Tests:** The stallions were tested with an incremental workload test before (test A) and after (test B) a four-week training period. The tests were carried out as follows:

1. Phase I: distance 1650 m; speed 350–450 m/min.
2. Eight minutes walk.
3. Phase II: distance 1650 m; speed 450–500 m/min.
4. Eight minutes walk.
5. Phase III: distance 1650 m; speed 550–600 m/min.

Speed and plasma lactate were measured after each workload and plotted as a curve (Fig. 1). The following performance parameters were obtained from this graph (Fig. 1):

La 550 = plasma lactate concentrate after a three-minute workload at a speed of 550 m/min.

V4 = speed that produces a plasma lactate concentration of 4 mmol/l during a three-minute workload.

**Training schedule:** The training consisted of three submaximal workloads (V4) of three minutes duration twice weekly and normal two-hour exercise sessions on the other days.

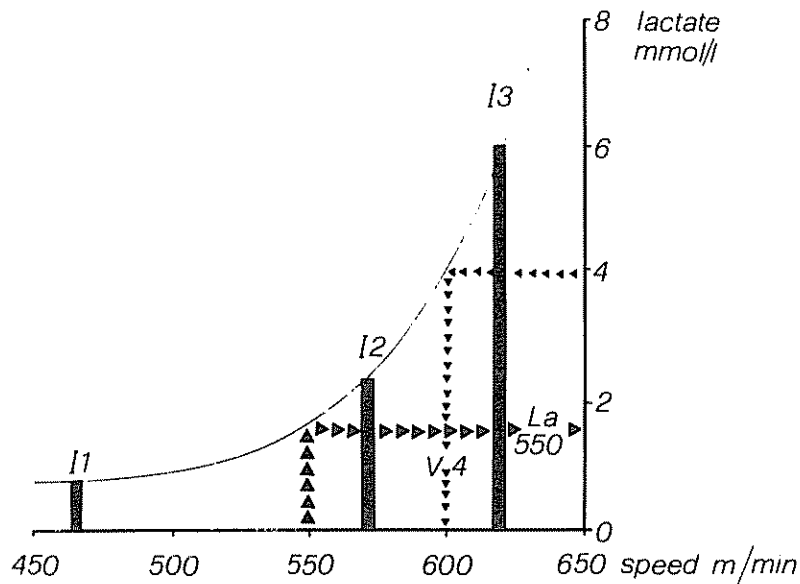


FIGURE 1. Diagram as used to determine V4 and La 550 (see text) from data obtained in the incremental workload test (curve manually fitted).

TABLE 1. Data from all horses before and after a four-week training period, ranked according to their V4, La 550, VMT and enzyme activities (units: V4, m/min; La 550, mmol/l; VMT, %; MDH, HAD and GAPDH, U/mg).

V4		La 550		VMT		MDH		HAD ( $\times 10^{-3}$ )		GAPDH	
horse no.	abs.	horse no.	abs.	horse no.	abs.	horse no.	abs.	horse no.	abs.	horse no.	abs.
	%		%		%		%		%		%
1	573	1	3.1	1	4.2	2	4.0	2	28.0	5	10.7
6	553	6	3.8	4	4.0	4	3.5	4	24.3	2	8.8
3	545	3	4.3	3	3.2	5	3.2	1	19.9	4	8.0
2	535	2	5.3	6	2.7	1	3.1	5	16.0	3	7.5
4	514	4	5.8	2	2.7	6	2.5	6	15.5	1	6.1
5	472	5	8.4	5	2.0	3	2.3	3	13.0	6	3.7
Activity changes between test A and test B											
5	+64	+13	-3.7	-44	+3.9	+144	+4.2	+168	+19.9	+128	+4.1
2	+48	+9	-3.1	-58	+2.7	+135	+0.9	+29	+10.2	+51	+2.2
6	+43	+7	-1.8	-37	+2.5	+92	+0.6	+17	-2.6	-16	+1.7
4	+36	+7	-1.4	-37	+1.1	+34	+0.5	+15	-3.6	-28	+1.4
1	0	0	0	0	+0.6	+15	+0.4	+17	-6.3	-26	+0.9
3	0	0	+0.1	0	-0.8	-19	-0.4	-10	-12.1	-43	-0.8
Test B											
6	596	2	2.2	6	6.6	6	6.7	6	35.4	2	11.0
2	583	6	2.4	2	5.2	4	4.1	1	30.1	5	9.9
1	573	1	3.2	5	4.7	1	4.0	4	18.0	4	9.4
4	550	4	4.0	4	4.6	5	3.7	2	15.9	3	8.4
3	542	3	4.3	3	4.3	2	3.6	5	13.4	1	7.8
5	536	5	4.7	1	3.4	3	2.7	3	9.4	6	7.8

Plasma lactate: The plasma lactate concentration was determined by Monotest (Boehringer), a modification of the method by Noll (1974).

Muscle biopsy and tissue preparation: Muscle samples were taken by needle biopsy as described by Bergström (1975) and modified by Straub (1981). For conventional EM morphometry the biopsy material was processed as indicated by Hoppeler *et al.* (1973).

Processing for morphometry of histochemically identified single muscle fibres was carried out as indicated by Hoppeler *et al.* (1983).

Morphometry: The volume of mitochondria per unit volume of muscle fibre, VMT, was calculated according to standard stereological procedures as indicated by Weibel (1979, Chapters 2 and 6).

Enzyme activities: The muscle tissue was homogenized according to Gysin (1982). Activities of the following enzymes were determined: glyceraldehyde 3-phosphate dehydrogenase, GAPDH, (E.C. 1.2.1.12) was determined according to Velick and Furfine (1963) and Wu and Racker (1963), malate dehydrogenase, MDH, (E.C. 1.1.1.37) according to Wolfe and Neilands (1956) and Kun (1963), and  $\beta$ -hydroxyacyl CoA dehydrogenase, HAD, (E.C. 1.1.1.35) according to Lynen and Wieland (1955). The specific activities were referred to the activity of 1 mg protein in the supernatant buffer solution of the homogenate after centrifugation. The protein concentration was determined according to Hartree (1973).

### Results

The performance values (V4 and La 550), the total volume of mitochondria (VMT) and the enzyme activity estimates (MDH, HAD and GAPDH) both before and after the training period are reported in Table 1. Also reported are the absolute and relative changes of all parameters measured. As the individual data are ranked for each parameter, Table 1 shows how the performance parameters compare to structural and biochemical variables measured in the skeletal muscle tissue. The ranking of the horses with regard to VMT is remarkably close to the ranking obtained either by V4 or La 550. Considerable differences in ranking are observed when animals are ranked according to enzyme activity estimates as compared to performance parameters. A significant negative correlation ( $r = -0.720$ ) was found between VMT and La 550 after the training period. The relationship between VMT and La 550 is illustrated in Fig. 2. VMT in histochemically identified muscle fibres ( $n = 5$ ) of horses 2 and 6 is reported in Table 2. The volume of

TABLE 2: VMT and  $\Delta$  VMT (units: %) as determined in histochemically identified muscle fibres (the semitendinosus muscle) of two horses before (A) and after (B) a four-week training period. Each value represents the mean of the estimates from five individual fibres.

Horses	Fibre type	VMT A	VMT B	$\Delta$ VMT
Horse 2	I	6.9	7.5	+ 8.7
	IIA	6.7	8.5	+26.9
	IIB	2.9	2.2	-24.1
Horse 6	I	7.8	12.8	+64.1
	IIA	5.9	9.4	+59.3
	IIB	3.7	4.5	+21.6

lipids per unit volume of muscle fibre was found to be less than 0.05% in all animals analyzed before and after the training period with no significant changes occurring during the training.

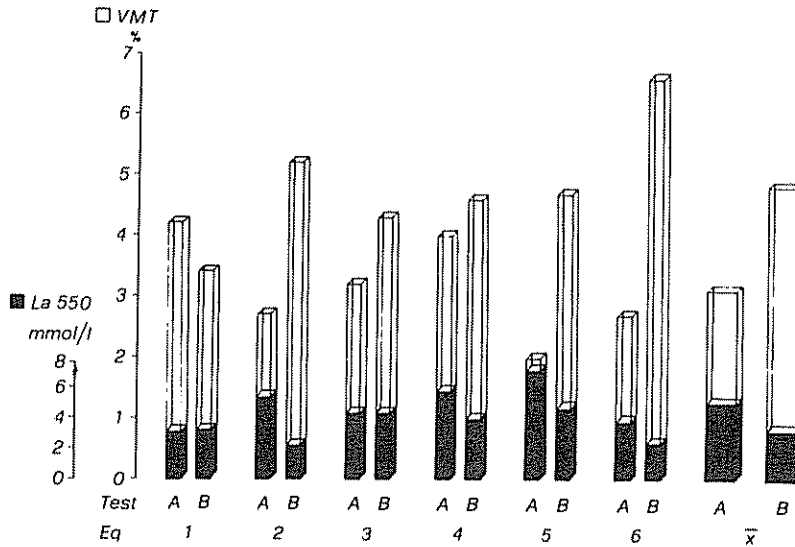


FIGURE 2. La 550 and volume density of mitochondria (VMT) in the semitendinosus muscle of six Halfbred stallions before (A) and after (B) a four-week training period.

### Discussion

Estimating plasma lactate concentration after standardized workloads is a useful indirect method for assessing aerobic capacity in horses. We therefore used the performance variables V4 and La 550 as reference parameters for comparison with the quantitative structural and biochemical analyses of the skeletal muscle tissue. The latter measurements were carried out in biopsies of the semitendinosus muscle because this muscle has a typical function in hind limb movement as well as being readily accessible for biopsies (Straub 1981).

Howald *et al.* (1975) showed that regular endurance exercise induces a considerable increase in the volume density of mitochondria and lipids in humans. Straub *et al.* (1975) showed that mitochondrial densities are higher in trained racehorses than in untrained ones. In the present follow-up study, six horses were tested before and after a four-week training period. Except for one horse, all horses showed remarkable increases in VMT during the relatively short training period (67% mean relative increase). The investigation also confirmed earlier findings of Straub *et al.* (1975) that there is almost no intracellular fat within equine skeletal muscle fibres. The intracellular fat deposits were also not changed significantly by our training protocol. This finding might indicate that the oxidation of intracellular fat stores is of less importance in our horses than in man. As the glycogen content of equine skeletal muscles is comparatively high (Lindholm *et al.*, 1974; Snow and Guy, 1979), it could be assumed that glycolysis is the main metabolic pathway for fuelling mitochondria in the horses analyzed in this study.

We measured the mitochondrial content in histochemically identified muscle fibres before and after the training period in two horses. With regard to fibre type, VMT reacted quite differently to the training stimulus in each horse (Table 2). It is obvious that we cannot draw any final conclusions as to how the different fibre types adapt to the particular training scheme used in this study, although the use of such an approach on a broader base seems promising.

GAPDH is localized in the cytoplasm of muscle cells. During training its activity increased in all stallions except for horse 5. According to Moesch and Howald (1975), endurance exercise in man induces an increase of HAD rather than of GAPDH. In this study we found a decrease of HAD in four out of six horses. These findings are in contrast to data reported by Snow and Guy (1979) who found HAD to be increased by some 100% after 15 weeks of training in six horses. Smaller increases in HAD, presumed to be due to exercise, are also reported by Essén *et al.* (1980) and Nimmo *et al.* (1982). In the studies where adaptations of HAD are reported, the training was carried out for much longer periods of time than in the present investigation. The discrepancy between the findings in horses and man with regard to training-induced changes of enzyme activities, when similar training periods are compared, might support the hypothesis of the importance of glycolysis in horses as the main source of energy for muscle contraction in the experimental situation of the present study. This aspect of our study certainly deserves some scrutiny in further investigations. The mitochondrial enzyme, MDH, increased its activity in all but one horse. Similar training-induced increases of MDH activity were found in man (Moesch and Howald, 1975), in rats (Holloszy *et al.*, 1975) and in horses (Straub *et al.*, 1976).

The correlation between the performance parameters and VMT and, to a lesser extent, between the performance parameters and the enzyme activity estimates, makes us believe that morphometry of the mitochondrial compartment of skeletal muscles and the measurements of the activities of some enzymes of the aerobic pathway may be valuable indirect methods for assessing the aerobic working capacity in horses.

### **Acknowledgements**

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# Skeletal Muscle Characteristics of Young Standardbreds in Relation to Growth and Early Training

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## Summary

*Muscle biopsies were obtained from both the left and right middle gluteal and semitendinosus muscles of ten Standardbred colts six to eight months of age. All ten colts were raised at the same farm during the following year and five of them (T-group) were subjected to regular controlled exercise four to five days per week, while the only physical activity of the other five foals (C-group) consisted of that usually occurring in a group of yearlings. Muscle biopsies were again obtained from all ten yearlings at four and ten months after the first biopsies had been taken. No difference in either fibre composition (type I, IIA, IIB) or oxidative and glycolytic enzyme activities were found when the two groups were compared at the time when the biopsies were taken. However, large variations in muscle characteristics existed among the yearlings. The type I/type II ratio did not change with age, but type IIA/type IIB ratio increased in both groups. Fibre areas increased by 30 to 70% in all fibre types. The oxidative enzyme citrate synthase showed a significant increase with age only in the T-group, while 3-OH-acyl CoA dehydrogenase was unchanged in both groups. The glycolytic enzymes, triose phosphate dehydrogenase and lactate dehydrogenase, were significantly decreased (20–40%) in both groups. No significant training effect was detected in this study since the yearlings in the T-group that were physically activated demonstrated an almost similar adaptation in muscle to the yearlings in the C-group. This does not exclude the possibility of other training régimes influencing muscle characteristics. Furthermore, the potential for adaptation in skeletal muscle may depend on hereditary factors since large variations in muscle characteristics existed among the foals even prior to training.*

## Introduction

In an attempt to maximize performance capacity many horses are subjected to increased levels of physical activity by 1.5 to 2 years of age. Training régimes which improve performance in man and animals have been shown to induce changes in the cardiovascular system and also in the skeletal muscles involved in exercise. Where