THE EFFECT OF TRAINING AND DETRAINING ON LACTATE DEHYDROGENASE ISOENZYMES IN THE HORSE

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Summary - In the horse, total LDH activity increased with training and the H and M subunit activity parallelled this increase. It is suggested that these increases are in response to a stimulus from the type of training program utilised. The first half of a detraining program decreased the activity of the H and M subunits as might be expected. A sharp rise in the total LDH and the M subunit activity occurred during the latter half of the detraining program. This unexpected increase may be due to relatively more hypoxic conditions prevailing in the muscle during the detraining period.

INTRODUCTION: Lactate dehydrogenase (LDH, E.C.1.1.1.27) is a tetrameric molecule made up of 4 subunits from 2 parent subunits designated M (muscle specific) and H (heart specific) which are under separate genetic control (4). The subunits combine to form 5 isoenzymes arranged M_4, M_3H, M_2H_2, MH_3 and H_4 with the M form being maximally active with high concentrations of pyruvate whilst the H form is most active at low concentrations (4). It has been suggested that this may be a method by which the cell regulates the production of lactate (10).

In the horse (7), in contrast to other species (8, 13, 15), LDH activity in skeletal muscle was found to increase with training. As well as studying total LDH activity, isoenzyme patterns have also been investigated, and it has been shown that training produces an increase in percentage of H subunits. It was therefore decided to further investigate the changes we found in total LDH activity by examining LDH isoenzyme patterns in samples obtained at the time of our original investigation (7).

Abbreviations: LDH - lactate dehydrogenase
SR - sarcoplasmic reticulum

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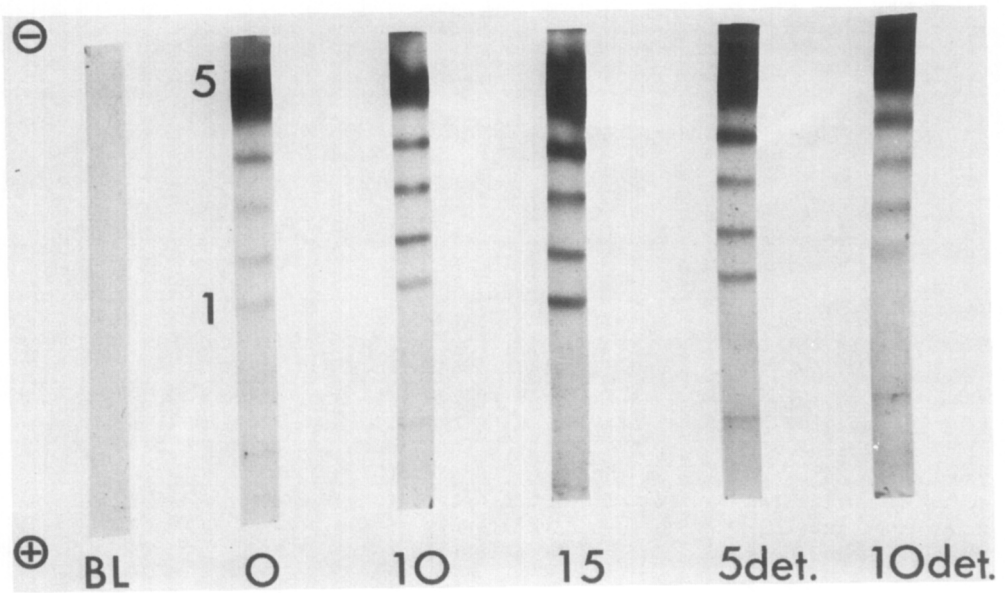

Fig (1) The effect of training and detraining on poly-acrylamide gel separations of LDH isoenzymes.
(BL = blank; 0, 10 and 15 = weeks of training; 5 det and 10 det = weeks of detraining).

MATERIALS AND METHODS: LDH isoenzyme studies were carried out on muscle samples obtained by percutaneous needle biopsy from the semi-tendinosus and middle gluteal muscles of 6 horses at 0, 10 and 15 weeks training and 5 and 10 weeks detraining. The training and detraining program used has already been described (7).

The total LDH activity was determined as described by Snow and Guy (14). Electrophoresis and staining of LDH isoenzymes was performed by a modification of the method of Dietz and Lubrano (5) as described by Anderson (1). Samples were prepared by diluting tissue homogenates in 0.1 M phosphate buffer pH 7.0 to activities around 500 umoles/ml/min and adding an equal volume of 40% sucrose.

The stain density can be used to evaluate the proportion of each isoenzyme, providing that all LDH isoenzymes have equal activity properties in the staining medium. Quantitation of the staining density of the separated LDH isoenzymes was performed densitometrically (Kipp and Zonen, Delft, Holland). The relative quantity of the H and M subunits in the different separations was calculated according to Thorling and Jensen (16).

Significance was determined by the "paired" t-test, $p < 0.05$ being considered significant.

RESULTS: Skeletal muscle LDH in this study was found to consist of 5 separate isoenzymes with by far the greatest activity being found in

TABLE 1

Effect of Training and Detraining on the % of LDH Isoenzymes in Horse Middle Gluteal Muscle [Mean ± S.E.M; 6 horses (4 horses week 15)]

	I	II	III	IV	V	M Subunits
0 weeks	0.90±0.14	1.83±0.37	4.20±0.88	7.70±2.00	85.37±2.12	93.68±0.96
10 weeks	1.76±0.43	3.13±0.91	6.82±1.62	9.27±1.18	79.02±3.50	90.33±1.99
15 weeks	2.51±0.71*	3.06±0.85	6.05±1.53	13.43±3.10	74.95±2.41	88.80±1.17*
5 weeks detraining	1.80±0.84	2.88±0.41	5.38±0.91	7.57±1.44	82.37±1.61	91.58±0.73
10 weeks detraining	0.92±0.19	2.10±0.47	5.30±0.46	8.90±2.66	82.78±2.48	92.65±0.71

* $p < 0.05$ vs 0 weeks

TABLE 2

Effect of Training and Detraining on the % of LDH Isoenzymes in Horse Semitendinosus Muscle [Mean ± S.E.M; 6 horses (4 horses week 15)]

	I	II	III	IV	V	M subunits
0 weeks	0.17±0.32	1.42±0.55	2.88±0.77	3.23±0.80	92.30±1.85	96.33±0.81
10 weeks	1.03±0.23*	2.75±0.77	6.68±1.43	9.08±1.42***	80.46±2.69***	91.30±1.11***
15 weeks	2.97±1.20**	4.58±1.16****	9.58±0.90*	11.85±1.35	73.02±2.62***	86.35±2.32***
5 weeks detraining	0.90±0.34	2.52±0.45	6.17±1.00**	8.51±1.43**	81.90±1.79**	92.00±0.91
10 weeks detraining	0.30±0.89	0.90±0.34	3.63±0.60	7.18±1.67	87.99±2.38	95.25±0.85

* $p < 0.05$ vs 0 weeks
** $p < 0.02$ " "
*** $p < 0.01$ " "
**** $p < 0.001$ " "

LDH_5, the most muscle specific isoenzyme (Fig 1). It was not always possible to detect densitometrically the activity of LDH_1. Training increased the percentage of $LDH_{1,2,3 \text{ and } 4}$ and decreased that of LDH_5 with a consequent increase in the percentage of H subunits (Tables 1 and 2). However, when activities of the subunits were examined, it

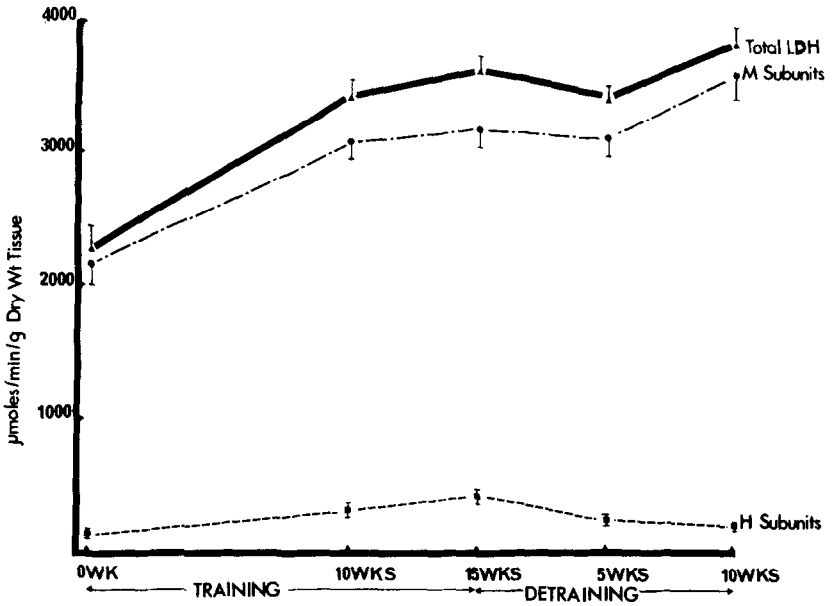

Fig (2) The effect of training and detraining on total LDH activity and the activity of the H and M subunits.

was found that the activity of both subunits increased, with the percentage increase being greater in the H subunit. As the changes in the activities of the H and M subunits and total LDH were similar for both muscles, the results have been combined as shown in Fig (2).

During the first 5 weeks of detraining, the activity of both subunits decreased, as did the percentage of H subunits compared to the M subunits. During the latter part of detraining, whilst the activity of the H subunit continued to decrease towards pre-training values, the activity of the M subunit and total LDH increased.

DISCUSSION: As in other studies, horse skeletal muscle was found to contain 5 multimolecular forms of LDH, with LDH_5 being present in the greatest amounts (1). The isoenzymes are found in definite regions of the cell - the isoenzymes of predominantly H subunits are found on the inner mitochondrial membrane whilst those of predominantly M subunits are located in the SR (2). Total LDH activity increases as the

percentage of fast twitch fibres in a muscle increases (7,9) and similarly, it has been shown that the H to M ratio decreases as the proportion of fast twitch fibres increases. The ratio of H to M found in our horses (approximately 0.1) is similar to that of human sprinters(13) and in both studies the proportion of fast twitch fibres was approximately 85%. In sprinters, the low proportion of H to M was attributed to the fact that there was a low proportion of high oxidative to fast contractile fibres and it has been suggested (12) that the isoenzyme pattern was determined by both the metabolic and contractile properties of the muscle fibres. It appears however that the contractile properties are more important in determining the isoenzymes composition as, in the horse over 60% of muscle fibres are high oxidative (7) and only a small percentage of H subunits are found, and similarly in the guinea pig the red lateral vastus is composed of almost 100% fast contractile high oxidative fibres (11) and only 24% of the subunits are H.

Previous reports have indicated that total LDH activity decreases with training and that the proportion of H subunits increase (8,13,14). In contrast, in this study, as well as having an increase in proportion of H subunits, total LDH activity increased markedly. As the alterations in the proportion of H and M subunits, with training do not describe the specific changes in activity of th H and M subunits, the effect of training in terms of total H and M subunit activity was also examined in this study and calculated from the data in other studies (Table 3). Due to the great variation in these activities between species, due as much to differing assay methods as possible species variation, changes in total subunit activity have been expressed as a percentage of the pre-training levels. In all cases except the horse, a decrease in M subunit activity occurred with generally an increase in H subunit activity. It has been suggested that the decrease in the amount of

TABLE 3
The Effect of Training on the Activity of the LDH M and H Subunits in Various Species (% of the activity of the Untrained Value)

	Man (13)		Pig (8)		Rat (17)		Horse	
	H	M	H	M	H	M	H	M
Untrained	100	100	100	100	100	100	100	100
Trained	155	55	133	71	88	56	216	148

M subunit is the reason for the decrease in total LDH activity (17).

The increase in H subunits with submaximal type training has been ascribed to a change in skeletal muscle metabolism towards that of cardiac muscle, i.e. a greatly increased oxidative capacity, which favours synthesis of the H subunit (17). As the H subunit is located on the inside of the mitochondrial membrane (2) it is not surprising that this increases, as it is known that the number of mitochondria and mitochondrial cristae increase with training (6). The reason for the species difference in the change in M subunit activities is not readily explicable but may be related to the intensity of exercise used in the various studies. Reductions in M subunit activity have generally been associated with endurance exercise programs. (8, 13, 17) which lead to an increased oxidative ability and a subsequent decrease in the requirement for LDH_5. A program with a large portion of anaerobic work might be expected to maintain or possibly increase the requirement for LDH_5. In contrast to our findings, anaerobic exercise in man was reported to have had no effect on the total LDH activity or the isoenzyme content (13). It may be, the type of program utilised in our study was a greater stimulus for the synthesis of both forms.

During the first half of the detraining program a decrease in activity of H and M subunits towards pre-training values occurred, as would be expected on the removal of the stimulus, in this case, exercise. The increase in total LDH activity, in the second stage of the detraining period, was due to an increase in M subunit activity, as H subunit activity was still tending to decrease. The reason for this possible resynthesis of M subunits is difficult to explain, but may be due to the introduction of a relatively hypoxic environment in the muscle due to lack of exercise. Hypoxic conditions have been reported to lead to an increased production of M subunits (4).

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