Chronic exercise training alters kinetic properties of rat skeletal muscle and myocardial lactate dehydrogenase

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10 weeks of treadmill running dramatically altered the kinetic properties of lactate dehydrogenase (EC 1.1.1.27) in skeletal muscle and myocardium in the rat. These changes, including V_{max}, K_m and optimal concentrations for pyruvate and lactate, indicate that exercise training induces the formation of H-isozyme in skeletal muscle and M-isozyme in the heart and represent metabolic adaptations of these tissues to chronic aerobic exercise.

Exercise training *Lactate dehydrogenase* *Kinetic property* *Isoenzyme* *(Skeletal muscle, Myocardium)*

1. INTRODUCTION

The isoenzyme patterns of lactate dehydrogenase (LDH, EC 1.1.1.27) are well known [1–4]. The H-isozyme, which predominates in the heart musculature of most vertebrates, and the M-isozyme, which predominates in many skeletal muscles, differ in biochemical and biophysical properties including amino acid composition, electrophoretic mobility and the relative rate of reaction with coenzyme NAD.

Numerous studies have been devoted to the influence of environmental and metabolic changes on the genetic expression of the two LDH isozymes. Exposure to high altitude, for example, has been reported to increase the H-subunits of LDH in skeletal muscle [5–7] whereas M-subunits were increased in heart associated with hypoxia [8], pulmonary stenosis [9], and anemia [10].

Chronic physical exercise has been used widely as a model to reveal the functional adaptation of LDH to metabolic stress. Kaplan and co-workers [11] studied the LDH isozyme forms in the breast muscle of 40 species of birds and demonstrated that the predominance of M-subunit in muscle was negatively correlated to the bird's habitual activity. Studies of LDH isoenzyme patterns in different muscle phenotypes also supported the hypothesis that the increase in synthesis of H-subunits in skeletal muscle is a result of chronic aerobic activity, e.g. in the slow-twitch red fibers [12]. However, Mole et al. [13] reported no change in the LDH isoenzyme patterns in rat skeletal muscle after 12 weeks of treadmill running. Thus, although most investigators agree that training decreases LDH total activity in skeletal muscle and increases LDH total activity in the heart [13–15], results concerning its isoenzyme pattern change in response to training are controversial. Recently, a study by Pette and co-workers [16], using chronic electric stimulation of rabbit skeletal muscle, showed that the proportion of M-subunits decreased whereas the H-subunits increased progressively with the duration of stimulation. Meanwhile, polyadenylated RNA translatable in vitro coding for M- and H-LDH was also decreased or increased, correspondingly. These data have provided strong evidence that even a relatively short period of increased physical activity is sufficient to elicit a shift of LDH genetic patterns.

Here, we studied the influence of 10 weeks of treadmill running on the kinetic properties of skeletal muscle and heart LDH in the rat.

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2. MATERIALS AND METHODS

The skeletal muscle and heart tissues in this experiment were derived from male Sprague-Dawley rats (age 2 months, body wt 250–275 g) randomly divided into trained and control groups. The details of animal care and training programs were essentially the same as described in [17]. After 10 weeks of training, trained rats were killed by decapitation 60 h after their last training sessions whereas control rats were killed at the same time of the day. The exsanguinated animal's abominal cavity was cut open and the heart was excised. One half of the heart, cut along the vertical axis, was immediately frozen in plates pre-cooled with liquid nitrogen and stored at −160°C. The entire musculature of one hindlimb was excised, freed of apparent fat and connective tissues and isolated in an ice-cold medium containing 10 mM Hepes buffer (pH 7.4). Muscle homogenization was performed in a Polytron (PT-10, setting 4) at 0°C. The homogenate was strained through a layer of medicine gauze to remove intramuscular fat connective tissues and centrifuged at 700 × *g* for 5 min. The supernatant was frozen at −30°C for LDH assays. The heart tissue was thawed and homogenized with a glass homogenizer in an ice-cold medium containing 10 mM Hepes, 2 mM EDTA (pH 7.4). This homogenate was centrifuged in an Eppendorf centrifuge at 10000 × *g* for 30 s and the supernatant was removed immediately before LDH assay.

The methods of Bergmeyer and Bernt [18] and Markert and Ursprung [19] were applied for the forward and reverse LDH reaction, respectively, in order to determine maximal enzyme activities (V_{max}) and Michaelis constants (K_m). Assays were carried out at 25°C.

Paired Student's *t*-test was used to analyze the data. α level was set at 0.05.

3. RESULTS AND DISCUSSION

Skeletal muscle and myocardial LDH activities in the trained and control rats at various concentrations of pyruvate or lactate as substrate are shown in figs 1 and 2, respectively. V_{max}, K_m and the optimal substrate concentrations are listed in table 1. Kinetic curves of LDH from untrained rat

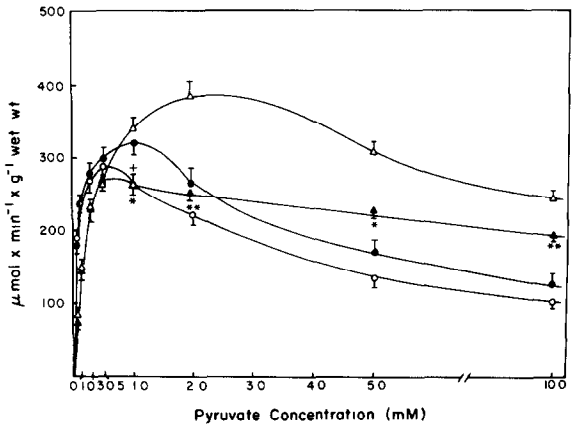

Fig.1. Effect of training on lactate dehydrogenase activity at various concentrations of pyruvate in skeletal muscle and heart. (△) Muscle LDH, control; (▲) muscle LDH, trained; (○) heart LDH, control; (●) heart LDH, trained. Initial reaction velocity is expressed as μmol/min per g wet tissue. Pyruvate concentrations in the assay system were 0.05, 0.1, 0.3, 0.5, 1.0, 2.0, 5.0, and 10.0 mM. Final NADH concentration was 0.2 mM. Assay was carried out in phosphate buffer, 50 mM, pH 7.5, at 25°C. Each point represents mean (± SE) from 9 animals. $*P<0.01$; $**P<0.001$, M-LDH; $^{+}P<0.05$, H-LDH; trained vs control.

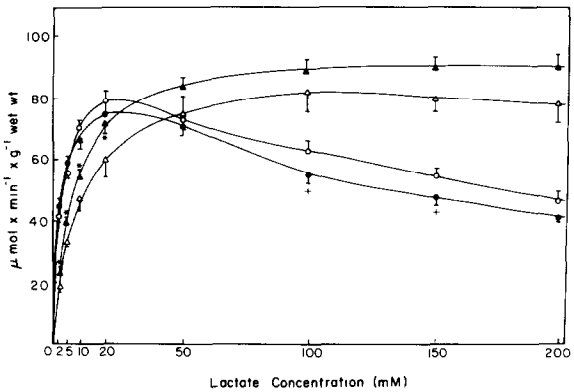

Fig.2. Effect of training on lactate dehydrogenase activity at various concentrations of lactate in muscle and heart. (△) Muscle LDH, control; (▲) muscle LDH, trained; (○) heart LDH, control; (●) heart LDH, trained. Initial reaction velocity is expressed as μmol/min per g wet tissue. Lactate concentrations in the assay system were 2, 5, 10, 20, 50, 100, 150, and 200 mM. Final NAD concentration was 0.66 mM. Assay was carried out in Tris-HCl buffer, 10 mM, pH 9.0, at 25°C. Each point represents mean (± SE) from 9 animals. $*P<0.05$, M-LDH; $^{+}P<0.05$, H-LDH; trained vs control.

Table 1

Kinetic properties of rat skeletal muscle and heart lactate dehydrogenase

Substrate	Heart		Muscle	
	Control	Trained	Control	Trained
V_{max} (μmol/min per g wet tissue)[a]				
Pyruvate	371	297	297	322
Lactate	82	91	81	77
K_m (mM)*				
Pyruvate	0.15	0.10	0.058	0.062
Lactate	7.1	6.0	2.0	1.5
Optimal concentration (mM)				
Pyruvate	2.0	0.7	0.5	1.0
Lactate	100	200	30	30–40

[a] Obtained from Lineweaver-Burk plots of the mean (9 experiments) enzyme activities at 8 different substrate concentrations

skeletal muscle and heart were typical of those for LDH M- and H-isozymes reported by Kaplan and Everse [4] and Battellino et al. [20]. The skeletal muscle LDH (M-LDH) assayed in the present experiment was obtained from mixed types of skeletal muscle, therefore it was a hybrid of both M- and H-subunits [4] and displayed kinetic properties characteristic of these two isozymes.

The important finding of the present investigation was that the kinetic patterns of rat LDH isozymes could be altered by a relatively short period of physical training. In the untrained rats, M-LDH maximal activity was reached at a pyruvate concentration of 2 mM. However, for the trained rats pyruvate concentration beyond 0.7 mM inhibited the enzyme, which is characteristic of heart LDH (H-LDH). Furthermore, maximal M-LDH activity in the trained rat was decreased by approx. 20%. As a result, the K_m (pyruvate) for M-LDH was shifted from 0.15 to 0.10 mM (table 1).

Although it has been reported by many authors that total LDH activity in skeletal muscle [13–16] and heart [14,20] decreases or increases, respectively, with training, this is the first demonstration that in addition to V_{max}, both K_m and optimal substrate concentration were changed by training. The reason that Mole et al. [13] failed to find any significant difference in the ratio of LDH activities at 0.33–10 mM pyruvate between the trained and control rats is probably because the assay method used, originally suggested by Wilson et al. [11], was not sensitive enough to detect the relatively small shift in kinetic properties with training. In the study by Wilson et al. [11], different ratios of LDH activity at low to high pyruvate concentration were found in various species of birds and this might be reflective of functional adaptations to habitual activity over the evolutionary course. Our data were also consistent with the findings of Seedorf et al. [16] that after 50 days of electric stimulation, the proportion of M-subunits in rabbit fast-twitch muscle descreased from 91 to 50% whereas H-subunits increased from 9 to 50%.

With lactate as substrate (fig.2), the M-LDH activity was significantly increased with training, especially at lactate concentrations below 25 mM, which is within the physiological range. V_{max} and K_m were also increased or decreased, respectively. The increased LDH activity for the reverse reaction, together with the decreased LDH activity for the forward reaction, accommodates metabolism to aerobic pyruvate utilization during strenuous exercise, which represents a well-known training adaptation in skeletal muscle.

In the heart, the effect of training on LDH kinetic properties was less dramatic than in skeletal muscle. Optimal pyruvate concentration for H-LDH was 0.5 mM for the untrained rats and higher concentrations inhibited the enzyme. This is typical of the H-LDH kinetics [4,20]. However, for the trained rat optimal pyruvate concentration was 1.0 mM. K_m for pyruvate was not changed substantially but there was a moderate shift of K_m for lactate from 2.0 mM in the control to 1.5 mM in the trained. York et al. [21] reported that there was a significant increase in M-subunits of LDH in myocardium of trained rats. Our data seem to be in agreement with their findings. It is conceivable that trained heart is more capable of utilizing lactate as a fuel, as indicated by a decreased K_m for lactate in the H-LDH after training. Nevertheless, the relatively small change in the H-LDH kinetics after training compared with skeletal muscle also suggests that heart has sufficient metabolic reserve to cope with the increased energy demand associated with physical exercise.

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