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Adaptations in lactate dehydrogenase and its isozymes in aging mammalian myocardium: interaction of exercise and temperature

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Abstract

The responses of the left and right ventricles (LV and RV) to physical conditioning in cold (25°C) and thermoneutral temperatures (35°C), with special reference to lactate dehydrogenase (LDH) and its isoenzyme profile, were studied in the 2-month (young)- and 12-month (middle-aged)-old rats. Moderate hypertrophy was a common observation irrespective of age, region and swim temperature. LV, however, hypertrophied to a significantly lesser extent in the middle-aged, than the RV. Blood Lactate (La) content showed a decline in the trained rather than their untrained counterparts. LDH activity decreased with age. Swim training induced elevations in the enzyme activity. The isoenzyme profile was suitably and efficiently altered in the LV and RV of trained animals to meet the arising O_2 demands. The above adaptations were best seen in the young and in the animals trained at thermoneutral temperatures. Thus it is suggested that young age is very apt for initiation of training programs although middle-age is not so late. Swimming in water near body temperature is emphasised as a more preferred environment to cold water, in order to derive maximal exercise-associated beneficial effects.

Keywords: Aging; Exercise; Hypertrophy; Isoenzymes; Lactate dehydrogenase; Myocardium; Temperature

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1. Introduction

Swim endurance training leads to an elevation in total lactic dehydrogenase (LDH) and H-LDH activity in the right ventricle (RV) of young as well as middle-aged rats (Anitha and Asha Devi, 1996). This response has proved beneficial since myocardial LDH activity has been reported to decline with age (Singh and Kanungo, 1968). The association of LDH as a key glycolytic enzyme in the myocardium and changes in its subunit composition under conditions of physical exercise cannot be exclusively investigated, due to its variations in the tissue (Fine et al., 1963; York et al., 1975; Murrell et al., 1993). The differential expression of multiplicity of LDH in the heart of swim trained rats can be complimented by studies dealing with regional variations and its association with swim temperature since swim endurance can be altered due to inability of animals to maintain body temperature (Dawson et al., 1968). Since the differences in the load sustained by left and right sides of the heart vary, the extent of adaptability can also differ with age (Canale et al., 1986). As the detrimental effects of aging appear first on the left side leading to occurrence of left side dysfunction and failure (Anversa et al., 1986), the possibility of ventricular remodelling in terms of better functional capacity can be anticipated through physical training.

In the present study, it was attempted to address the following: can swim temperature act as one of the major determinant in invoking metabolic adjustments in the myocardium during physical training? If the above is true, then the next question is, will the responses of left ventricle LV and RV vary with training? Finally, the relative intervention of age as a factor during physical conditioning is sought. The aforesaid possible metabolic changes through variations of myocardial LDH isoenzymes in the aging heart were examined.

2. Materials and methods

2.1. Chemicals

Acrylamide, bis, APS, NADH, NAD, NBT, PMS, sodium lactate, lithium lactate and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). All other biochemicals were of reagent grade. Albumin standard was obtained from Pierce (Rockford, IL).

2.2. Animal care and training program

Female pathogen free albino rats of Wistar strain were obtained from the Central Animal Facility (CAF, I.I.Sc, Bangalore, India). The animals thus obtained were of two age groups, 2 months (young) and 12 months (middle-aged). They were housed three per cage in polypropylene cages (13.5 in. $\times$ 9 in. $\times$ 7 in.). The animals were maintained at a temperature of 28.5 ± 1°C, relative humidity

of 77.5 ± 1% and on a 12 h light/12 h dark cycle with free access to tap water and standard commercial diet (Lipton India, Calcutta). During the investigation, animals of both age-groups were allocated into the following groups: (A) sedentary controls (SE-C); (B) trained swimmers at 25°C; (C) untrained swimmers at 25°C (U-25); (D) trained swimmers at 35°C (T-35); and (E) untrained swimmers at 35°C (U-35).

The training programme was the same as described elsewhere (Anitha and Asha Devi, 1996). Briefly, swim training was carried out in a glass tank (77 × 39 × 38 cm) and the rats exercised initially for 5 min/day with a progressive increase to 30 min/day for 35 days at 25°C (cold) and 35°C (thermoneutral). Untrained swimmers were allowed to swim at their respective temperatures, at a stretch for 30 min, 24 h prior to sacrifice while SE-C were restricted to cage activity. During training, careful observations were made for any symptoms of fatigue or pathological disorders. Under such circumstances, exercise was immediately terminated. Animals were sacrificed by etherization.

2.3. Tissue and blood sampling

The heart tissue was washed in ice-cold Tyrode's solution several times, trimmed of excess fat and freed of blood vessels. The regions of study viz, LV and RV were isolated in cold and diced in the same media. A 20% homogenate was prepared in ice-cold 0.25 M freshly prepared sucrose solution (Fine et al., 1963). Homogenization was carried out in a glass homogenizer fitted with a teflon pestle and the homogenate was centrifuged in a refrigerated centrifuge (Sorvall RC 5C) at 4°C for 20 min at 280 000 × *g*. The procedure was repeated thrice and the combined supernatants were used for LDH assay and electrophoretic separation of LDH isoenzymes. Blood was deproteinised using 5% TCA at 1:10 dilution.

2.4. Assays

Blood and tissue La was quantitated by the method of Barker and Summerson, (1965). Glucose and other interfering materials of the protein-free filtrates were removed and an aliquot of this was heated with H_2SO_4 to convert La to CH_3CHO which was then determined by reaction with p-hydroxyphenyl in the presence of copper ions. Lithium lactate was used as a standard.

LDH (E.C.1.1.1.27) activity was determined by the method of Cabaud et al., (1965). Na-Pyruvate, in the presence of $NADH_2$ was used as the substrate. 2,4-DNPH was added and the hydrazone formed was determined using a standard prepared with pyruvate buffer.

LDH isoenzymes were separated on mini polyacrylamide slab gels (Celis, 1964). Gels were scanned using a LKB laser ultro scan-XL which determined relative peak heights as percentage. Activities of individual isoenzymes were calculated in terms of percentage relative area.

2.5. Statistical methods

All data are represented as mean ± S.D. of three animals. Significance of variables were tested by applying student's t-test. Differences are considered statistically significant if the probability (P) value was less than 0.1% ($P < 0.001$). Changes in weekly body weights (BW) of SE-C and swim trained rats were analysed by Karl Pearson correlation coefficient (r).

3. Results

Growth curves for total BW are shown in Figs. 1 and 2 for the trained young and middle-aged, respectively. The changes in BW as compared to that before the initiation of the training program recorded a steady increase in all the groups of animals, the increase being maximum in SE-C. T-25 and T-35 of both age groups showed reduced BW as compared to SE-C. In the 2-month-old rat, T-25 showed a greater decrease ($r = +0.97$) in BW than T-35 ($r = +1.1$), while in the 12-month-old rat the trend reversed. Cardiac hypertrophy was evident in the trained groups as the heart weights (HW) of these animals were greater than their respective sedentary and untrained counterparts, though the magnitude varied with swim

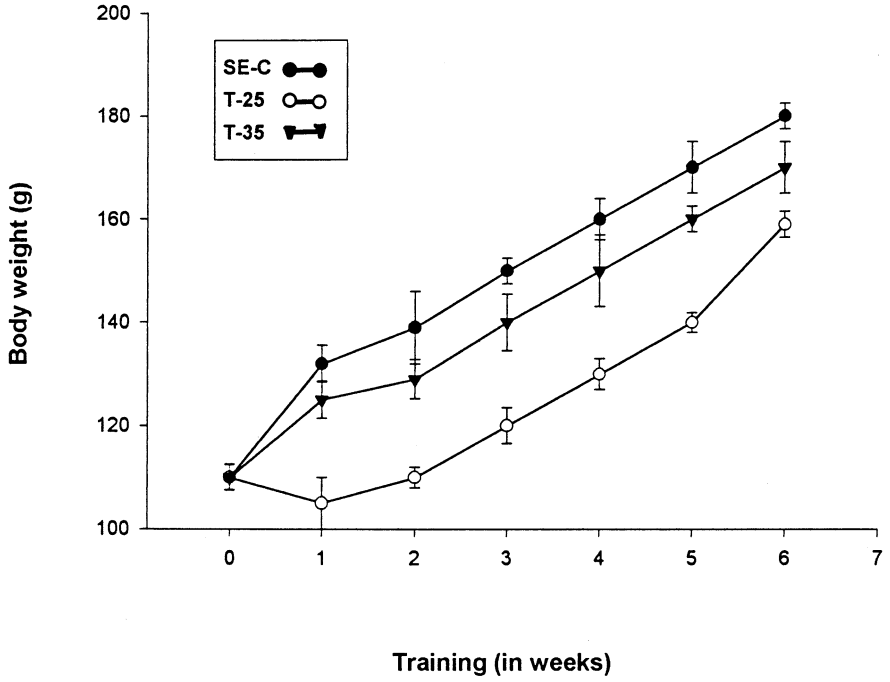

Fig. 1. Weekly variations in the body weights (g)of young sedentary (SE-C) and trained rats at 25°C (T-25) and 35°C (T-35). For more details see text.

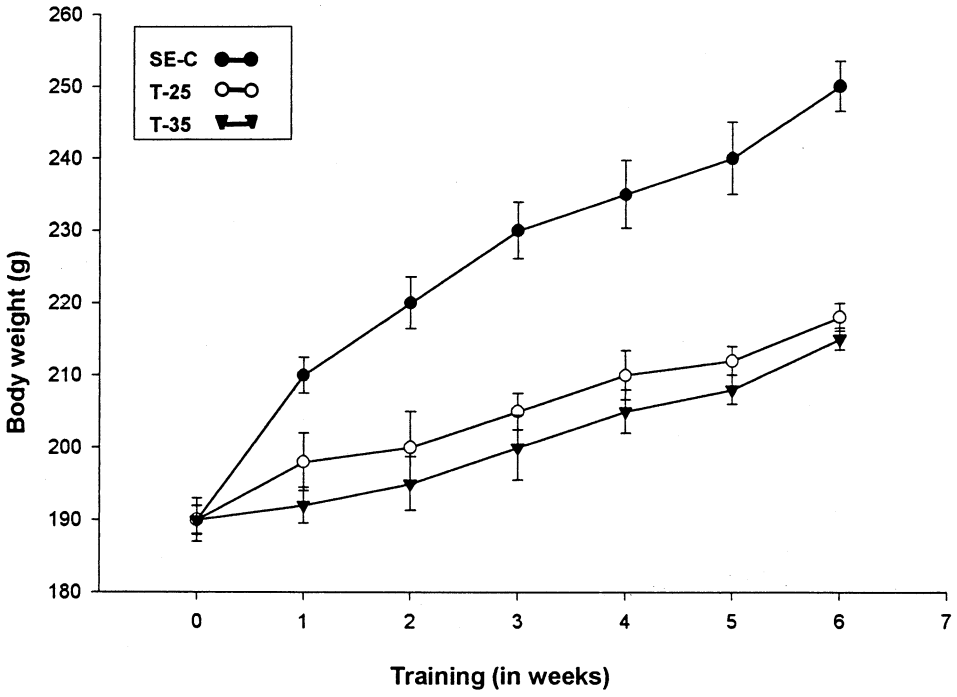

Fig. 2. Weekly variations in the body weights (g) of middle-aged sedentary (SE-C) and trained rats at 25°C (T-25) and 35°C (T-35). For more details see text.

temperature (Table 1). In the young, maximum elevation in heart mass was seen in T-25 (18%, $P < 0.001$) than in T-35 (8%) unlike in the middle-aged wherein T-35 rats showed greater hypertrophy (11%, $P < 0.001$) than T-25 rats (6.1%, $P < 0.001$). Besides the total heart mass, regional variations were also noticeable in the extent of hypertrophy. In the young, LV hypertrophied to a greater extent (30%, T-25; 17%, T-35) than RV (24%, T-25; 10%, T-35), while in the middle-aged it was the RV which exhibited greater hypertrophy (30%, T-25; 45%, T-35) than the LV (24%, T-25; 31%, T-35).

3.1. Blood lactate (La)

Young and middle-aged untrained swimmers exhibited higher La than their trained counterparts. Young T-25 showed a 35% decrease while T-35 had reduced La of 68% over the controls. Among the middle-aged rats, the extent of depletion in blood La was similar in cold and thermoneutral temperatures in trained as well as the untrained (80%, $P < 0.001$) (Table 2).

Table 1
Changes in heart weight as a function of physical training at different temperatures[a]

Age at the onset of exercise (months)	Groups	Total HW (mg)	LV (mg)	RV (mg)	Age at the termination of exercise (months)
2	SE-C	630.0 ± 5.0	247.0 ± 6.4	122.0 ± 2.5	3–3.5
	T-25	745.0 ± 3.0***	321.0	151.0	
			± 3.6***	± 2.8***	
	U-25	630.0 ± 2.0	243.0 ± 2.6	122.0 ± 2.5	
	T-35	682.0 ± 2.5***	290.0	134.0 ± 4.0	
			± 5.0***		
	U-35	635.0 ± 5.0	241.0 ± 2.3	128.3 ± 3.5	
12	SE-C	703.0 ± 4.7	306.0 ± 11.5	130.0 ± 10.0	13–13.5
	T-25	746.0 ± 4.4.7***	380.0	170.0	
			± 20.0**	± 10.0**	
	U-25	676.0 ± 25.1	316.6 ± 5.7	130.0 ± 1.1	
	T-35	780.0 ± 20.0**	400.0	189.3	
			± 2.0***	± 1.1***	
	U-35	661.6 ± 12.5	302.0 ± 2.3	131.0 ± 3.6	

[a] Values represented are mean ± S.D. of three animals/group. SE-C, sedentary control; T-25 and T-35, trained swimmers at 25 and 35°C, respectively; U-25 and T-35, untrained swimmers at 25 and 35°C, respectively; HW, heart weight; LV, left ventricle; RV, right ventricle.

** Significantly different from SE-C at: $P < 0.01$.;

*** $P < 0.001$.

Table 2
Changes in blood lactate as a function of age and physical training at different temperatures[a]

Age at the onset of exercise (months)	Groups	Blood lactate (mg %)	Age at the termination of exercise (months)
2	SE-C	4.64 ± 2.0	3–3.5
	T-25	8.40 ± 2.5	
	U-25	13.0 ± 6.0	
	T-35	3.0 ± 1.0	
	U-35	9.6 ± 4.0	
12	SE-C	2.73 ± 0.19	13–13.5
	T-25	2.30 ± 0.09***	
	U-25	9.60 ± 0.10	
	T-35	1.60 ± 0.14	
	U-35	8.00 ± 1.20	

[a] Values represented are mean ± S.D. of five animals/group. For abbreviations see Table 1.
*** Significantly different from untrained at $P<0.001$.

3.2. LDH

Myocardial LDH activity showed a decline with age (Table 3) Training, however, resulted in elevated activities, irrespective of age, region and swim temperature. In the young, T-25 showed a greater increase in LDH activity in both the regions (LV, 19%; RV, 27%) than T-35 (LV, 7%; RV, 10%). T-35 rats of middle-age exhibited higher levels of LDH activity than their respective counterparts at 25°C. These results could be correlated to the extent of hypertrophy that were noticed in these regions (Table 4). Separation of LDH isoenzymes have been indicated through representative plates (Figs. 3 and 4). In the young, relative increase in the LDH-IV (HM_3) band with a decrease in the LDH-I (H_4) and LDH-II (H_3M) bands were

Table 3
Lactate dehydrogenase (LDH) activity in young rats as a function of physical training at different temperatures[a]

Groups	LDH activity (U/mg protein)	
	LV	RV
SE-C	209.6 ± 6.65	165.3 ± 3.51
T-25	260.0 ± 0.0***	210.6 ± 1.15***
U-25	183.0 ± 6.24	122.0 ± 2.00
T-35	243.3 ± 3.05*	182.6 ± 3.00**
U-35	125.6 ± 6.02	119.0 ± 3.60

[a] Values expressed are mean ± S.D. of three animals/group. For abbreviations see Table 1.
* Significantly different from SE-C at: $P<0.02$;
** $P<0.01$;
*** $P<0.001$.

Table 4
Lactate dehydrogenase (LDH) activity in middle-aged rats as a function of physical training at different temperatures[a]

Groups	LDH activity (U/mg protein)	
	LV	RV
SE-C	157.6 ± 5.85	119.6 ± 1.52
T-25	210.6 ± 3.05***	122.3 ± 5.08***
U-25	120.6 ± 3.50	100.0 ± 2.00
T-35	249.6 ± 2.51***	175.6 ± 3.05***
U-35	140.6 ± 3.05	116.0 ± 2.0

[a] Values expressed are mean ± S.D. of three animals/group. For abbreviations see Table 1.
*** Significantly different from SE-C at: $P < 0.001$.

noticeable in the LV of swimmers trained at 25°C. Between the exercise groups, cold induced insignificant alterations in LDH-I and LDH-V (M_4) as seen in Table 5 (Fig. 3C, D). Untrained animals exhibited depleted LDH-I and LDH-II activities in the LV. RV showed increased LDH-I in the trained as well as untrained myocardium at 25°C. However, the magnitude was greater amongst the untrained (Table 5, Fig. 3A, B). Besides these alterations, LDH-IV was significantly elevated at 25°C in the untrainees. Changes in the LDH isoenzymes in rats exercised at 35°C has been indicated in Fig. 4. LDH-I and LDH-II activities were greater in the

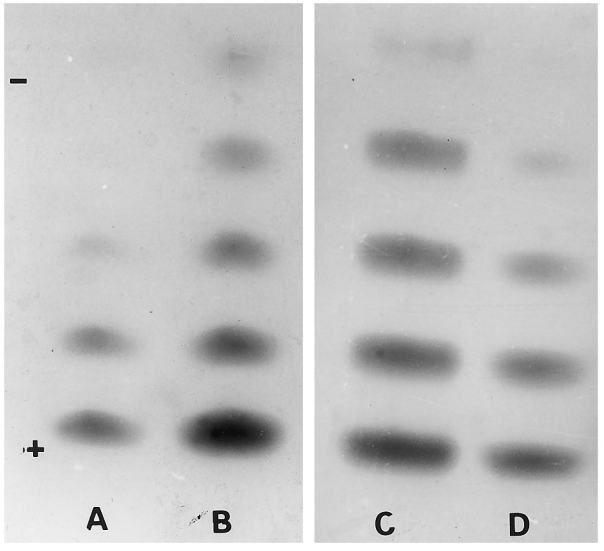

Fig. 3. Lactate dehydrogenase (LDH) isozymes; a representative plate showing electrophoretic migration of bands for LDH activity. (A) right ventricle (RV) of untrained swimmers at 25°C (U-25); (B) RV of trained swimmers at 25°C (T-25); (C) left ventricle (LV) of U-25; (D) LV of T-25; +, anode; −, cathode; I (H_4), II (H_3M), III (H_2M_2), IV (HM_3), V (M_4); Y, young.

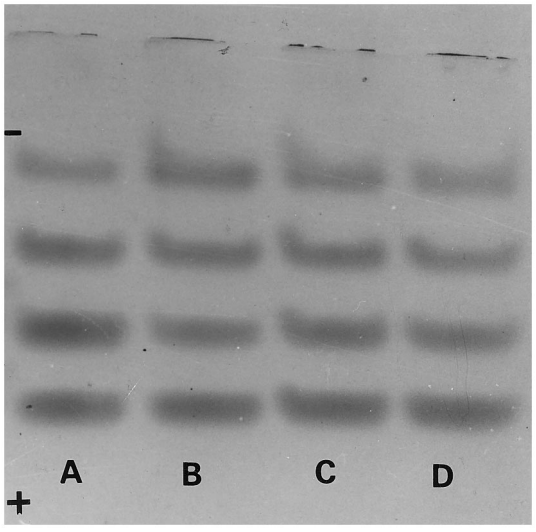

Fig. 4. Lactate dehydrogenase (LDH) isozymes; plate showing electrophoretic migration of bands for LDH activity. (A) left ventricle (LV) of trained swimmers at 35°C (T-35); (B) LV of untrained swimmers at 35°C (U-35); (C) right ventricle (RV) of T-35; (D) RV of U-35. Refer to Fig. 3 for all other abbreviations.

untrained LV as compared to the trained, while LDH-III (H_2M_2), LDH-IV and LDH-V were greater in the trained than the untrained (Table 6, Fig. 4A, B). Untrained RV exhibited significant decreases in LDH-I, -II, -III and -IV activities in the trained and was accompanied by a tremendous increase in LDH-V. Training middle-aged in cold induced changes in the LV through elevated LDH-I and LDH-V while LDH-II and LDH-III activities reduced (Table 7) and this was in comparison with the SE-C. Untrained, however, exhibited elevated LDH-I and -II activities, while all the other three isoenzymes showed reduced activities. The LV of T-35 showed increased activities in LDH-I, -IV and -V over the SE-C (Table 8). RV of middle-aged showed decreased LDH-I, -II and -V activities when trained at 25°C, and these changes were similar at 35°C too except that LDH-V increased at this temperature. However, no significant differences in isoenzyme activities were noticeable between the trained and untrained middle-aged rats.

4. Discussion

Results of the present study indicate a decrease in BW and an increase in HW of animals that were swim trained irrespective of age, region and temperature. This is in concordance with previous results (Bhagavathi and Asha Devi, 1993). This also upholds the fact that physical training of rats by regular swimming increases the HW if the exercise is sufficiently strenuous (Rockstein et al., 1981), and thereby suggesting the fact that the training regimen employed herein is stressful enough to

evoke changes in the myocardium. The LV and RV increased their mass along with the total HW from 2 to 12 months. These results indicate development of hypertrophy with age and is in agreement with the studies of Capasso et al. (1990) who reported that LV in rats increased by 48% while RV augments by 115% and thereby implying a 64% greater overall growth in RV than LV with aging. These responses for 20–29-month-old rats are interpreted in terms of a greater growth response of RV. In the present study, hypertrophying of LV exceeded that of RV at both swim temperatures but in the middle-aged the extent of hypertrophy in LV significantly decreased. This indicates that at an early age, the LV which possesses a fairly sufficient compensatory mechanism but with aging the growth reserve of RV exceeds markedly that of LV. These differences could be due to a greater load sustained by LV throughout life which in turn may exhaust the compensatory capacity of LV at an earlier age (Anversa et al., 1990).

In the present study it was observed that both young and middle-aged animals had lower swim velocity in cold water (young, 1.8/min; middle-aged, 1.2/min) than their counter parts that swam in thermoneutral water (young, 2.8 m/min; middle-aged, 2.2 m/min). As indicated by Dawson et al. (1968), this is suggestive of the fact that swim endurance in small animals such as rats is reduced when water temperature is below body temperature. It is also reported that such a reduction in performance may be due to inadequate oxygenation of muscles consequent to a reduced circulation.

The lower blood La level observed in the present study in the trained animals as compared to the untrained could be attributed to a decreased rate of lactate formation in the exercising muscle which is reported for skeletal muscle studies by Donovan and Brooks (1983) and is interpreted as due to: (1) arterio venous lactate differences across the muscles being smaller in trained; and (2) greater depletion of glycogen in the trained rather than in the untrained.

Table 5
Lactate hydrogenase (LDH) isoenzymes in the left and right ventricles of young trained and untrained rat hearts at 25°C[a]

Group	Region	LDH-I	LDH-II	LDH-III	LDH-IV	LDH-V
SE-C	LV	50.28 ± 0.99	31.68 ± 0.62	11.10 ± 0.22	2.5 ± 0.05	4.32 ± 0.09
T-25		44.50 ± 0.24***	21.10 ± 0.10***	15.80 ± 0.08***	11.40 ± 0.06***	6.6 ± 0.03***
U-25		42.89 ± 0.68***	22.59 (0.22***	15.81 ± 0.23***	10.04 ± 0.15***	8.57 ± 0.13***
SE-C	RV	26.43 ± 0.68	31.22 (0.85	22.60 ± 0.45	13.65 ± 0.27	5.25 ± 0.20
T-25		38.56 ± 0.71***	34.94 ± 0.69***	19.79 ± 0.40***	5.65 ± 0.10***	0.95 ± 0.02***
U-25		34.24 ± 0.70***	26.77 ± 0.53*	20.77 ± 0.42*	15.33 ± 0.31***	2.32 ± 0.04***

[a] Value are mean $\pm$ S.D. of three observations. Activity is expressed in terms of relative area (%). For abbreviations see Table 1.

* Significantly different from SE-C at: $P < 0.02$;

*** $P < 0.0001$.

Table 6
Lactate dehydrogenase (LDH) isoenzymes in the left and right ventricles of young trained and untrained rat hearts at 35°C[a]

Group	Region	LDH-I	LDH-II	LDH-III	LDH-IV	LDH-V
SE-C	LV	50.28 ± 0.99	31.68 ± 0.62	11.30 ± 0.22	2.5 ± 0.05	4.32 ± 0.09
T-35		20.76 ± 0.16***	29.35 ± 0.21*	28.95 ± 5.86	12.92 ± 0.09*	6.54 ± 0.05***
U-35		26.57 ± 1.3	33.62 ± 1.68	22.54 ± 1.13	9.96 ± 0.39***	7.96 ± 0.05**
SE-C	RV	26.43 ± 0.68	31.22 ± 0.85	22.60 ± 0.45	13.65 ± 0.27	5.25 ± 0.20
T-35		33.80 ± 0.55***	25.76 ± 0.50***	21.93 ± 5.20	17.05 ± 8.10	1.46 ± 2.0
U-35		26.41 ± 6.00**	21.17 ± 2.4*	14.86 ± 1.5*	15.27 ± 1.6***	22.29 ± 2.3***

[a] Value are mean ± S.D. of three observations. Activity is expressed in terms of relative area (%). For abbreviations see Table 1.

* Significantly different from SE-C at: $P<0.02$;

** $P<0.01$;

*** $P<0.001$.

The heart is an organ with predominant aerobic metabolism and several studies have examined the LDH activity and isozyme distribution in exercise. LDH is a key regulatory enzyme of glycolysis and dependent on NAD for the interconversion of lactate and pyruvate. They are tetramers of two different polypeptides, i.e. H and M subunits, and it is possible to identify five electrophoretically different LDH isozymes in the heart. H-LDH is responsible for the conversion of lactate to pyruvate which is distributed in tissues with predominant aerobic metabolism, whereas the M-type subunit which converts pyruvate to lactate is more abundant in anaerobic tissues. In tissues like the heart where a mixture of the two subunits is present, adaptive changes to varying energy requirements can be expected (Everse and Kaplan, 1973). The decline in the total LDH activity with age in the heart as reported by Anitha and Asha Devi (1996) and Singh and Kanungo (1968) agrees with the present finding wherein swim endurance training resulted in an increase in total LDH activity, the extent being greater in the young than in the middle-aged. Similar effects have been reported in swim trained adult male rats (York et al., 1975; Gollnick et al., 1976; Anitha and Asha Devi, 1996) and confirming the fact that increased enzyme activity is to meet the demands of the exercise used. LV exhibited increases in LDH to a lesser extent than RV implying that LV is more intolerant to anaerobic conditions. Elevated LDH enzyme activity in response to endurance swimming regardless of how it has been induced could give the heart a greater capacity for lactate utilization and the increased activity in trained rats could complement the oxidative system by supplying additional substrate in the form of pyruvate (Gollnick and Hearn, 1961). In addition the trained heart has the capacity to increase the content of mitochondria, increase activities of dehydrogenases such as LDH and SDH along with phosphorolytic enzymes. These alterations will eventually lead to increased generation of ATP similar to that seen in the skeletal muscle (Holloszy and Coyle, 1973). The levels of LDH activity may be

Table 7
Lactate dehydrogenase (LDH) isoenzymes in the left and right ventricles of middle-aged trained and untrained rat hearts at 25°C[a]

Group	Region	LDH-I	LDH-II	LDH-III	LDH-IV	LDH-V
SE-C	LV	22.59 ± 0.90	34.75 ± 1.39	26.29 ± 1.04	10.04 ± 0.42	5.44 ± 0.22
T-25		39.59 ± 0.68***	22.22 ± 0.20***	11.39 ± 2.10***	10.58 ± 0.15***	15.23 ± 0.28***
U-25		27.50 ± 0.70***	35.39 ± 0.80	23.52 ± 1.0***	8.82 ± 0.30***	3.39 ± 0.15
SE-C	RV	30.60 ± 0.64	27.30 ± 0.54	22.46 ± 0.45	14.15 ± 0.29	5.09 ± 0.10
T-25		27.29 ± 1.06***	18.06 ± 0.64***	31.29 ± 9.3***	20.04 ± 4.0***	3.10 ± 0.38***
U-25		32.13 ± 0.64***	22.58 ± 2.8*	23.41 ± 2.45***	17.27 ± 0.40***	4.39 ± 0.08***

[a] Values are mean ± S.D. of three observations. Activity is expressed in terms of relative area (%). For abbreviations see Table 1.

* Significantly different from SE-C at: $P < 0.02$;

*** $P < 0.001$.

especially sensitive to the extent of the stress of endurance training, i.e. whether the training protocol is strenuous and prolonged or moderate and long.

Results of the present investigation reveal exercise associated changes in LDH isoenzyme composition of both ventricles. Although the young rats trained at 25 and 35°C exhibited elevated LDH-IV and -V activities with reduced -I and -II activities in the LV, these increases were significantly lesser than the extent of shifts reported during other cardiac hypertrophic stresses like altitudes exposure (York et al., 1976) suggesting the preponderance of M_4 isozyme and unlimited O_2 delivery to the heart during swim training. The T and UT RV showed significantly greater LDH-I activity at both the temperatures but the fact that LDH-IV also increased in the RV alone simultaneously and by a larger magnitude suggests that the untrained are more under an O_2 stress. Thus the presence or absence of hypoxia is more critical in determining the magnitude of changes in LDH subunit composi-

Table 8
Lactate dehydrogenase (LDH) isoenzymes in the left and right ventricles of middle-aged trained and untrained rats at 35°C[a]

Group	Region	LDH-I	LDH-II	LDH-III	LDH-IV	LDH-V
SE-C	LV	22.59 ± 0.90	34.75 ± 1.39	26.19 ± 1.04	10.44 ± 0.42	5.44 ± 0.22
T-35		28.49 ± 0.30***	26.60 ± 0.30***	22.32 ± 0.01***	13.91 ± 010***	0.22 ± 8.08***
U-35		14.91 ± 0.30***	26.58 ± 0.50***	23.02 ± 0.50**	17.47 ± 0.35***	17.84 ± 0.36***
SE-C	RV	26.43 ± 0.68	31.22 ± 0.85	22.60 ± 0.45	13.65 ± 0.27	5.25 ± 0.20
T-35		24.89 ± 0.22***	21.96 ± 0.40***	22.79 ± 0.46***	19.09 ± 0.4***	10.52 ± 0.20***
U-35		25.06 ± 0.43***	21.00 ± 0.40***	22.68 ± 4.5	18.81 ± 0.20***	11.25 ± 2.5***

[a] Values are mean ± S.D. of three observations. Activity is expressed in terms of relative area (%). For abbreviations see Table 1.

** Significantly different from SE-C at: $P < 0.01$;

*** $P < 0.001$.

tion, than mere changes in HW. Situations that result in increases of exclusively M-subunit may not always reflect an anaerobic adaptation in the heart but rather an aerobic one where changes in M-LDH provide a mechanism by which LDH activity is alterable over the long term in the presence of varying concentrations of peripherally supplied lactate.

Increased LDH-III, -IV and -V activities were accompanied by increased LDH activity in LV of young rats trained in cold and thermoneutral temperatures while LDH-I and -II were decreased compared to the SE-C. The source of LDH-V in the hypertrophied young trained LV ventricle, regardless of temperature may be interpreted based on the reports that embryonic and growing myocardial muscle, which is less susceptible to hypoxic effects than adult muscle, do contain more of M-LDH than adult myocardium (Dawson et al., 1968). Hence it is reasonable to think that those portions of the hypertrophied myocardial cell that are newly formed following training are similar to embryonic tissue and contributes to the increased M_4 activity. This further leads to the speculation that metabolism in LV is shifted towards formation of lactate and less towards pyruvate in the trained ones. Myocardial lactate production indicates anaerobic metabolism and lactate extraction has been used as an index of myocardial oxygenation (Gertz et al., 1980). When the O_2 supply is inadequate, excess glycolysis occurs and lactate is produced. RV also showed a similar trend in the untrained (50% in U-35; 38% in U-25). A marginal elevation was noticeable in RV of 25 and 35°C trained and untrained animals. In the trained young RV, however, both swim temperatures induced marked decreases in M_4-LDH activity and thereby indicating that the reduction of pyruvate by using NADH is low and the NADH that is produced during glycolysis is utilized to generate ATP via an intact electron transport pathway. The decline in M_4-LDH may indicate an aerobic metabolism in the trained RV.

Thus temperature of the water in which the animals swam significantly influenced the activity of M_4-LDH. Young animals trained in cold water did show adaptations to the possible conditions of anaerobiosis by increasing their M_4-LDH activity in the LV but not in the RV, which still remains aerobic as evidenced by increased H_4-LDH. In the middle-aged, however, animals adapted to the stress of swimming in cold waters by increasing their LDH-V in the LV but not in the RV. Reduced LDH-I, -II and -V activities were evident in the cold-trained middle-age RV and was unlike the LV of T-35 and T-25 rats wherein LDH-V increased. These changes are indicative of adaptation towards La synthesis. Changes in LDH with respect to temperature reveal a higher activity in young rats which were swim trained at 25°C. However, the condition reversed with respect to middle-aged rats. During high intensity exercise in cold, the enhanced heat flux from active muscles and from the body core to the skin is offset by a much higher metabolic heat production (Falk et al., 1994). The increase in metabolism during cold exposure was probably not sufficient to maintain the core temperature among the middle-aged rats unlike in the young. Thus, a diminished ability to maintain core temperature during rest and exercise in cold, occurs with age. Based on the experimental evidences on adaptation of LDH isozymes and its redistribution in response to varying O_2 that could

arise in the trained and untrained states, it is attractive to suggest that LDH is a rate-limiting enzyme in energy production in the cardiac muscle in terms of lactate and pyruvate production. Further experimental evidence is required to precisely define the mechanisms through utilization of an ATP consuming system.

It can be concluded that the compensatory capacity of LV diminishes with age. Prolonged endurance training in cold and thermoneutral temperatures can make the LV in the young and middle-aged less vulnerable to stressful conditions by redistribution of isoenzymes in terms of elevated LDH-V activity and improving the capacity of compensation to changing situations of O_2 demands and thereby the risk of cardiovascular diseases in the aged, a major portion of which is contributed by LV dysfunction. In contrast to the LV, the RV elevates its LDH-V activity only when the middle-aged rats are trained at 35°C. Thermoneutral temperature is suggested to be a more preferred environment for endurance training programs like swimming irrespective of age because cold environment acts as a stress and thus reducing their extent of adaptability. Preferential conditions for swimming with respect to temperature could be better evidenced by extending studies relating to heat too.

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