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LDH isoenzymes in muscle diseases

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The LDH isoenzyme pattern was investigated in biopsy material of muscles of children suffering from progressive muscular dystrophy and other neurogenic muscular disorders. An absence or pronounced activity loss of LDH 5 was observable in most of the cases. The proportion of subunits was the same in the diseased and the control muscles. Comparing the measured isoenzyme patterns with those expected from a random coupling of the subunits, a lower percentage of LDH 5 and a higher one of LDH 4 was detected. The cause of the absence of LDH 5 is not a disturbance of M subunit synthesis but the post-translational inhibition of the tetramer forming process of the synthetised M subunits.

The pattern of LDH isoenzymes has been investigated in diseases of the skeletal muscles by several authors [8, 12, 14, 19]. The slowest migrating fraction, LDH 5, was found to be absent or to show a considerable loss of activity. It has been concluded that this is an aspecific sign of muscle diseases [12, 14, 19], but some authors regard it as a diagnostic characteristic of some muscular disorders [8]. The origin of the phenomenon has not been clarified.

The enzyme LDH (E. C. 1.1.1.27) is formed by the coupling of two different protein chains as subunits. The isoenzyme molecules consist of four chains of the two different types of subunit, H and M [17]. The mechanism of the process has been clarified by Millar by isolating LDH monomers (H and M), dimers (two homologue types, HH and MM, and a heterologous one, HM) and tetramers from the cells synthetising LDH [17]. The tetramers are the well-known isoenzymes LDH 1 = HHHH; LDH 2 = HHHM; LDH 3 = HHMM; LDH 4 = HMMM; and LDH 5 = MMMM. Millar's conclusion was that the isoenzymes are in the first step formed by connecting with the same or the other subunit, and these dimers form tetramers with the possible three types of dimer [17].

Several authors studied the regulation of the LDH pattern by synthetising the subunits; their coupling follows randomly according to the actual subunit pattern. So the pattern of the isozymes follows the binomial distribution [5, 15, 18]. All these investigations were made *in vitro*. In experiments *in vivo* a non-binomial distribu-

tion was found in both normal and pathological cases [4, 7, 23]. This suggests that the LDH pattern is regulated by some post-translational modifying effects.

The isoenzymes which are rich in M subunits (LDH 4,5) are characteristic of the glycolytic white fibres, while the types rich in H subunits are found in higher amounts in the oxidative red fibres. There is some functional difference between the isozymes. The types migrating more quickly at electrophoresis catalyse the lactate-pyruvate reaction under aerobic circumstances, while the slower ones catalyse the pyruvate-lactate reaction and permit the reoxidation of the reduced coenzymes originating from anaerobic glycolysis [13].

The absence of LDH 5 in the muscle diseases cannot originate from the subunit-selective outflow of the enzyme, because the molecular weight of the two subunit types is the same [5, 8, 14, 18]. In this case — taking into consideration the shorter half-life of the M subunits — at least traces of LDH 5 ought to be found in the serum. Still, LDH 5 could not been detected in the serum of patients suffering from muscle diseases [21].

Thus, the absence of LDH 5 may only be due to a disturbance of M subunit synthesis or an inhibition of the tetramer forming process of the synthetised M subunits [8, 14, 19].

In the present work the subunit proportion has been investigated in different muscle diseases to clarify the effects responsible for the specific LDH isozyme pattern.

Material and Methods

The material studied was obtained by needle biopsy of the gastrocnemius muscle of 8 children suffering from progressive muscular dystrophy (DMP), one each with polyomyelitis, Werdnig-Hoffmann disease, Friedreich ataxia, familiar cardiomyopathy, and 3 having some unclarified neurogenic muscular disorder. From the material a homogenate was prepared with cooled distilled water at a concentration of 30 mg/ml and centrifuged at 10 000 *g* for 30 min. LDH activity in the clear supernatant was determined by an optical method. At the substrate concentration employed the isoenzymes having different subunit structure participated in the reaction with the same activity (22). The individual isoenzymes were separated by electrophoresis on 5.5% polyacryamide gel (6). The percentage distribution of the isoenzymes was determined in a K-Z densitometer-integrator. Knowing their subunit structure, the proportion of H and M subunits was calculated (2).

In the case of random coupling of the subunits, the expected isozyme distribution can be calculated with the help of the formula

$$LDH_{1-5} = \frac{n}{r} H^n M^{n-r} =$$

$$= \frac{n!}{(n-r)!\, r!} H^n M^{n-r} ,$$

where H and M are the proportions of the subunits, n is the number of H subunits in the isoenzyme, and r the number of all subunits in the isoenzyme.

By comparing the result to those obtained in the course of measurements, it was possible to decide the presence or the absence of some post-translational modifications.

As a control, biopsy material from the oblique external abdominal muscle of healthy children operated upon for inguinal hernia, was studied.

Results

Total LDH activity in the muscle of sick children generally exceeded the control level (Tables I, II, III). The highest value was found in some DMP cases, while in others the activity was hardly higher than in normal individuals. Scattering was higher in the neurogenic muscle disorders, sometimes with an activity identical to the control or lower than it. The isozyme pattern in the normal cases showed a small alteration depending on age (Table I). It was pronounced in the decrease of LDH 1,2 and in the increase in activity of isozymes of slower migration.

The mean values are presented for summarizing the main tendencies. Their statistical evaluation was not possible in view of the different symptoms, the different age of the children and the different stage and severity of the illness. The isozyme patterns expected from the calculated ratio of subunits and the measured one are freely comparable.

In the control cases, the proportion of LDH 5 was slightly higher than expected (Table I). In 5 cases of the neurogenic muscular disorders there was a complete absence of LDH 5. The ratio of the subunits did not differ from the ratio observed in the controls, the proportion of M subunits was even a little higher than in healthy muscles (Table II). The proportion of LDH 5 was lower than the predicted level, in 3 cases pronouncedly and in 2 other cases minimally. In all 5 cases the proportion of LDH 4 was higher than expected.

In the DMP children LDH 5 was totally absent except for one case (Table III). The M subunit proportion was not lower than in the controls, in some cases it was even higher. The proportion of LDH 5 was essentially lower, that of LDH 4 markedly higher, than calculated by the subunit pattern.

No correlation was observed between the age or the duration of the disease and the degree of the observed alterations in the LDH isozyme pattern.

Discussion

The differential diagnostic value of LDH isoenzymes in muscular disease is doubtful [12, 14, 19], but the phenomenon described may lead to a better understanding of the biochemical events. The selectivity of the increase in permeability and the following enzyme outflow, described by Richterich et al [21], cannot be the cause of the absence of LDH 5, hence the molecular weight of the subunits is the same [5, 20, 25].

On the basis of the functional differences occurring among the LDH isozymes one may explain the shift of muscle metabolism towards oxidative metabolism: this would lead to an LDH 1,2 predominancy, a heart-type pattern, yet such a distribution was not detected in any of the cases [2, 10, 13].

The genetically determined or acquired defect of M subunit synthesis,

TABLE I

The pattern of LDH isozymes and subunits

Sex, Age, yr	LDH activity mU/mg v/w	Measured LDH isozyme pattern				
		1	2	3	4	5
m 1	11.10	52.52	32.84	8.92	8.53	0.0
m 1.5	20.03	31.45	31.43	17.56	14.92	4.62
m 2	20.75	22.68	27.16	27.68	19.66	2.77
m 2.5	18.24	20.22	43.97	25.19	9.36	1.27
m 3.5	20.03	26.73	35.35	18.94	16.55	2.39
m 8	16.45	15.02	23.59	31.57	26.29	3.52
Mean	17.76	28.10	32.36	21.64	15.89	2.43

TABLE II

The pattern of LDH isoenzymes and subunits in the muscles

Sex Age	Diagnosis	LDH activity mU/mg v/w	Measured LDH isozyme pattern				
			1	2	3	4	5
f newborn	cardiomyopathy	83.72	7.53	18.25	31.19	43.14	0.0
m 6wk	Werdnig-Hoffmann	26.56	26.75	16.65	28.71	28.00	0.0
m 2 yr	neurogenic muscle disease	21.82	35.80	36.19	20.38	7.59	0.0
f 2 yr	neurogenic muscle disease	64.89	4.91	18.0	28.57	30.61	17.87
m 7 yr	neurogenic muscle disease	76.12	0.0	10.23	30.25	38.91	20.57
f 8.5 yr	Poliomyelitis	3.92	32.08	34.13	25.14	8.65	0.0
m 13 yr	ataxia Friedreich	69.78	14.00	22.40	48.24	14.75	0.0
	Mean	49.54	17.4	22.26	30.35	24.52	5.49

suggested by several authors, was based on the M subunit consisting only of LDH 5 [12, 14, 19]. In our material the M subunit content in the diseased muscle was not lower than in the controls of the same age group, indicating that the cause of the absence of LDH 5 was not at the level of subunit synthesis.

The random coupling of subunits has been shown *in vitro* [5], and some authors accepted it to be the case *in vivo*, too [5, 15, 18]. There are some results obtained in animal muscles where the LDH pattern did not correspond to the binomial distribution either in normal or in pathological cases [4, 16, 23]. There was a slight

in the muscles of control children

Subunit pattern		Predicted LDH isozyme pattern				
H	M	1	2	3	4	5
83.71	16.29	49.10	38.22	11.15	1.4	0.0
67.53	32.47	20.79	39.29	28.84	9.2	1.11
60 38	39.62	13 2	34.7	34.15	14.9	2.4
67.99	32.01	20.1	38.52	27.69	8.86	1.06
66.84	33.16	18.9	39.34	29.3	9.5	1.2
55.06	44.94	9.15	29.88	36.71	19.9	4.07
66.92	33.08	21.87	36.66	27.97	10.63	1.64

of children suffering from neurogenic muscular disorders

Subunit pattern		Predicted LDH isozyme pattern				
H	M	1	2	3	4	5
47.58	52.42	5.12	22.58	37.32	27.41	7.55
60.58	39.42	13.46	35.05	34.20	14.84	2.41
75.02	24.98	31.67	42.18	21.07	4.67	0.38
40.32	59.68	2.64	15.64	34.72	34.26	12.8
32.52	67.48	1.11	9.28	28.89	39.97	20.7
72.4	27.6	27.47	41.89	23.95	6.08	0.58
58.6	41.4	11.79	33.32	35.3	16.63	2.93
55.29	44.71	13.32	28.56	30.78	20.55	6.75

deviation from the binomial pattern also in our own normal controls; mostly the isozyme LDH 1 characteristic of the embryonic development of muscle [3, 24] showed a higher than the calculated percentage. The isozyme pattern found by Pearson and Kar in normal adult muscles showed a higher percentage of LDH 5 than that expected from the subunit pattern [19]. This observation indicated the presence of some post-translational modifying effect which may promote the conversion of the isozyme pattern beside the genetic control of subunit synthesis [3, 9, 24].

The LDH 5 deficit in the diseased muscles agrees well with the higher

TABLE III
The pattern of LDH isoenzymes and subunits

Sex	Age, yr	LDH activity mU/mg v/w	Measured LDH isozyme pattern				
			1	2	3	4	5
m	2	536.0	3.9	18.25	38.91	38.6	0.0
m	5	102.4	9.13	20.86	40.43	29.56	0.0
m	6	912.6	3.97	16.29	44.03	35.69	0.0
m	6.5	177.7	37.44	43.1	7.99	10.63	0.98
m	6.5	214.6	21.18	32.51	12.96	32.71	0.0
m	7	4.61	39.26	30.57	29.92	0.0	0.0
m	9	179.63	16.04	27.65	33.9	22.43	0.0
f	11	97.6	5.24	12.73	25.65	38.76	17.41
	Mean	278.14	17.02	25.25	29.23	26.05	2.3

percentage of LDH 4, because the synthetised M subunits seem to have the possibility to form tetramers only as LDH 4 isozyme. The dissociation and reassociation of the subunits is influenced by the molecular microenvironment [16, 18]. So it seems that the alteration of the ionic-permeability characteristics in the muscle [11] does not permit the coupling of homologue MM dimers to form LDH 5, and these dimers form with heterologous HM dimers LDH isozymes [17].

The role of the changes in ionic strength and charge characteristics is supported by the observation of Katz and Kalow [14] who in dystrophic muscles could not find the slowest migrating positive isozyme of isocitrate-dehydrogenase (ICDH).

Fine et al. suggested that the cause of the non-binomial distribution is an inhomogeneity of the cells under study [9]. The LDH 1,2 predominance in the red fibres, and the LDH 4,5 preponderance in the white fibres may lead to an absence of LDH 5 and to a higher percentage of LDH 4 by the inhomogeneous mathematical distribution.

Our results indicate a disturbance of the tetramer forming process in muscular diseases. It is more pronounced in DMP than in neurogenic disorders, but its differential diagnostic value must be verified in further cases.

As the complete evolution of the skeletal musculature coincides with the higher importance of anaerobic metabolic pathways [3, 24], the functional role of LDH 5 becomes continuously more significant and its absence more crucial; this may parallel the progressive character of the disease.

in the muscles of children suffering from DMP

Subunit pattern		Predicted LDH isozyme pattern				
H	M	1	2	3	4	5
46.68	53.32	4.74	21.69	37.16	28.3	8.08
52.37	47.63	7.52	27.36	37.33	22.63	5.44
47.11	52.89	4.92	22.11	24.82	27.88	7.82
76.33	23.67	33.94	42.1	19.58	4.04	0.31
60.84	39.19	13.7	35.27	34.05	14.61	2.35
77.14	22.86	35.4	41.97	18.65	3.69	0.0
59.32	40.68	12.38	33.96	34.93	15.97	2.73
37.29	62.71	1.93	13.00	32.81	36.78	15.46
57.13	42.77	14.32	29.68	29.92	19.24	5.27

References

1. Boyd, J. W.: The disappearance of 14C labelled isoenzyme 5 of L-lactate dehydrogenase from plasma. Biochem. biophys. Acta (Amst.) **146,** 590 (1967).
2. Cahn, R. D., Kaplan, N. O., Levine, E., Zwilling, E.: Nature and development of lactic dehydrogenases. Science **136,** 962 (1962).
3. Clausen, J., Hustrulid, R.: The foetal development of lactate-dehydrogenase isoenzymes, glucose-6-phosphate and 6-phosphogluconate dehydrogenase from human striated muscle. Biochem. J. **111,** 219 (1969).
4. Davis, R. M., Baetz, L. A., Hubberth, W. T., Graham, C. K.: Age related changes of LDH activity and isozyme pattern in the bovine fetus. Biol. Neonate **22,** 64 (1973).
5. Dawson, D. M., Goodfriend, T. L., Kaplan, N. O.: Lactic dehydrogenase functions of the two types. Science **143,** 929 (1964).
6. Dietz, A. A., Lubrato, T.: Separation and quantitation of LDH isozymes by disc electrophoresis Anal. Biochem **20,** 246 (1967).
7. Dux, L., Dux, E., Mazareán, H., Guba, F.: A non-neural regulatory effect on the metabolic differentiation of the skeletal muscles, effect of castration and testosterone administration on the skeletal muscles of the rat. Comp. Biochem. Physiol **64***A* 177 (1979).
8. Emery, A. E. H., Sherbourne, D. H., Dusch, D. H.: Electrophoretic pattern of muscle lactic dehydrogenase in various diseases Arch. Neurol. **12,** 251 (1965).
9. Fine, I. H., Kaplan, N. O., Kuftinec, D.: Developmental changes of mammalian lactic dehydrogenases. Biochemistry **2,** 116 (1963).
10. Fondy, T. P., Kaplan, N. O.: Structural and functional properties of the H and M subunits of lactic dehydrogenases. Ann. N. Y. Acad Sci. **119,** 888 (1964).
11. Herman, B. A., Fernandez, S. M.: A microfluorimetric study of membrane dynamics during development of dystrophic muscle in vitro. Arch. Biochem. Biophys. **196,** 430 (1979).
12. Kar, N. C., Pearson, C. M.: Electrophoretic patterns of several dehydrogenases and hydrolases in muscles in human myopathies. Amer. J. clin Path. **47,** 594 (1967).
13. Karlsson, J., Frith, K., Sjödin, B., Gollnick, P. D., Saltin, B.: Distribution of LDH isozymes in human skeletal muscles. Scand J. clin. Lab. Invest **33,** 307 (1974).
14. Katz, A. M., Kalow, W.: Abnormal isoenzyme pattern in human myopathies. Nature (Lond.) **201,** 1349 (1966).
15. Markert, C. L., Ursprung, H.: The ontogeny of isozyme pattern of lactate-dehydrogenase in the mouse. Develop. Biol. **5,** 363 (1962).
16. Markert, C. L., Massaro, E. J.: Lactate dehydrogenase isoenzymes: Dissociation and reassociation by dilution. Science **162,** 695 (1968).

17. Millar, D. B.: Mechanism of isozyme formation as related to subunit interactions. In: Isozymes I. Molecular structure (ed. Markert C. L.) Academic Press, New York 1975, pp 169—190.
18. Nadal-Ginard, B.: Regulation of lactate-dehydrogenase levels in the mouse. J. biol. Chem. **253**, 170 (1978).
19. Pearson, C. M., Kar, N. C.: Isoenzymes: General considerations and alterations in human and animal myopathies. Ann. N. Y. Acad Sci. **138**, 293 (1966).
20. Richterich, R.: Enzymopathologie. Springer Verlag, Berlin—Göttingen—Heidelberg 1958, 401.
21. Richterich, R., Gautier, E., Egli, E., Zuppinger, K., Rossi, E.: Progressive Muskeldystrophie I. Mitt. Die Heterogenität der Serum Lactat — Dehydrogenase. Klin. Wschr. **39**, 346 (1961).
22. Schweitzer, E. S., Farron, F., Knox, W. E.: Distribution of LDH and its subunits in rat tissues and tumors. Enzyme **14**, 173 (1973).
23. Shaklee, J. B.: The role of the subunit interactions in the genesis of non-binomial lactate-dehydrogenase isozyme distribution. In: Isozymes I. Molecular structure (ed. Markert C. L.) Academic Press, New York 1975, pp 101—118.
24. Takasu, T., Hughes, B. P.: Lactate dehydrogenase isoenzymes in developing human muscle. Nature (Lond.) **212**, 609 (1966).
25. Wieme, R. J., Herpol, J. E.: Origin of the lactate dehydrogenase isozyme pattern found in the serum of patients having primary muscular dystrophy. Nature (Lond.) **194**, 287 (1962).

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