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INFLUENCE OF DYNAMIC LOADS DIFFERENT IN DURATION ON CHANGES IN THE ACTIVITY OF LACTATE DEHYDROGENASE ISOENZYMES IN INTERVERTEBRAL DISC CELLS

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Purpose: to determine the effect of long-term dynamic loads on changes in the activity of isoenzymes lactate dehydrogenase (LDH) in the cells of the intervertebral discs in the age aspect.

Material and Methods: the study was performed on male Wistar rats of three age groups: 1, 3 and 12 months. A total of 90 animals of the experimental series and 90 animals of the control series were observed. Dynamic loads were created by running in a horizontal treadmill. Using histochemical methods, the analysis of changes in the activity of lactate dehydrogenase isoenzymes in the cells of the intervertebral discs of Wistar rats after 20- and 90-day experimental run was performed.

Results: it is experimentally established that changes in the activity of lactate dehydrogenase isoenzymes depend on the age of the animals and the duration of dynamic loads. There was a decrease in the activity of LDH-1 and LDH-2 isoenzymes in the cells of the intervertebral disc, but an increase in the activity of LDH-3 and LDH-4 isoenzymes.

Conclusions: the identified age features of changes in enzyme activity are proposed to be used as a histochemical test to objectively assess the degree of destructive effect of prolonged running on the fibrous cartilage of the intervertebral disc.

Keywords: lactate dehydrogenase, intervertebral disc, treadmill, experimental running.

Introduction

Lesions of the musculoskeletal system, especially large joints and spine have a high social significance. The basis of clinical manifestations of back pain in most cases is the defeat of the intervertebral discs [8]. Such cases have been reported spinal overload [17,18], as well as after inadequate physical activity in sports [7; 11].

Therefore, finding out the adaptive capacity of cartilage during exercise is an urgent task.

An indicative model for studying the processes of anaerobic and aerobic metabolism of motor activity in different modes is the reaction of lactate dehydrogenase. There are some reports of the activity of this reaction in the serum of athletes [9]. The value of histochemical studies lies in their ability to localize the biochemical reaction and assess the activity of the reaction in cell structures.

Connection of research with scientific programs, plans, topics. The research was performed within the framework of the departmental theme of research work "Medical and biological substantiation of rehabilitation measures and the appointment of physical rehabilitation for young people of different levels of training".

Purpose of the study: to determine the change in the activity of isoenzymes lactate dehydrogenase (LDH) in the cells of the intervertebral discs during prolonged dynamic loads in the age aspect with histochemistry methods.

Material and Methods of the research

The study was performed on male Wistar rats of three age groups: 1, 3 and 12 months, which corresponds to immaturity, puberty and old age [5]. Work with laboratory animals was carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [16]. A total of 90 animals of the experimental series (ES) and 90 animals of the control series (CS) were observed.

The animals were trained to run in a horizontal treadmill using an electronic counter to determine the length of the run. Rats of the I group ran for 20 days 10560 m, and of the II group, for 90 days 2 17280 m.

For all age groups of the experimental series, the maximum running speed was calculated and the equal treadmill speed was selected – 40 m / min. This speed allowed to use a long-distance running in experiments. Animals in the control series were in normal vivarium conditions.

Studies were performed by using macro-microscopy, histology (hematoxylin-eosin) and histochemistry.

Histochemical reactions are performed on enzymes that reflect the state of anaerobic energy supply systems in cells: lactate dehydrogenase (LDH) (K.F.1.1.1.27), its isoenzymes. LDH is an enzyme of anaerobic glycolysis that catalyzes the conversion of pyruvate to lactate [2, 19]. The method of spectrophotometry usually determines the activity of LDH and its five isoforms. By Histochemical methods Four LDH isoenzymes were detected: LDH-1, LDH-2, LDH-3, LDH-4. The reactions were performed on frozen in the cryostat sections of the MX disc, held parallel to the base of the vertebral bodies. The thickness of the sections was 4-5 μm. The setting of histochemical reactions was carried out in compliance with the same conditions [1] and taking into account the recommendations for processing and evaluation of the results of histoenzymological studies [10]. Control sections were incubated under the same conditions without substrate.

Quantitative evaluation of enzyme activity was performed on a two-beam scanning cytophotometer MUF-5. Measurements were performed by the plug method using a probe with a diameter of 200 μm and a lens of 50 and an operating wavelength of 546 nm. Extinction rates were determined at five points in the cytoplasm of each cell, after which the average value was calculated. The suitability of the material for photometric works with registration of the absorption spectrum on objects of different density was determined befor measurements.

The obtained digital material of biometric and cytophotometric studies was processed by the method of variation statistics.

Results of the research

After a 20-day run of the animals in the treadmill, a change in the orientation of the collagen fibers and cells of the fibrous ring of the intervertebral discs was observed. After a 90-day run, changes in the shape, structure and internal organization of the intervertebral discs were detected. On the territory of the fibrous ring there was a defibering of bundles of collagen fibers, disruption of fibrous plates course and cell orientation. In the ventral parts of the fibrous ring, blood vessels appeared, which were oriented along the fibrous plates. Signs of dystrophic changes were especially pronounced in the intervertebral discs of animals aged 1 and 12 months.

Under conditions of hyperkinesia, the morphology of fibrous ring cells and enzyme activity changed. LDH activity varied, but the degree of change was determined by the age of the animals and the level of physical activity (Fig. 1).

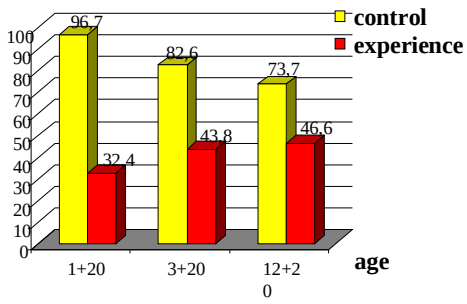

Fig.1. Indicators of total LDH activity in fibrous ring cells after 20 days of hyperkinesia. Age of animals: 1, 3 and 12 months + 20 days of running

In series 1 + 20 hyperkinesia, dark brown granules of diformazan densely located around a kernel were found in cells. The cells of the inner layer of the fibrous ring were located less orderly than in the control, and in places - as part of small isogenic groups. At the same time reduction to 80% of number of active cells is noticed. LDH activity decreased compared to control by 66.49%.

In the 3 + 20 series of hyperkinesia, densely spaced granules of diformazan filled most of the cytoplasm. LDH activity decreased by 46.97%.

In a series of 12 + 20 hyperkinesia, a change in LDH activity was detected against the background of impaired cell orientation and polymorphism of difformazan granules: large, small, and dusty forms appeared among them. LDH activity decreased by 37.6%.

As for LDH isoenzymes after a 20-day run, their changes were specific (Fig. 2).

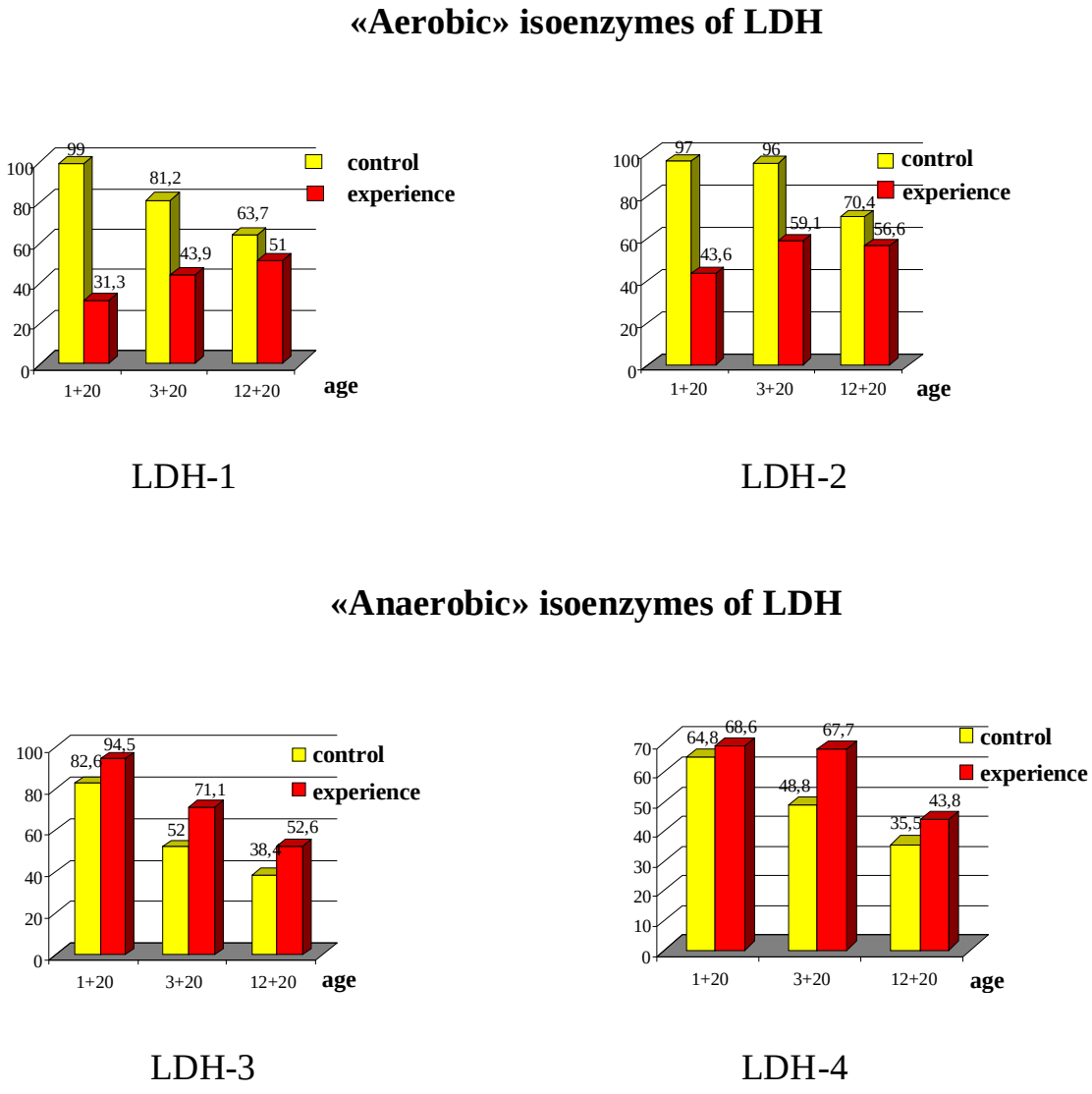

Fig. 2. Indicators of LDH isoenzyme activity in fibrous ring cells of the disc after a 20-day run. Age of animals: 1, 3 and 12 months + 20 days

In series 1 + 20 of hyperkinesia, the activity of LDH isoenzymes varied differently. Significantly decreased the activity of LDH-1 (by 68.3%) and LDH-2 (by

55%). At the same time, the activity of LDH-3 increased (by 12.6%) and even less, the activity of LDH-4 (by 5.53%).

As a result, the gradient of activity of LDH isoenzymes in the intervertebral disc cells of the of 1-month-old rats after a 20-day run was: LDH-3 – LDH-4 – LDH-2 – LDH-1

In the series 3 + 20 hyperkinesia, among LDH isoenzymes decreased: the activity of LDH-1 (by 45.9%) and LDH-2 (by 38.4%), while the activity of LDH-3 and LDH-4 increased (by 26.9 and 27.9% respectively).

The gradient of activity of LDH isoenzymes in disc cells in 3-month-old rats after a 20-day run was LDH-3 – LDH-4 – LDH-2 – LDH-1.

In the 12 + 20 series of hyperkinesia, the change in the activity of LDH isoenzymes was manifested by a decrease in the activity of LDH-1 and LDH-2 (by 19.9% and 19.6%, respectively) and an increase in the activity of LDH-3 and LDH-4 (by 26.9 and 18, 9% respectively).

As a result, the gradient of LDH activity of 12-month-old animals after a 20-day run was: LDH-2 – LDH-3 – LDH-1 – LDH-4.

A long 90-day run caused a change in LDH activity, which had age differences. At the end of the experiment, the animals reached the age of 4, 6 and 15 months. A peculiarity was in the increase in LDH activity in the intervertebral discs of animals trained in the treadmill from 1 month of age. In the intervertebral discs of older animals, LDH activity decreased (Fig. 3).

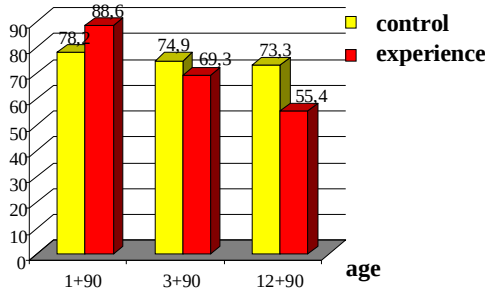

Fig.3. Indicators of total LDH activity in the fibrous ring cells of the disc after a 90-day run. Age of animals: 1, 3 and 12 months + 90 days

In series 1 + 90 hyperkinesia, in the outer layer cells of the fibrous ring were dark brown granules of diformazan, tightly arranged around the nucleus. The inner layer cells of the fibrous ring were arranged in a less orderly manner, in places as part of small isogenic groups. LDH activity increased by 11.7%. *In series 3 + 90 hyperkinesia*, LDH activity reduced by 75%. Large difformazan granules appeared in the cytoplasm, scattered among the small granules that predominated in the ectoplasm. *In the series 12 + 90 hyperkinesia*, LDH activity decreased by 24.4%. Diformazan granules densely filled the endoplasm and clearly delineated the contours of the nucleus; most of the granules differed in dark brown color and medium size. The distribution in the fibrous ring of enzyme-labeled cells was uneven, and the characteristic orientation of the cells along the fibrous plates, which is inherent in the control, was absent. Changes in the activity of LDH isoenzymes also had age differences (Fig. 4).

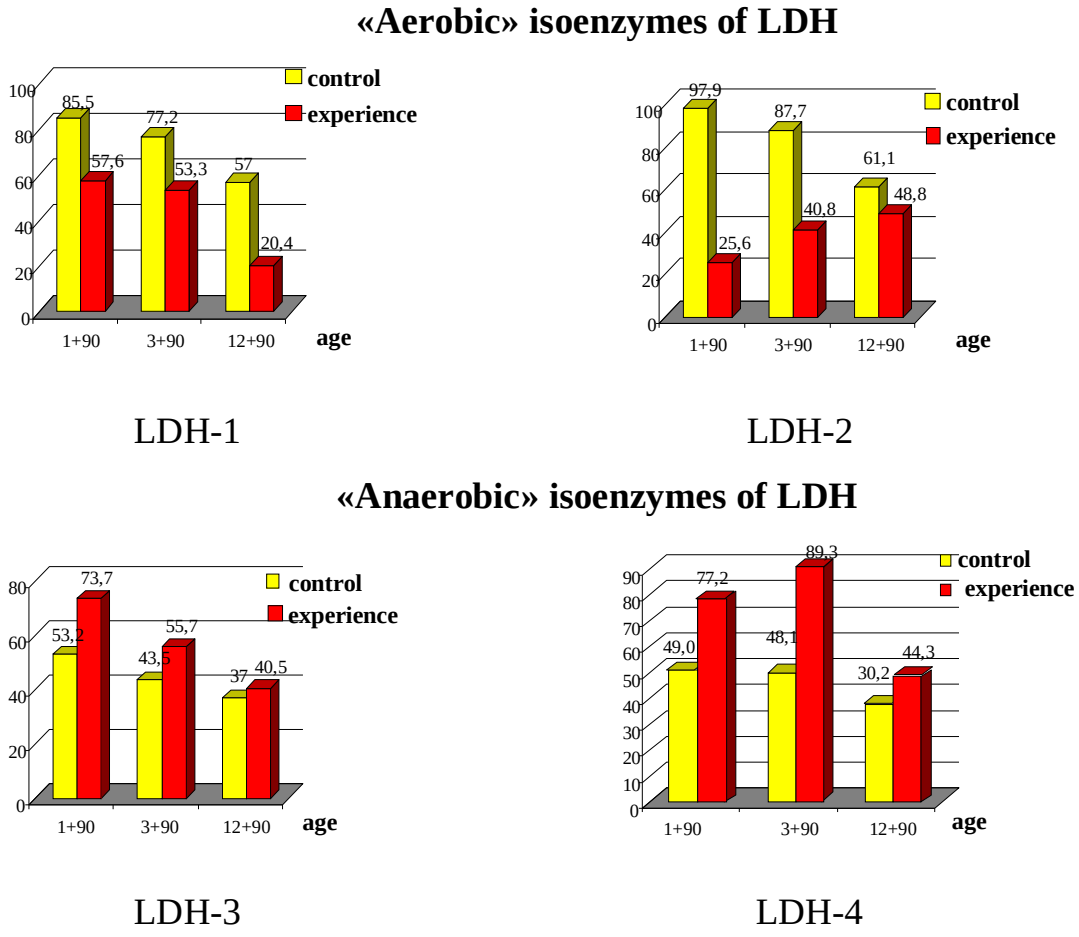

Fig.4. Histogram of LDH isoenzyme activity indicator in fibrous ring cells after 90-day run. Age of animals: 1, 3 and 12 months + 90 days of running

In series 1 + 90 of hyperkinesia, the activity of LDH isoenzymes varied differently. A decrease in the activity of LDH-1 isoenzyme (by 32.6%) and LDH-2 (by 73.8%), but an increase in the activity of LDH-3 and LDH-4 isoenzymes (by 27.8 and 6.6%, respectively).

The activity gradient of LDH isoenzymes (in descending order) to the cells of the intervertebral discs of 1-month-old rats after a 90-day run had the following sequence: LDH-4 – LDH-3 – LDH-1 – LDH-2.

In the series 3 + 90 hyperkinesia, it was revealed changes in the activity of LDH isoenzymes: the activity of LDH-1 decreased by 30.9%, LDH-2 – by 53.5%; at the same time, the activity of LDH-3 increased by 21.9%, and LDH-4 – by 46.1%.

The activity gradient of LDH isoenzymes of 3-month-old rats after 90-day run: LDH-4 – LDH-3 – LDH-1 – LDH-2.

In the series of 12 + 90 hyperkinesia, the change in the LDH isoenzymes activity after prolonged hyperkinesia was expressed by a decrease in the activity of LDH-1 (64.8%) and LDH-2 (20.1%) isoenzymes and an increase in LDH-3 activity (8.6%).) and more significantly – LDH-4 (by 31.8%).

As a result, the gradient of activity of LDH isoenzymes of 12-month-old animals changed: LDH-2 – LDH-4 – LDH-3 – LDH-1.

Therefore, under conditions of long-term dynamic loads, the decrease in LDH activity in adult animals is accompanied by a change in the ratio of the activity of its isoenzymes.

In the cells of the intervertebral discs of young animals after a 90-day run, there is a clear tendency to reduction of the activity of isoenzymes LDH-1 and LDH-2 and growth of the activity of isoenzymes LDH-3 and LDH-4.

In animals of older age groups, in conditions of hyperkinesia there was the most significant decrease in LDH activity. The analysis of the activity of isoenzymes revealed a decline in the activity of LDH-1, and then LDH-2 with increasing LDH-4 and less significantly – LDH-3. Thus, animals of different ages have similarities in the response of cells to high dynamic loads, but with some differences in the quantitative expression of these reactions.

Among the isoenzymes LDH-1 and LDH-2 belong to the more "aerobic" isoforms, LDH-3 and LDH-4 – to the "anaerobic". The increase in the activity of anaerobic LDH isoenzymes can be compensation for the decrease in the activity of total LDH under conditions of prolonged dynamic loading and serve as a marker of reduced aerobic oxidation in the glycolysis system.

Thus, in the conditions of hyperkinesia there were changes in the activity of total LDH, the general direction of which was its reduction in animals of mature and older groups with a subtle response of isoenzymes to changes in the structure and function of intervertebral discs. Only in immature animals, the activity of total LDH, during prolonged running, increased.

The decrease in the activity of total LDH was accompanied by a decrease in the activity of more "aerobic" LDH-1 and LDH-2, but an increase in the activity of more "anaerobic" LDH-3 and LDH-4 isoenzymes of LDH.

In this case, changes in carbohydrate metabolism in conditions of prolonged hyperkinesia are combined with the activation of apoptosis of cells of the intervertebral disc and hyaline cartilage [4]. Activation of apoptosis – a normal, genetically programmed process of cell death during the development of the organism, indicates the acceleration of involutive processes in the intervertebral discs under prolonged dynamic loads, and the spread of dystrophic processes. This confirms the high mechanosensitivity to physical exercise not only hyaline [13] but also fibrous cartilage.

Evaluation of LDH activity, and especially its isoenzyme spectrum, has a high diagnostic value and is used in the diagnosis of a number of diseases [14, 15]. However, most studies are based on biochemical analysis of serum and give only indirect information about intracellular metabolism in tissue cells. The presented histoenzymological data can be used in the analysis of biopsy material, the possibility of obtaining which is currently increasing due to the expansion of methods for diagnosing pathology of the spine [3, 8].

Conclusions / Discussion

Histoenzymological and cytophotometric methods are sensitive and accurate methods for determining the activity of enzymes [12]. The fibrous ring of the intervertebral disc is made of dense conjunctive and fibrous cartilaginous tissue, the cells of which have high glycolytic activity. The analysis showed that the dynamic load contributes to the biochemical changes in the activity of LDH in the cells between the vertebral discs.

After a 20-day run, the activity of total LDH in fibrous ring cells decreased in animals of all ages with a maximum decrease in young rats. After a 90-day run, the total LDH activity was more stable. A significant decrease in its activity was found only in older rats. Regarding LDH isoenzymes, the change in their activity was multidirectional. A characteristic response to dynamic loads was a change in the cells of the ratio of the activity of "aerobic" and "anaerobic" isoenzymes of LDH, which depended on the age of the animals and the duration of the run.

Normally, the intervertebral disc has the highest activity of LDH-2, then - LDH-1, ie aerobic isoforms of LDH. LDH-4 isoforms have the lowest activit. At the same time, the activity of all LDH isoforms decreased with age.

Prolonged physical activity caused a decrease in the activity of aerobic isoforms – LDH-1 and LDH-2 and especially, more than twice, in young animals after a 20-day run.

The 90-day run also conduced to reduction of activity of aerobic isoforms and especially LDH-2 in young animals. Regarding anaerobic isoforms of LDH, their activity increased in rats of the studied age groups, and especially after a 90-day run.

It is known that when LDH activity decreases, metabolic acidosis develops, which contributes to the disruption of tissue structure [6]. Damage to the tissues of the intervertebral disc is a prerequisite for damage to the motor spinal segment as a whole. Therefore, the obtained indicators of LDH activity and, personally, its isoenzymes can serve as a histochemical test for an objective assessment of the degree of damaging effect of prolonged running on fibrous cartilage at different ages.

Prospects for further research. Further research can be aimed at elucidating the change in the activity of enzymes of the redox cycle under dynamic loads to increase the reliability of the diagnosis of the condition of the intervertebral disc under exercise.

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