

Structural rearrangements of the spinal-motor segment with prolonged dynamic loads

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Purpose: to study structural rearrangements of intervertebral discs and adjacent structures of the spine of rats and the possibility of their preservation under conditions of prolonged dynamic loads in the experiment.

Material & Methods: study was performed in an experiment with 90 male rats of the Wistar line, using a training run in a linear treadmill for 20 and 90 days. On the histotopographic sections, after injection into the aortic arch of the animal mascara-gelatin mass, the number of capillary glomeruli in the subchondral parts of the vertebral bodies was counted. Using standard histology methods, structural changes in metaphyseal cartilages, apophyses of vertebral bodies and intervertebral discs of the lumbar spine.

Result: The running regimes are defined that cause damage to various structures of the spine and, on the contrary, increase their reliability.

Conclusion: it is established that with age the number of blood vessels in the bodies of the vertebrae and capillary glomeruli in their subchondral regions gradually decreases, which is accompanied by a tightening of the intervertebral disk and contiguous structures. Different in intensity and duration of physical activity cause a corresponding change in the number of blood vessels in the vertebrae and, as a consequence, the level of their blood supply.

Keywords: running of animals in the treadmill, vertebral-motor segment, intervertebral disc, capillary glomeruli of subchondral parts of vertebral bodies.

Introduction

The issues of adaptation of biosystems to physical loads are given great attention in physical culture and sports [1; 2; 4]. Physical load is the most important exogenous factor of activation of metabolic processes in the cell and a powerful stimulus of adaptive rearrangements of bone and cartilage [2; 8; 16]. In record sports, training loads of maximum intensity are often used, considering them to be the best way to improve results. Nevertheless, the myth about the benefits of such training is gradually debunked [9; 15]. One of the severe complications of physical overload is dystrophic joint damage [7; 10] and intervertebral (IV) discs [6; 12].

Preservation and improvement of structural reliability of the ventral spine in conditions of dynamic loads is actual for physical culture and sports.

Among the diseases that affect the vertebral-motor segments, degenerative diseases occur much more often than neoplasms, inflammatory diseases and pathologies of development, and therefore degenerative diseases of the spine become of primary clinical importance [3; 5; 18], including in sports [4; 6]. At the same time, it is avascular cartilaginous structures of the spine and braditrophic structures of the intervertebral disc that are especially damaged during physical overloads [13] and especially in young individuals [12; 17]. As for the comparative reaction of IV discs to different modes of dynamic loads, these studies are few [11; 12].

IV disk is the central link of the vertebral-motor segment, the defeat of which triggers the dystrophic processes of contiguous spinal structures and leads to the development of osteochondrosis [3; 10; 19]. Osteochondrosis of the spine

is a multifactorial destructive-dystrophic disease, primarily affecting the intervertebral disc, and then other elements of the spine [3; 5; 10]. At the same time, the IV disk is constantly adapted to new types and regimes of motor activity [3; 4]. Practically important is the problem of "fatigue" of fibrous structures and cartilage [7]. With increased loads on the locomotor apparatus, the strength of IV disks, capsules of joints, ligaments and other braditrophic structures decreases, which causes their dystrophic lesions [6; 10]. Symptoms of overstrain of the structures of the spine are the cause of pain in the back, and in sports – the reason for the decrease in athletic performance [1; 4; 9; 13]. Overloads and, as a result, accelerated aging of joints and IV discs in athletes of a number of sports specializations often cause premature departure from sports [4; 6; 9].

Relationship of research with scientific programs, plans, themes. The research was carried out within the framework of the department theme of the research work "Medico-biological justification for carrying out rehabilitation activities and the appointment of physical rehabilitation to young people of different levels of fitness"

Purpose of the study: to study the structural rearrangements of IV discs and adjacent structures of the spine of rats and the possibility of their conservation under conditions of prolonged dynamic loads in the experiment.

Material and Methods of the research

The study was carried out on 90 white male rats of three age groups: 1, 3 and 12 months. Work with laboratory animals was carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrates, which are

used for experimental and other scientific purposes" [14].

Two groups of rats, three ages, 30 animals in each group participated in the experiment. The control group consisted of 30 animals of the same age. The optimum speed of movement of the treadmill tape ($40 \text{ m}\cdot\text{min}^{-1}$), which allowed using long running for all age groups in the experiment, was chosen experimentally. The load increased stepwise for 6 minutes of daily running in the first week with a sequential increase of 6 minutes in each following week of training. As a result, rats of the 1st group ran for 10 days 10560 m, and rats of the second group in 90 days – 17280 m.

The material was studied by the methods of macro-microscopy and standard histology with the coloring of preparations by hematoxylin-eosin and picrofuxin according to Van Gieson.

To estimate the condition of the sources of diffusion nutrition of the avascular structures of the spine (IV disks, metaphyseal cartilages, cartilaginous apophyses of the vertebral bodies), the blood supply of the subchondral divisions of vertebral bodies adjacent to the IV has been studied. The method of filling the vessels with a 5% solution of carcass with gelatin was used. The pour was made by a syringe in the arch of the aorta after opening under ether anesthesia of the thorax. Capillary glomeruli were counted on the enlightened sections of the lower lumbar motor segment (in rats this is the L-5-L-6 segment). Number of ink-stained capillary glomeruli was counted in the subchondral parts of the vertebral bodies in the four zones of the subchondral parts of the bodies of adjacent vertebrae:

- 1 zone – the ventral part of the body of the cranial vertebra;
- 2 zone – dorsal area of the body of the cranial vertebra
- 3 zone – the ventral part of the body of the caudal vertebra
- 4 zone – dorsal area of the body of the caudal vertebra.

The evaluation of statistical differences was determined using the Student's test.

Results of the research and their discussion

Age changes are traced in the lumbar IV discs and adjacent structures of the ventral spine. A specific feature of the rat disc is the preservation of the nucleus pulposus represented by the dorsal chord fragment in all the age groups studied.

In immature 1-month-old rat rats, the disks had a voluminous gelatinous nucleus and a lamellar fibrous ring. The gelatinous nucleus contained clusters of notochordal cells among the copious matrix. In vertebral bodies, a large number of vessels injected with mascara-gelatin mass were detected. At the base of the vertebral bodies, the vessels terminated with terminal microvessels in the form of capillary glomeruli. Neither the gelatinous nucleus nor the fibrous ring of the IV discs of the blood vessels contained. The greatest number of glomeruli was observed in 1-month, the smallest – in 12-month-old animals.

Under the influence of varying physical activity, a change in the number of vessels in the structures of the spine was observed.

After a 20-day run, the animals of the 1st group, in comparison with the control group, determined the increase in the number of capillary glomeruli at the base of the vertebral bodies. The structure of the apophyses of vertebral bodies, growth plates and MP disks remained unchanged. In the paravertebral muscles of the lumbar level, the number of blood vessels that followed the direction of the muscle fibers increased. Vascular injuries not detected.

In 1-month-old animals, the number of capillary glomeruli in the subchondral parts of the vertebral bodies was greatest. At the same time, their higher content was noted in the 1 and 3 zones, which corresponds to the ventral sections of adjacent vertebrae. The number of capillary glomeruli in the 1st zone was more than in the 2nd by 8,07%; and in the third zone is more than in the 4th by 10,41%.

In 3-month-old animals, compared with 1-month-old animals, the number of capillary glomeruli decreased in all zones equally. The difference in the number of glomeruli in the four zones was small. In the 1st zone it is 3.87% more than in the 2nd zone, in the third zone it is 3,32% more than in the 4th zone.

In 12-month-old animals, a decrease in the number of capillary glomeruli was more significant. Compared to 1-month-old animals, the number of glomeruli decreased in the 1st zone in 3-month-old rats by 16.68%, in 12-month rats – by 53,22%.

After a 20-day run, an increase in the number of capillary

Table 1

Changes in the number of capillary glomeruli in the subchondral parts of vertebral bodies in the age aspect under conditions of 20-day hyperkinesia, $\bar{X} \pm m$

Series experiments		1 (n=10) Control	2 (n=10) Experiment	Assessment of statistical significance		
				t	p	
1+20	Zones in the bodies of the vertebrae	1	31,0±1,019	35,8±1,13	$t_{1,2}=4,95$	$p_{1,2}<0,001$
		2	28,5±1,056	33,0±1,46	$t_{1,2}=2,49$	$p_{1,2}<0,05$
		3	28,8±1,302	32,5±2,10	$t_{1,2}=1,48$	$p_{1,2}<0,2$
		4	25,8±1,195	28,5±1,58	$t_{1,2}=1,35$	$p_{1,2}<0,2$
3+20	Zones in the bodies of the vertebrae	1	25,8±2,309	28,5±1,40	$t_{1,2}=0,99$	$p_{1,2}>0,05$
		2	24,83±0,703	27,7±0,88	$t_{1,2}=2,52$	$p_{1,2}<0,05$
		3	25,0±1,602	30,0±1,46	$t_{1,2}=2,31$	$p_{1,2}<0,05$
		4	24,17±1,602	26,8±1,57	$t_{1,2}=1,17$	$p_{1,2}>0,05$
12+20	Zones in the bodies of the vertebrae	1	14,5±2,93	19,0±1,06	$t_{1,2}=1,44$	$p_{1,2}<0,2$
		2	11,06±0,919	18,2±1,22	$t_{1,2}=4,65$	$p_{1,2}<0,001$
		3	13,66±1,116	17,8±3,69	$t_{1,2}=1,08$	$p_{1,2}>0,05$
		4	9,5±0,763	17,5±0,99	$t_{1,2}=6,40$	$p_{1,2}<0,001$

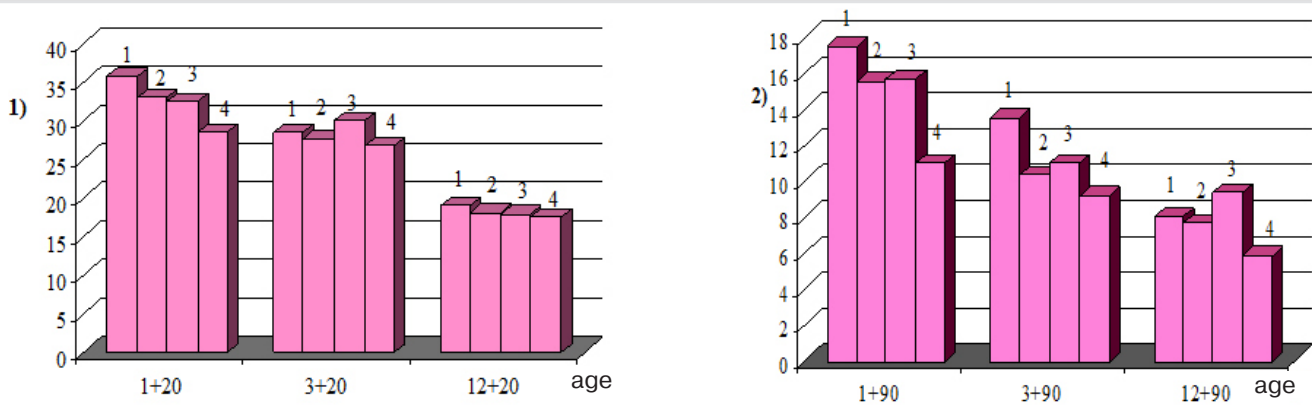


Figure 1. Indicators of the number of capillary loops in the subchondral areas of the vertebral bodies adjacent to the IV discs, respectively, four zones under running conditions: 1 – after a 20-day run, 2 – after a 90-day run

glomeruli in the 1st zone was observed in rats of the series 1+20 and 3+20 hyperkinesia, and in rats of the 12+20 series in all the investigated zones (Table 1).

After a 90-day run, the number of capillary glomeruli in animals of all age groups, and especially in old animals, on the contrary, decreased. The maximum decrease in the number of glomeruli was noted in the dorsal zones of the vertebral bodies (zones 2 and 4).

In general, compared with the control series, the number of capillary loops in each age group increased after a 20-day run, but decreased after a 90-day run (Figure 1).

Typical was the appearance of newly formed blood vessels in the fibrous ring, especially in the ventral areas of the discs. Here networks of blood vessels were determined, some of which followed between fibrous disc plates.

In accordance with the change in the level of diffusion power, the structure of the discs changed. Dystrophic lesions of the MP tissues were preceded by ingrowth into the avascular IV of the blood vessels, which disrupted the fine structure of the disc and potentiated dystrophic changes in its tissues.

On the inner layer of the fibrous ring, numerous gaps were found, surrounded by acellular regions and damaged by a matrix. At the same time, signs of intradisk dislocation of the gelatinous nucleus, protrusion of the inner sections of the fibrous ring to the surface of the disk, and ruptures of collagen fibers in the ventral regions of the fibrous ring.

These changes in the IV disk were accompanied by rearrangements of adjacent cartilaginous structures of the ventral spine. Metaphyseal cartilage (growth plates) lost the characteristic zonality of the structure, in them the cell-free areas expanded. Apophyses of vertebral bodies underwent deforma-

tion and uneven ossification. In the paravertebral muscles of the lumbar level, the natural geometry of the vessels' motion was disturbed, their diameter changed.

Conclusions

Diffusive nutrition of the MP disk comes from the capillary glomeruli of the blood vessels of the vertebral bodies. With age, the number of blood vessels in the bodies of the vertebrae and capillary glomeruli in their subchondral areas gradually decreases, which is accompanied by a tightening of the intervertebral disk and adjacent structures. Different in intensity and duration of physical activity cause a corresponding change in the number of blood vessels in the vertebrae and, as a consequence, the level of their blood supply.

After a 20-day run, the number of capillary glomeruli increased, and most of all in the 1st zone of the vertebral bodies (at the ventral surface), and in the 12+20 hyperkinesia animals, the number of vessels increased in all the investigated zones. Under these conditions, the metaphyseal cartilage retained the zoning of the structure, and the MP disc its structural integrity, without signs of damage.

After a 90-day run, the number of capillary loops compared to the control series decreased in animals of all age groups, and it was uneven in different parts of the base of the vertebral bodies. The most significant decrease was found in the dorsal parts of the bases of vertebral bodies (2 and 4 zones). These changes were combined with the spread of dystrophic processes in the tissues of MP disks and adjacent avascular structures, which indicates a decrease in the level of their diffusive nutrition.

Prospects for further research. The purpose of further research is to find ways of reparative regeneration of the structures of the spinal column.

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References

1. Laputin, A.M. (2001), *Biomekhanika* [Biomechanics], Olimpiiska literatura, Kyiv. (in Ukr.)
2. Kornilov, N.I. & Avrunin, A.S. (2001), *Adaptatsionnye protsessy v organakh skeleta* [Adaptation processes in the organs of the skeleton],

MOR-SAR AV, SPb. (in Russ.)

3. Kremer, Yu. (2013), *Zabolevaniya mezhpozvonkovykh diskov* [Intervertebral disc diseases], trans. with English, V.A. Shirokova (red.), MYeDpress-inform, Moscow. (in Russ.)
4. Kolesnichenko, V.A. & Straude V.A. (2005), "Vertebrogenic aspects of athletic selection and orientation of athletes", *Sportivna meditsina*, Vol. 1, pp. 171-174. (in Russ.)
5. Kolotusha, V.G., Rudenko, A.Ye. & Kadyrova, L.A. (2005), *Osteokhondroz poyasnichnogo otdela pozvonochnika* [Osteochondrosis of the lumbar spine], Kiev. (in Russ.)
6. Levenets, V.N. (2002), "Sports injuries – diagnosis, clinic and treatment", *Mater. I Vseukr. z'izdu fakhivtsiv iz sportyvnoi medytsyny i LFK z mizhnarodnoiu uchastiu* [Materials I Allukr. the Congress of Sports Medicine Specialists and Excellence with International Participation], Odesa, pp. 32-35. (in Russ.)
7. Mikhaylov, A.N. (2013), "Organizational and clinical aspects of the prevention and diagnosis of fatigue (stressful) bone fractures in athletes", *Voprosy organizatsii i informatizatsii zdravookhraneniya*, No. 2, pp. 63-71. (in Russ.)
8. Pikalyuk, V.S. & Chernov, A.T. (2005), "Changes in bone growth indexes in rats of different age groups under the influence of hypergravity", *Ukrainskiy medichniy almanakh*, Vol. 8, No. 1, pp. 137-142. (in Russ.)
9. Platonov, V.P. (2004), *Sistema podgotovki sportsmenov v olimpiyskom sporte. Obshchaya teoriya i ee prakticheskie prilozheniya* [System of training athletes in the Olympic sport. General theory and its practical applications], Olimpiyskaya literatura, Kiev. (in Russ.)
10. Radchenko, V.O., Petrenko, D.Ye., Golubeva, I.V. & Bengus, L.M. (2008), "Actual problems of arthrology and vertebralogy" (based on the materials of the International Conference dedicated to the 100th anniversary of the establishment of the State Institution "Institute of Spine and Joint Pathology named after Prof. M.I. Sitenko of the Academy of Medical Sciences of Ukraine", *Ortopediya, travmatologiya i protezirovanie*, No. 1, pp. 111-117. (in Russ.)
11. Sak, A.Ye. (2005), "Age differences in the response of the spinal column to high dynamic loads", *IX Mizhnarodniy naukoviy kongres "Olimpiyskiy sport i sport dlya vsikh" 20–23 veresnya 2005 r.* [IX International Scientific Congress "Olympic Sport and Sport for All" September 20–23, 2005], Kiiv, pp. 830. (in Russ.)
12. Sak, A.E. (2007), "Peculiarities of the reaction to physical load of the spine structures with different levels of blood supply", *Biomedical and Biosocial Anthropology*, No. 9, pp. 268-269. (in Ukr.)
13. Chertenkova, Ye.V. (2006), "Mechanosensitivity of cartilage", *Ortopediya, travmatologiya i protezirovanie*, No. 3, pp. 124-129. (in Russ.)
14. Council of Europe (1986), *European convention for the protection of vertebral animals used for experimental and other scientific purpose*, 18.03.1986, Strasbourg.
15. Petibois, C., Cazorla, G., Poortmans, J.R. & Deleris? G. (2002), "Biochemical aspects of overtraining in endurance sports", *Sports Med*, Vol. 32 (13), pp. 867-878.
16. Weineck, J. (1996), *Sportbiologie, Balingen: Perimed-spitta*, Med. Verl. Ges.
17. Weinstein, S.L. (2003), *The Pediatric Spine. Principles and Practice*, Raven Press, New York.
18. Iorio, J.A., Jakoi, A.M. & Singl, A. (2016), "Biomechanics of Degenerative Spinal Disorders", *Asian Spine J*, No. 10(2), pp. 377-384.
19. Hui Li, Jia-zhi Yan, Yong-jie Chen, Wei-bo Kang & Jia-xi Huang (2017), "Non-invasive quantification of age-related changes in the vertebral endplate in rats using in vivo DCE-MRI", *Journal of Orthopedic Surgery and Research*, Nov No. 12(1), pp. 169.

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