Disturbances of structure and function of paravertebral muscles are one of the risk factors of lumbar spine degenerative diseases. It is proved that muscles play an important role in spine fusion and in other bone fusion. Objective: to analyze the relationship between the results of the posterolateral lumbar fusion after stabilization of L2–L3 vertebrae with the use of transpedicular screws and structural features of m. multifidus caused by different levels of physical activity. Methods: 20 laboratory rats were divided in four groups, each of five animals: I — have swum before and after surgery; II — have swum before surgery only; III — have swum after surgery only; IV — have not swum. All animals underwent posterolateral spinal fusion, transpedicular construction was mounted at L2–L3 level. X-ray examination was performed directly after surgical procedure, and 3 months later. Histologic study was performed for evaluating of m. multifidus and spine fusion area. Results: using X-ray examination the signs of formed posterior spine fusion were observed 3 months after operation in 80 % animals of I group, in 60 % of II, in 40 % of III, in 20 % of IV. Histologically it was established that the degree of muscles changes directly correlates with the quality of spine fusion. Minimal signs of muscle fibers destructive changes, as well as maximum observations of formed spine fusion, were registered in the I group under increased physical activity regimen, while most evident destructive changes and the worst results — in IV group with low physical activities. We have established weak but statistically significant correlations between increased physical activity and reduction of the content of adipose (p = 0.010512) and fibrous (p = 0.019142) tissue in the muscles of operated rats. Conclusion: physical activity has a positive influence upon functional and adaptive muscle capacity and forming of posterior spine fusion. Key words: spine fusion, vertebral fusion, m. multifidus, histology, vertebrae stabilization, in vivo study.

Key words: spine fusion, vertebral fusion, m. multifidus, histology, vertebrae stabilization, in vivo study
**Introduction**

Problem of degenerative spine diseases treatment remains the issue of the day due to substantial number of people suffering from low-back pain. That is why physicians all over the world make great efforts to study pathogenesis and methods of the treatment of this pathology.

Paravertebral muscles disturbance is considered one of the risk factors of lumbar spine degenerative diseases [1–4]. Paravertebral muscles, as stabilizers of spine, are of primary importance in spine mechanical stability support, and protect spine structures from destruction caused by load bearing.

Changes in paravertebral muscles with age [5], as a result of injuries or degenerative processes, unavoidably lead to their dysfunction [7, 8], which may cause chronic low back pain [8–10].

Specialists pay certain attention to paravertebral muscles effect on spine fusion development, which remains the «gold standard» in treatment of various diseases and traumatic injuries of the spine [11–14]. However, according to different authors, the non-union numbers come up to 40 % [15]. In some cases the reasons of healing failures are clear, and can be accounted for known factors: for example, diabetes mellitus or tobacco smoking, which interferes with neurovascular processes [16–18]. But more often the causes of unsatisfactory results of vertebral fusion are missing. That is why a lot of scientific works are devoted to the study of biological mechanisms of spine fusion [17–20].

It is proved that muscles play an important role in spine fusion and in other bones fusion. Muscles provide vascular invasion into bones fusion area, serve as a source of oxygen and growth factors, create favourable conditions for cellular migration [18, 21, 22]. M. Bawa et al. [18] have studied porous or nonporous barrier sheets in spinal arthrodesis area, and have established that nonporous barrier sheets in spinal arthrodesis area, or nonporous barrier sheets in spinal arthrodesis area, produce vascular invasion into bones fusion area, and have established that nonporous barrier sheets in spinal arthrodesis area, as a result of injuries or degenerative processes, unavoidably lead to their dysfunction [7, 8], which may cause chronic low back pain [8–10].

Objective: to analyze the relationship between the results of the posterolateral lumbar fusion after stabilization of L₁–L₅ vertebrae with the use of transpedicular screws and structural features of m. multifidus caused by different levels of physical activity.

**Material and methods**

Experimental study is approved by the Bioethics Committee of Sytenko Institute of Spine and Joint Pathology (report № 101, May 14, 2012.), according to the rules of «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» and «On Animals Protection of Cruelty» Ukrainian Law regulations [25].

**Animals**

Experimental modeling is performed on 20 laboratory rats (5 months old, weight from 430 to 500 grams) from Sytenko Institute of Spine and Joint Pathology experimental-biological clinic. Animals were divided in four groups, each of five rats:

- I — have swum before and after surgery;
- II — have swum before surgical procedure only;
- III — have swum after surgical procedure only;
- IV — have not swum.

Swimming regimen before surgery is aimed to refine structural and functional muscle state. Swimming after surgical procedure is considered as physical and functional rehabilitation. Experimental rats were swimming five days in a week for 40 minutes each day. The regimen without swimming is regarded as hypodynamia caused by lack of motion and strenuous activity because of small cage sizes.

**X-ray examination** in anteroposterior and lateral plain is performed for each animal directly after surgical procedure, and 3 months later.

**Surgical procedures** were performed under aseptic conditions and general anesthesia (aminazine — 10 mg/kg, ketamine — 50 mg/kg). Two facial incisions were made bilaterally, the paraspinous musculature was exposed and blunt dissection was used.
to separate the longissimus and multifidus muscles of two adjacent vertebrae.

The transpedicular construction and the fixation technique of lumbar spine vertebrae were used, which had been developed and patented [26, 27].

Screws were transpedicularly implanted into adjacent vertebrae bodies, and after this the authoring construction was mounted at L₄₋₅ level (fig. 1).

Autologous grafting was performed during the operation: posterior parts of facet joints are resected to make place for screws installation and to fulfill decortication, but facet joints bone tissues are left in the wound. After the antibacterial agent application the wound is sutured up layer-by-layer. The rats were killed 3 months after surgery with ether.

**Histology**

For the histologic study *m. multifidus* was exposed from each animal on three levels: cranial (L₃₋₄), at the level of surgical intervention (L₄₋₅), and caudal (L₅₋₆) to the operating area. The lumbar spine (L₃₋₆) was also examined after decalcification. After routine histological tissue follow-up procedures, specimens (*m. multifidus* and lumbar spine) were embedded in celloidine. Histological sections (7 µm in thickness) were prepared using microtom and stained with hematoxylin and eosin (H&E), van Gieson Stain (mixture of Picric Acid and Acid Fuchsin). Histological slides were evaluated under Olympus BX-63 (Japan) light microscope and photographed with a «CellSensDimension» ver. 510 image analysis system.

The result of surgery was evaluated as satisfactory, if histological study detected new formed bone tissue at the levels of grounds spinous processes of vertebra, facet joints and interlaminar space.

**Histomorphometric study** of *m. multifidus* was performed with «CellSensDimension» software, version 510 (Olympus Soft Imaging Solution GmbH, 2013) for Olympus BX-63 microscope. Miofibrils mean diameter is gauged pursuant to described methodology [24, 28]. Relative areas of adipose, connective and muscular tissues were also measured.

Posterolateral lumbar fusion was assessed using clinical, radiological and histological methods.

According to clinical approach, the fusion was regarded as formed, if the consistent osseous tissue could be detected macroscopically at the level of operated spinal segment, and the mobility of operated segment was absent after transpedicular contraction removal.

Using X-ray we proved the completely formed fusion occurs in a case when proper construction positioning was obvious in anteroposterior and lateral plain roentgenogram after 3 months surgical aggression, in addition to blurriness of closing plates contours and operated segment inter-body gap due to bone deposition of.

In compliance with histological methods, the fusion was considered as (completely) formed, if new formed osseous tissues are detected at the levels of spinous processes of vertebra, facet joints and interlaminar space.

**Statistical Analysis**

Kruskal-Wallis ANOVA was applied to ascertain significant differences in morphometric indices (i. e. muscle fibers mean diameter, and muscular, connective and adipose tissue relative areas) between experiment series (Kruskal-Wallis test statistic is denoted in paper by K-WH). Further pairwise comparisons are performed using Mann-Whitney test (Mann-Whitney test statistics is denoted in paper by M-WZ) with Benjamini–Hochberg correction procedure for multiple comparisons.

**Fig. 1.** Transpedicular construction and its implementation tools (a); rat’s operating wound with transpedicular construction at L₄₋₅ level (b)
Statistical significance of dependences between qualitative features (a physical activity regimen and a surgery outcome) was assessed using contingency tables analysis. Fisher exact test was applied due to small sample sizes. To estimate the significance of differences in one-way frequency tables for separate groups of animals, one-sided binomial test was evaluated. To estimate the strength of relationship between physical activity regimen and surgery outcome, or morphometric indices values, rank Gamma (G) correlation coefficients are evaluated.

All calculations are performed with $P = 95\%$ confidence probability, so statistical significance is established at $p$-level $< 0.05$.

**Results**

A satisfactory arrangement of the structure was observed in all animals immediately after surgical intervention and 3 months later using X-ray (fig. 2).

In carrying out *m. multifidus* microscopic analysis for *I series* specimens (animals swimming before and after surgery), the following structural features at LIV–LV level (the level of surgical procedure) were detected. Most of muscle fibers had a normal structure, remained polygonal shape in transverse sections, and had transverse streaks in longitudinal sections.

Elongated nuclei located uniformly at the periphery of the myofibers. Muscle fibers which were covered by perimysium adhered one to another closely. However, isolated swollen muscle fibers with homogeneous sarcoplasm and flattened pyknotic nuclei were present. The increased nuclear density caused by reparative processes was observed in certain areas.

The vessels of typical structure were located in endomysium and in perimysium (fig. 3), but the thickened vascular walls were noticed in some places, which is an indicator of vessels sclerosis. Muscle fibers replacement with adipose tissue was observed in certain areas.

The histological analysis performed on animals of *series II* (which have swum only before surgical intervention), reveals that *m. multifidus* fibers located at the level of surgical intervention are mostly characterized by retained polygonal form in transverse sections, and had transverse streaks in longitudinal sections. That is, like in *I series* animals, the muscle structure complies with the regular one in the major part of area.

But there also were areas, where muscle fibers have a swollen form, containing homogeneous sarcoplasm and flattened nuclei, which is an indicator of degenerative process. In addition, unlike *I series* animals, the excrescence of fibrous tissue in perimysium was observed between muscle fiber bundles at all observable levels, and it was most evident at the level of operation. The thickened vascular walls in perimysium occur more often than in *I series* animals, which may result in blood supply disturbance of muscle and of spinal fusion area (fig. 4).

It is statistically confirmed, that mean diameter of muscle fibers at the level of surgical invasion is significantly less in *II series* animals, than in *I series* ones ($M-WZ = 3.087233, p = 0.002020$). But there is no significant difference between *I* and *II series* animals in percentages of muscular, adipose and connective tissues (table).

Reparative processes are connected with nuclei hyperplasia, which were oblong, of oval shape, and hypochromic in such areas.

Animals, which have swum after surgical operation only (*III series*). *M. multifidus* changes similar to *II series* rats were demonstrated. However, their destructive changes were more marked at the level of surgical procedure. Particularly, connective tissue spreads out not only in perimysium, but also in endomysium. Among the muscle fibers there were those in which the nuclei migrated from the periphery to the center, which also depicts muscle fibers destruction. The vessels with thickened vascular walls
were detected in perimysium, which is the evidence of blood circulation disturbance (fig. 5). The mean diameter of muscle fibers at the level of operation was significantly less in III series animals, than in those of I and II series (table; in comparison with I series: M-WZ = 6.945576, p = 3.7763·10^{-12}; in comparison with II series: M-WZ = 6.435580, p = 1.231759·10^{-10}).

During histological analysis of *m. multifidus* in non-swimming rats (*IV series*) at the level of surgery there were significant number of muscle fibers which had irregularly stained sarcoplasm and features of destructive changes, which are manifested by fiber swelling and sarcoplasm homogenization. The loss of muscle fibers polygonal form was detected in transverse sections. In swollen fibers nuclei were mostly located in the center (internal nuclei), which reflects the destructive processes. The transverse streaks were observed in isolated fibers in longitudinal sections. Degenerative disorders were also connected with the presence of separate fibers with the features of wax-like necrosis.

There were some atrophic changes of muscle fibers, which were shown by their thinning and longitudinal bundle, which may be associated with the restructuring as a result of reduced muscle function. Significantly less values of mean diameter of muscular fibers at the level of operation comparative to all the rest experimental series can be proved statistically (table; in comparison with I series: M-WZ = 9.397529, p = 5.6209·10^{-2}; in comparison with II series: M-WZ = 12.17904, p = 0.000; in comparison with III series: M-WZ = 6.956756, p = 3.4886·10^{-12}).

The great majority of nuclei are in a state of pyknosis, but muscle fibers with increased density of large hypochromic nuclei are found, which indicates repair processes.

Muscle fibers replacement with adipose tissue was observed in substantial areas which indicates the adipose degeneration. Statistical analysis has proved the significant extension of adipose tissue at the level of surgical intervention in comparison with I series animals (table; W-WZ = –2.45537, p = 0.014074).

Connective tissue ex crescence and endomysium edema are noticed. Statistical analysis of aggregated data taken not only at the level of surgical intervention, but also at caudal and cranial levels in addition, prove significant difference in relative content of connective tissue between experimental animals of IV and I series (K-WH (3, 116) = 8.0873, p = 0.0442; M-WZ = –2.605, p = 0.0082). Besides this, muscular fibers of IV series animals were characterized by various shapes and sizes in transverse sections: there orbicular turgid fibers of enlarged diameter are detected among them, as well as small angled fibers, which reflects dystrophic processes course. The abundance of vessels with thickened vascular walls and narrowed internal space was observed in perimysium and endomysium (fig. 6).

Spinal fusion in different experiment series animals

The degree of changes in muscles directly correlates to spondylosyndesis quality. Maximum number of observations with formed spine fusion was met in the group of animals with increased muscle activity (which had swum before and after operation; I series). The results indicate positive effect of muscular exercises on back spine fusion forming.

The signs of formed posterior spine fusion were observed 3 months after operation in 80 % of I series animals (which had swum before and after surgical intervention). In addition to X-ray data, the density of bone tissue was instrumentally and visually assessed in wound at the operated segment level during transpedicular constructions deinstallation.

### Table

Rats’ muscles morphometric parameters at the level of surgery depending on motion activity (experiment series): mean muscle fibers diameter (µm) and tissues relative area (%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment series (M ± m)**</th>
<th>Significance of Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Diameter</td>
<td>58.01 ± 1.66 (II, III, IV)*</td>
<td>52.14 ± 0.65 (II, III, IV)*</td>
</tr>
<tr>
<td>Muscle tissue</td>
<td>95.43 ± 1.84</td>
<td>92.83 ± 1.17</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.59 ± 0.32 (IV)*</td>
<td>1.54 ± 1.54</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>3.98 ± 1.84</td>
<td>5.59 ± 1.57</td>
</tr>
</tbody>
</table>

* The difference is significant in comparison with: I, II, III, IV experiment series

** M — sample mean, m — standard error of mean
Fig. 3. The vessels of conventional structure (arrows) in I series animals' endomysium. The polygonal form of muscle fibers persists. Van Gieson. ×400

Fig. 4. The thickened vascular wall (arrow) and usual structures in perimysium observed in II series animals. Connective tissue in perimysium. Van Gieson. ×400

Fig. 5. The thickened vascular walls (arrows) in perimysium for III series animals. Endomysium edema. The excrecence of connective tissue in perimysium and endomysium. Muscle fibers polygonal shape loss. Van Gieson. ×400

Fig. 6. The thickened vascular walls (arrow) in perimysium and in endomysium for IV series animals. Adipose degeneration. The loss of muscle fibers polygonality. Nuclei migration. Van Gieson. ×400

Fig. 7. Macro image. Formed «bone block» of adjacent vertebrae posterior parts. 3 months after surgery

Fig. 8. Micro image. Fragment of rat’s lumbar spine at LIV–LV level: spinous processes (Sp) bone tissue growth in interlaminare spaces and facet joints areas before their joining (a), arcs edges and spinous processes (Sp) are not joined (b). H&E. ×20
Mobility in operated segment specimen was absent after transpedicular construction removal (fig. 7).

Considerable growth of bone tissue of spinous processes, which spreads to interlaminar spaces and joins vertebrae this way (fig. 8, a) is observed during lumbar spine histological examination of rats, in which the "bone block" formation was clinically and X-ray established. This also is a sign of formed spinal fusion in our and other researches opinion [29].

At the same time the dense net of thickened bone trabecuclae with brightly stained osteocytes at their surface was observed. Intertrabecular spaces contained red bone marrow.

The signs of generated spine fusion were registered in 60 % of II series animals (which had swum before surgical intervention) 3 months after operation.

The signs of formed spine fusion were established in 40 % of III series rats (which had swum after operation) 3 months after surgery.

In 80 % of animals with low level of physical activity (IV series, which had not swum) an adequate vertebrae bone junction was not noticed. The histological examination of these animals did not reveal a vertebrae joining by bone tissue growth in spinous processes and facet joints areas, as well as in interlaminar spaces (fig. 8, b).

**Discussion**

Lumbar spine fusion is the widespread type of surgical intervention in treatment of various diseases and traumatic injuries of spine [11–14]. Unfortunately, results of the manipulation are not always successful, the part of nonunions may come from 10 up to 40 %; low back pain, and instability (laxity) appear [15, 30, 31]. A lot of factors exert spine fusion generation; they are patient’s age, concomitant diseases, surgical tools implemented [16–18]. However, to avoid unsatisfactory results of spondylosyndesis, profound researches of the biological mechanisms of its formation are needed.

Paravertebral muscles play an important role in spine mechanical stability support, protecting its structure from destruction caused by weight loading. Changes in paravertebral muscles caused by aging, traumatic injuries or degenerative disorders, exert negative effect on their functioning, that may cause lumbar spine chronic pain [5–10]. Therefore the assumption about paravertebral muscles participation in spine fusion processes is well-grounded.

In present research the animal model (laboratory white rats) is used to ascertain the m. multifidus structural features caused by different physical activity levels influence upon posterior interbody fusion after transpedicular construction fixation at LIV–LV level and bone autograft implementation. Posterior spine fusion ("bone block") formation is registered in 3 months term with the help of clinical, X-Ray and histological methods. Essential growth of spines bone tissue is established. The bone tissue spreads in interlaminar spaces, facet joints and connects vertebrae in such way, that it is the histological proof of formed posterior spine fusion.

Similar histological features were obtained in experiments with rabbits, which have been implanted autograft bone chips with or without recombinant bone morphogenetic protein-2 into the area between interspinous processes of vertebral bodies at LIV–LV level, to achieve fusion. Based on clinical, X-ray, histological and biomechanical examinations, authors have proved the bone mass bridging [29].

However, even in experimental conditions it is not always possible to come up to the bone mass bridging of vertebral bodies. The instability was registered with the help of clinical and X-Ray methods after 3 months after surgery in 16.7 % of rabbits, which were transplanted by ilium autograft in LIII, LIV vertebral bodies’ transverse processes area to provide side spine fusion. Rabbits were kept in separate hutch without additional physical activity [32]. 68.7 % of positive results (formed spine fusion) were established roentgenologically in 9 weeks after autograft implantation in spite of analogous conditions [33]. We reckon, that such difference in results occurs due to different regenerative capacity of rats’ bone tissue in comparison with rabbits’ one. Moreover, the X-Ray method of estimating spine fusion quality turns out to be less convincing and evidential.

Based on the animal model (New Zealand white rabbits) it was proved, that blood vessels germinate, and cells migrate from muscles to spine fusion area, which is a necessary condition for bone regeneration [18]. By using different materials in spine fusion area the authors have shown, that the degree of obstruction of vessels germination into union area determines the quality of spine fusion. In present research we have also established, that the degree of changes in muscle nutrient vessels differs depending on the level of animal’s physical activity. In animals with low level physical activity, the hypotrophic and degenerative changes in muscles were observed together with vascular walls thickening and luminal narrowing, which impedes blood supply and venous outflow in fusion area, which is an important and dominant cause of spine fusion hampering. The relationship between level of physical activity and paravertebral muscles structure, as well as its
The obtained results indicate that muscular activity and the worst results — in the series with low physical activity regimen (the animal, which had swum before and after surgical intervention), while most evident destructive changes and the worst results — in the series with low physical activities (the animals, which had not swum). The obtained results indicate that muscular activity (swimming regimen) has a positive influence upon functional and adaptive muscle capacity, and posterior spine fusion.

**Conflict of interest.** The authors declare the absence of conflict of interest.

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РОЛЬ МНОГОРАЗДЕЛЬНЫХ МЫШЦ В ОБЕСПЕЧЕНИИ ЗАДНЕГО СПОНДИЛОДЕЗА ПРИ ТРАНСПЕДИКУЛЯРНОЙ ФИКСАЦИИ

В. А. Радченко 1, А. Г. Скиданов 1, Н. А. Ашукина 1, З. Н. Данищук 1, М. Н. Нессонова 2, Д. В. Мороценко 3, Н. А. Скиданов 3

1 ГУ «Институт патологии позвоночника и суставов им. проф. М. И. Ситенко НАМН Украины», Харьков
2 Национальный фармацевтический университет, Харьков, Украина
3 Харьковский национальный медицинский университет, Украина

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