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THE PECULIARITIES OF REMODELLING MUSCLE TISSUE OF RATS UNDER THE VIBRATION INFLUENCE

Nazar M. Kostyshyn[1], Liybov P. Kostyshyn[2], Marta I. Servetnyk[3], Mechyslav R. Grzegotsky[1]

[1] Department of Normal Physiology, Danylo Halytsky Lviv National Medical University – Lviv, Ukraine
[2] Department of Toxicological and Analytical Chemistry, Danylo Halytsky Lviv National Medical University – Lviv, Ukrainey
[3] Department of Pathological Anatomy and Forensic Medicine, Danylo Halytsky Lviv National Medical – Lviv, Ukraine

Corresponding author: Nazar Kostyshyn, e-mail: kostyshyn.nm@gmail.com

ABSTRACT

Study of the influence of vibration oscillations of different frequency, amplitude and vibration acceleration on the structural and functional state and mechanisms of muscle tissue remodelling. An experimental study was conducted on sexually mature male rats. The rats of the four experimental groups were subjected to vertical vibration oscillations of 15, 25, 50 and 75 Hz, respectively. It has been established that pathological changes in muscle tissue in the form of different variants of damage and remodelling tend to increase, which correlates with the frequency of vibration, amplitude and vibration acceleration level, as in the 2nd group, where the maximum permissible vibration levels did not exceed the established allowable norms, and in other groups of animals, where the permissible levels of total vibration were exceeded. By increasing vibration acceleration for more than 1.25 m/s2 (0.13 g, frequency more than 25 Hz and amplitude of 2 mm), severe damages are observed in the form of alterative changes of muscle fibres with the disappearance of transverse strain, homogenization of sarcoplasm, fragmentation with dissociation fibres on separate beams, partial and subtotal myocytolysis, and necrosis of separate fibres. Inflammation is rapidly increasing with the increase in the frequency of vibration and the level of vibration acceleration for more than 5.0 m/s2 (0.51 g).

Keywords: muscle tissue, remodelling, whole body vibrationx

INTRODUCTION

Skeletal muscles are a specialized tissue that can change its structural and functional state in response to mechanical stimuli [1, 2, 3, 5, 7, 10, 11, 12]. According to the scientific literature, myogenic effects that occur under the influence of vibration are poorly understood, and the information described is often controversial. In particular, in 1990, Griffin described the high sensitivity of muscles to mechanical influences and determined the ability of vibration to alter muscle remodelling in humans. The series of experiments, conducted by Bosco et al. established that intensive constant physical training with an additional effect of vibration on the whole body (vertical vibrations were modelled using a vibrating platform, with a frequency of 26 Hz, an amplitude of 10 mm and vibration acceleration of 5.5 g) can lead to increased muscle strength and accelerate hypertrophy of the athletes' leg muscles. Later, Xie et al. (2008) confirmed the development of compensatory hypertrophy of the flounder muscle in rats exposed to long-term influence of low-amplitude high-frequency vibration, comparing them with the control group. Based on scientific studies, these scientists have argued that low-amplitude high-frequency vibration is safe and can be used as an effective way to improve muscle tone, in order to prevent its atrophy. On the other hand, Murfee et al. have shown that under the influence of vibration, the number of functioning arterioles and venules is steeply reduced, especially in distal parts of the limb muscles, which is the result of maladaptation.

According to modern scientific ideas, high-frequency vibration can cause muscle damage, which is manifested by the oedema of muscle fibres. In particular, experimental studies of Falempin and InAlbon demonstrated that the effects of vibration with a frequency of 120 Hz and an amplitude of 0.3 mm during the 14 days have a tonic effect on muscle tissue. This, in its turn, leads to spontaneous tonic contractions of muscle fibres due to afferent impulses, which is called tonic vibration reflex. However, such an effect may lead to failure of adaptation (maladaptation) and gradual exhaustion of muscle tissue in the future.

Thus, nowadays, various sources of literature provide a large amount of information on the impact of vibration oscillations on muscle tissue, but none estimates the degree of changes in muscle function depending on frequency, amplitude, and acceleration [4, 13]. Therefore, the elucidation of physiological mechanisms of remodelling of muscle tissue under the influence of various vibration parameters is an actual direction of research.

PURPOSE

The purpose of our research is to study the effects of vibration oscillations of different frequency, amplitude and vibration acceleration on the structural and functional state and mechanisms of muscle tissue remodelling.

MATERIAL AND METHODS

An experimental study was conducted on 60 sexually mature male rats weighing 180-220 g. The animals were divided into 5 groups, each of 12 members. The rats of the four experimental groups were exposed to vertical vibration oscillations of 15, 25, 50 and 75 Hz, respectively, 2 times a day for 20 minutes within 28 days. Six animals from each group were withdrawn from the experiment on the 28th day of the study by decapitation and taking biological material for histological examination. The rest of the animals continued to be kept under the standard vivarium within the next 28 days, without exposing them to the effects of vibration. On the 56th day, a re-evaluation of the condition of the experimental animals' muscle tissue was carried out by means of histological examination. Experimental animal studies were conducted in compliance with the principles of bioethics, in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 86/609 / EEC (1986).

Vertical vibrational oscillations were modelled using an APC Rain-60 vibrating pump. A vibrating platform with a container, where a test group of rats was located, was attached to the vibration pump stem. Taking into account that there are no elastic suspensions of resonant adjustment in this type of vibration pumps, an electric frequency regulator of the voltage AFC-120 was introduced into the electric circuitry of their drive electromagnets, which allows to change the frequency of drawing an anchor of an electromagnet to its starter, thus changing the frequency of oscillations attached to the stock anchor platform. For the study groups, the vibration acceleration level was established as the following: 0.45 m/s2 (0.05 g) for the 1st group; 1.25 m/s2 (0.13 g) for the 2nd; 5.0 m/s2 (0.51 g) and 11.3 m/s2 (1.15 g) for the 3rd and 4th groups, respectively.

For histological examination, the fragments of the rats' quadriceps muscle were taken, fixated with 10% formalin solution for 24 hours in a sealed container for the biological material storage and poured with a paraffin wax mixture. The longitudinal and transverse sections of tissues in the thickness of 7 μm were carried out with a microtubule snuff MS-2. Standard solutions of haematoxylin and eosin were used to colour the resulting micro-particles. The description of the histological preparations fixated on the glass slides was performed on the Nikon Eclipse E200 microscope; photographs were taken by the Nikon D5000 camera.

RESULTS

Based on our histological studies of the four-headed muscle of the experimental rats, we obtained the following results. On the 28th day of the experiment, in the animals in the first experimental group which were exposed to vibration at a frequency of 15 Hz, single, focal contracture appears: fibres acquire wavy configuration and fragmentation, visualized in longitudinally located muscle fibres. In the preparations, the expressed damage of myofilaments, fragmentation, local dissociation on separate filaments is visualized. The transverse strain is preserved centrally (Fig. 1). The histological preparation of skeletal muscle of the second experimental group is represented by numerous contractions of muscle fibres and their fragmentation with capture up to 20 fibres. There are typical areas of subtotal fibres separation on 8-10-15 coarse, and bright eosinophilic homogeneous beams. In selected areas, fibres with vacuolation in the central zones are determined (Fig. 2). On the 28th day of the experiment, in the preparations of the third experimental group, numerous contractions of muscle fibres were determined and their fragmentation was already with capture up to 20-30 fibres. There were areas of subtotal separation of fibres on 8-10-15 coarse, and bright eosinophilic homogeneous beams. Also, in considerable areas, there were fibres with a pronounced vacuolation in the central zones. Distributed focal lympho-macrophage infiltration was common in the intermuscular spaces (Fig. 3). In the experimental animals of the fourth group, exposed to vibration with a frequency of 75 Hz, loss of transverse strain was determined, steeply expressed alterative changes were observed, which indicated damaging processes in muscle fibres. In this group of preparations, contractions and contractile necrosis of muscle fibres, their numerous fragmentation, covering up to 20-30 fibres, were characteristic. In addition, there were distributed areas of total separation of fibres into rough, totally homogeneous, bright eosinophilic beams, and some of them even with signs of hyalinosis. The total number of myoblasts was slightly reduced. In considerable areas, fibres with pronounced vacuolation in the central zones were determined. Distributed focal lympho-macrophage infiltrates were observed in the intermuscular spaces (Fig. 4).

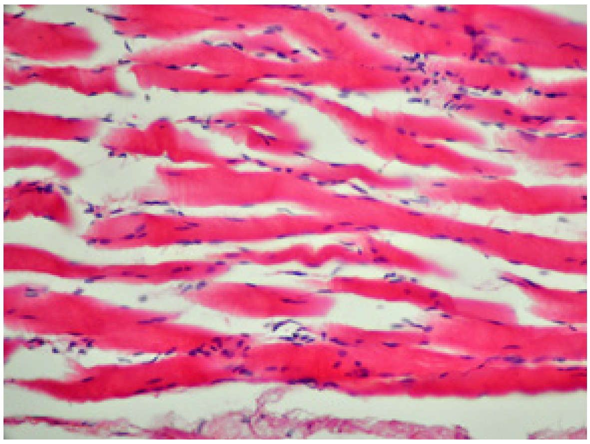

Fig. 1. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle of a typical structure (longitudinal section). Sporadic contracture of muscle fibres.*

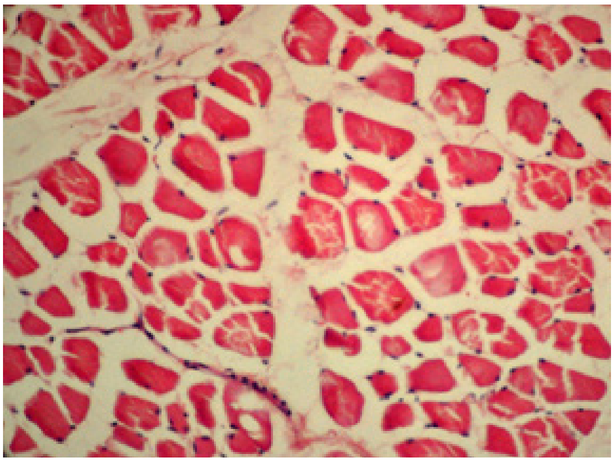

Fig. 2. Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (cross-section). Reduced myoblasts. Centralized zones of partial myolysis with vacuolation. Diffuse interstitial oedema.

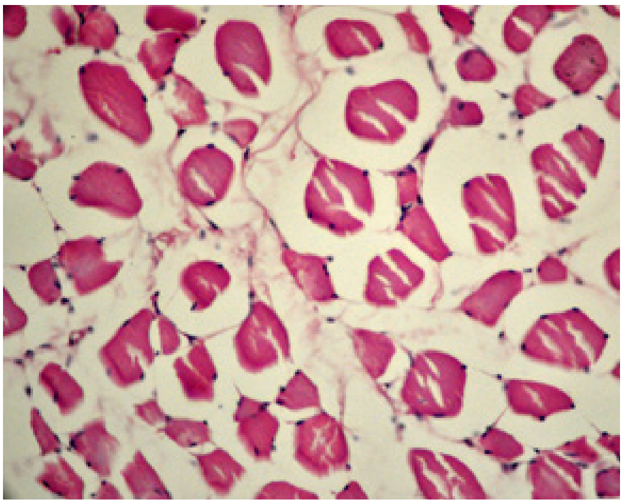

Fig. 3. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (cross-section). Reducing the number of myoblasts, Multiple zones of separation of muscle fibres into individual homogeneous, bright eosinophilic beams. Diffuse, interstitial oedema*

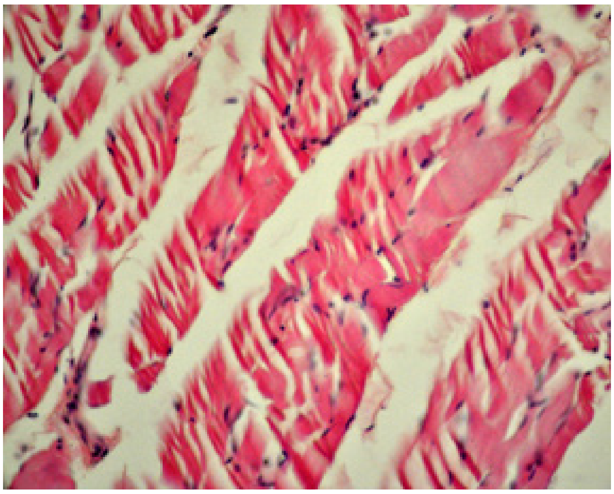

Fig. 4. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (elongated section). Pronounced alterative changes in muscle fibres: the disappearance of the transverse band, the overall decrease in the number of myoblasts, diffuse interstitial oedema*

On the 56th day of the experiment, changes in the histological preparations of the experimental rats' muscles were as follows: in the first group, on the transverse section, muscle fibres forming multicellular syncytium, had a typical, unaltered form of polygonal structures. Their dimensions were slightly increased (signs of hypertrophy). Syncytium was formed by merging individual, small myoblasts. The nuclei of muscle fibres were monomorphic, oval, occasionally flattened, located along the periphery, directly under the plasma membrane of each muscle fibre, in the amount of 3-5 pieces. In the part of fibres, there was a small area of enlightenment in the central zone, and on the periphery, closer to the sarcolemma, - a zone of more intense eosinophilic colour, which may be such due to the preservation of the morphological features of the initial manifestations of the muscle syncytium fragmentation. Intermuscular space was enlarged as a result of diffuse interstitial oedema (Fig. 5). In the histological preparations of the second experimental group, hypertrophy of muscle fibres was pronounced, numerous contractions of fibres and their single fragmentation were observed. In the longitudinally located muscle fibres there were focuses preserved after the damage of partial myocytolysis with folding of the distal parts of the sarcoplasm. There were also areas of focal separation of fibres into individual, coarse, bright eosinophilic homogeneous bundles. In separate sections of the transverse location, fibres with vacuolation in the central zones were determined. In response to irreversible damage to muscle fibres, polymorphic and inflammatory infiltrates appeared, varying in cellular composition and localization. Single minor-focal infiltrates were localized perivascularly. In intermuscular spaces, inflammatory infiltrates that were located around the damaged muscle fibres were more common in the number and distribution of cells. These infiltrates consisted mainly of polymorph-nuclear leukocytes - neutrophils, eosinophils and macrophages (Fig. 6). In the third experimental group, there was a pronounced hypertrophy of muscle fibres, their very compact location with narrowing of the intermuscular spaces, numerous contractions of fibres and their sporadic fragmentations. In the longitudinally located muscle fibres, in their total homogenization, the areas of enlightenment, as well as the partial myocytolysis preserved after the damage with the folding of the distal sections of the sarcoplasm and the sporadic fragmentations into the bright eosinophilic homogeneous bundles were also found. The nuclei of the myoblasts were elongated and flattened yet defined, and enlarged with centrally located nucleoli, due to hypertrophy of fibres (Fig. 7). In all studied preparations of the fourth experimental group, the animals of which were exposed to vibration with a frequency of 75 Hz, significant altered changes in muscle fibres were defined - the disappearance of transverse strain, contracture, the zone of fragmentation, distributed necrotic changes in muscle syncytium in the form of subtotal myocytolysis with fragmentary preserved sarcolemma and pronounced cellular inflammatory reaction in response to irreversible damage - necrosis of muscle fibres. In the intermuscular space, numerous, prevalent polymorphic-inflammatory infiltrates, containing neutrophilic leukocytes, are determined. In separate focuses, neutrophils appear in the middle of necrotic altered muscle fibres (Fig. 8). In other areas, there were also adaptive (compensatory-adaptive) changes, namely - pronounced hypertrophy of muscle fibres, their compact position and narrowing of the intermuscular space.

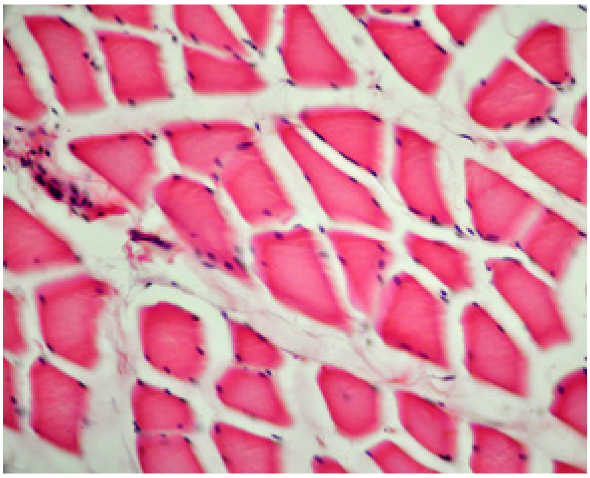

Fig. 5. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (cross-section). Hypertrophy of muscle fibres. Oedema of intermuscular interstitial*

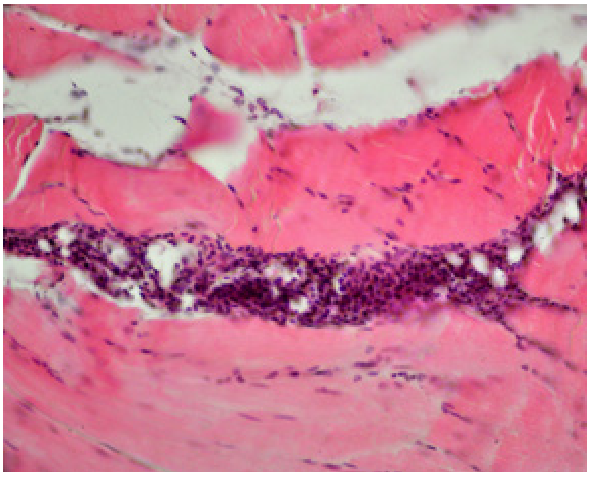

Fig. 6. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle with longitudinal orientation of fibres. Pronounced hypertrophy of muscle fibres, their compact location, homogenization, single contracture, fragmentation into separate eosinophilic beams*

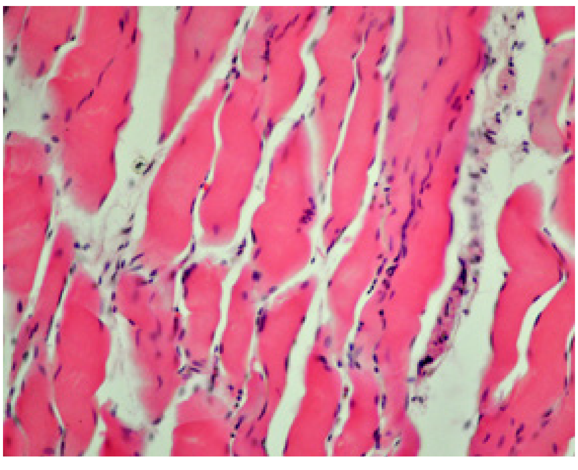

Fig. 7. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (longitudinal section). Pronounced hypertrophy of all muscle fibres, sporadic fragmentations and contractures, homogenization of sarcoplasm, increase in the size of the myoblastic nuclei, their moderate hyperchromia, the appearance of sporadic basophilic nucleoli*

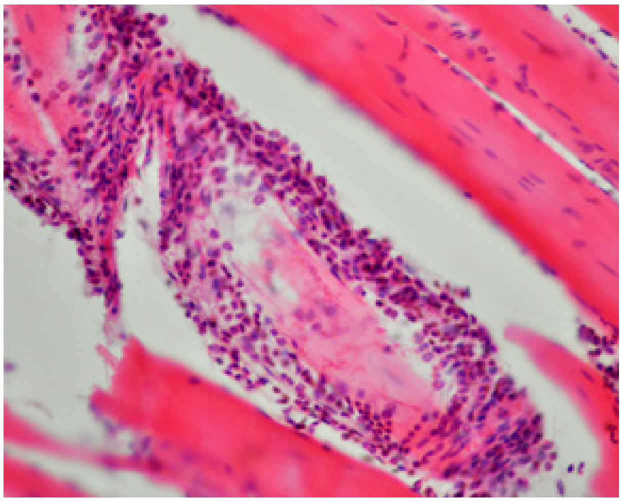

Fig. 8. *Colouring with haematoxylin and eosin. Magnification x 400. Skeletal muscle (longitudinal incision). Hypertrophy of muscle fibres, lack of transverse strain, homogenization of sarcoplasm. In the centre - muscle fibre with signs of subtotal myolysis and necrosis, and with fragmentary preserved sarcolemma. There is an intense, dense, polymorphocytic inflammatory infiltrate around the damaged fibre*

DISCUSSION

International hygienic standards for total vibration (ISO 2631/1) indicate a safe level of 20 to 90 Hz with a vibration acceleration level of less than 0.56 g. The effect of low-frequency vibration with a vibration acceleration level of < 0.9 m/s2 does not lead to steep or chronic tissue damage, and the probability of development of muscle pathology in the distant period remains very low [1, 6, 8, 9, 10, 11, 12, 14, 15]. However, we have found that pathological changes in muscle tissue in the form of diverse variants of damage and remodelling tend to increase, which correlate with the frequency of vibration, amplitude and level of vibration acceleration, as in the first group of research, where the maximum permissible vibration levels did not exceed the established tolerable norms, and in other groups of animals where the permissible levels of total vibration were exceeded. And with increasing vibration acceleration more than 1.25 m/s2 (0.13 g, frequency more than 25 Hz and amplitude of 2 mm), severe damages are observed in the form of alterative changes of muscle fibres with the disappearance of transverse stratification, homogenization of sarcoplasm, fragmentation with dissociation fibres on separate beams, partial and subtotal myocytolysis, necrosis of separate fibres. In response to irreversible damage to the muscles, a typical protective reaction (inflammation) has been developed, increasing rapidly along with rising vibration frequencies and a vibration acceleration of more than 5.0 m/s2 (0.51 g). Also, focal and/or prevalent inflammatory cell infiltration with predominance (in their composition) of neutrophilic and eosinophilic leukocytes was detected. The intensity and density of the infiltration location increases in correlation with the vibration acceleration level. Obviously, the effects of cross-linked muscle damage observed in preparations on the 56th day of the experiment, took place due to the prior influence of vibration and were represented by various pathological processes, and, in particular, as increasing manifestations of compensatory and adaptive reactions in the form of pathological hypertrophy of muscle fibres with the formation of compactly located, enlarged in size and modified in the form muscle fibres, reduction of the intermuscular spaces leading to a violation of hemoperfusion of muscles.

CONCLUSION

1. Long-term effects of general vibration can negatively affect muscle tissue and the body as a whole, potentiating the development of pathological processes caused by external influences. We have noted that changes in muscle remodelling directly depend on the strength of the vibration stressor.

2. The degree of the severity of damage to skeletal muscles, which increases from the 1st to the 4th group, depends on the parameters of vibration. Alterative changes in muscle fibres are manifested by the gradual loss of transverse strain, progressive damage to myoblasts with signs of karyolysis, contractions, fragmentation, local and total dissociation of fibres into separate filaments, the appearance of sporadic and widespread minor-focal lymphocytic infiltrates.

3. On the 56th day of the experiment, signs of compensatory and adaptive reactions in the form of pathological hypertrophy of muscle fibres with the formation of extremely compactly spaced, enlarged in size and modified muscle fibres, reduction of intermuscular spaces, violation of perfusion and inflammation of the muscles were noted.

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Резиме

ОСОБЕНОСТИТЕ НА РЕМОДЕЛИРАЊЕ НА МУСКУЛНИТЕ ТКИВА НА СТАОРЦИ ПОД ВЛИЈАНИЕТО НА ВИБРАЦИИ

Назар М. Костишин[1], Љубов П. Костишин[2], Марта И. Серветник[3], Мечислав Р. Гжегостки[1]

[1] Катедра за нормална физиологија, Национален медицински универзитет во Лавов „Данило Халитски“ – Лавов, Украина

[2] Катедра за токсиколошка и аналитичка хемија, Национален медицински универзитет во Лавов „Данило Халитски“ – Лавов, Украина

[3] Катедра за патолошка анатомија и судска медицина, Национален медицински универзитет во Лавов „Данило Халитски“ – Лавов, Украина

Студија за влијанието на вибрациските осцилации со различна фреквенција, амплитуда и забрзување на вибрациите врз структурната и функционалната состојба и механизам на ремоделирањето на мускулните ткива. Беше изведена експериментална студија врз сексуално зрели машки стаорци. Стаорците од четирите експериментални групи беа подложени на вертикални вибрациски осцилации од 15, 25, 50 и 75 Hz, соодветно. Беше утврдено дека патолошките промени во мускулното ткиво во форма на различни варијанти на оштетувањето и ремоделирањето имаат тенденција на зголемување, што е во корелација со фреквенцијата на нивоата на вибрациите, амплитудите и вибрациите, како и во втората група, каде што максимално дозволените нивоа на вибрации не ги надминаа утврдените дозволени норми, а и во други групи животни, каде што беа надминати дозволените нивоа на вкупни вибрации. Со зголемување на забрзувањето на вибрациите за повеќе од 1,25 m/s2 (0,13 g, фреквенција поголема од 25 Hz и амплитуда од 2 mm), се забележуваат сериозни оштетувања во форма на алтернативни промени на мускулните влакна со исчезнување на попречното напрегнување, хомогенизација на саркоплазмата, фрагментација со дисоцијациски влакна на одделни носачи, делумна и суптотална миоцитолиза, и некроза на одделни влакна. Воспалението брзо се зголемува со зголемувањето на фреквенцијата на вибрации и на нивото на забрзување на вибрациите за повеќе од 5,0 m/s2 (0,51 g).

Клучни зборови: мускулно ткиво, ремоделирање, вибрации на целото тело