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ORIGINAL ARTICLE

EFFECT OF MECHANICAL STIMULI ON REMODELING AND MINERAL DENSITY OF THE SPINE

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ABSTRACT

The aim: The aim of our study was to determine effect of whole body vibration on the bone tissue remodeling, determine the correlation between bone mineral density, markers of bone metabolism and level of vibration stimulus.

Materials and methods: Four experimental groups Wistar rats were exposed to vertical whole body vibration during 28 days. Blood intake and QCT-scanning of the lumbar spine was performed on the 28th and 56th day of study.

Results: The largest loss of trabecular bone was observed in experimental groups III (0.51g) and IV (1.15g), which was decreasing to 12% ($p<0.05$) and 14% ($p<0.05$), respectively, in comparison with the control group. After 56th day of the experiment, bone loss dynamics was the following: in the 1st group ≤ 10% ($p <0.05$), in the 2nd ≤ 12% ($p <0.05$), in the 3rd ≤ 17 % ($p <0.05$), and 4th ≤ 22% ($p <0.05$) compared with the control group. Changes in the level of hydroxyproline in the first experimental group were not statistically significant ($p>0.05$), in the second group – increased by 19.3% compared with the control rates, in the third – by 65.3%, and in the fourth – the level doubled ($p<0.05$). Increase of free hydroxyproline indicates violations in the balance between destruction and compensatory acceleration of collagen biosynthesis, which gradually decreases up to the 56th day. It has been determined that with the increase in vibration frequency, elevation of osteocalcin level in rats' blood is observed.

Conclusions: Acceleration 0.51g increases the rate of bone metabolism, causes collagen metabolism disorders, loss of bone mineral mass, which further leads to osteoporosis.

KEY WORDS: bone, whole body vibration, mineral density, bone remodelling

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INTRODUCTION

Physiological remodeling of bone tissue occurs in continuous replacement of bone plates and formation of new tissue at resorption site. These converse processes are provided due to activity of osteoclasts and osteoblasts [1]. The study of influence of different experimental exogenous factors on bone tissue metabolism remains an urgent issue, since the number of people working in unfavorable conditions, as exposure to vibration, noise and influence of other stressful factors, increases every year [2-4]. The latest investigations show that the majority of mechanized instruments and machines, which generate vibration, work at vibration acceleration rate that significantly exceeds normal ranges [5-7]. Levels of transport vibration in industry are significantly higher and often exceed 1 g. In hygienic standards on general vibration (ISO 2631/1, Geneva, Switzerland, 1985), safe level constitutes 20 - 90 Hz and vibration acceleration level is below 0.56 g [8-10]. Such vibrations do not result in acute or chronic damage to the tissues and have anabolic or anticatabolic influence on bone tissue. Physical exercises also show positive effect on mineral density and bone strength. Vibrations occurring during physical exercises slow down osteoclastogenesis and have anabolic effect on bone tissue [11-12].

THE AIM

To study influence of different vibration frequencies on the process of bone remodeling in rats, determination of correlation between mineral density of bone tissue, markers of bone metabolism and the level of vibration stimulus.

MATERIALS AND METHODS

Experimental investigation was performed on 60 pubertal (2 month old) Wistar male rats weighing 180-200 g. The animals were distributed into 5 groups, 12 in each group. The animals of control and all experimental groups were kept in the same conditions of the vivarium. The investigation on animals was performed following bioethics principles. Experimental animals of four groups were exposed to vertical vibrations with 15, 25, 50 and 75 Hz frequencies, respectively, twice a day for 20 minutes, 5 days a week during 28 days. The following four weeks (28 days), animals of all experimental groups were kept in standard conditions of the vivarium and were not exposed to vibrations [13]. Vertical vibrations were modeled using vibration pump APC Rain-60, power 250 watt with maximum pressure 7 bar. Vibration platform with a container, where experimental group of rats was placed, was attached

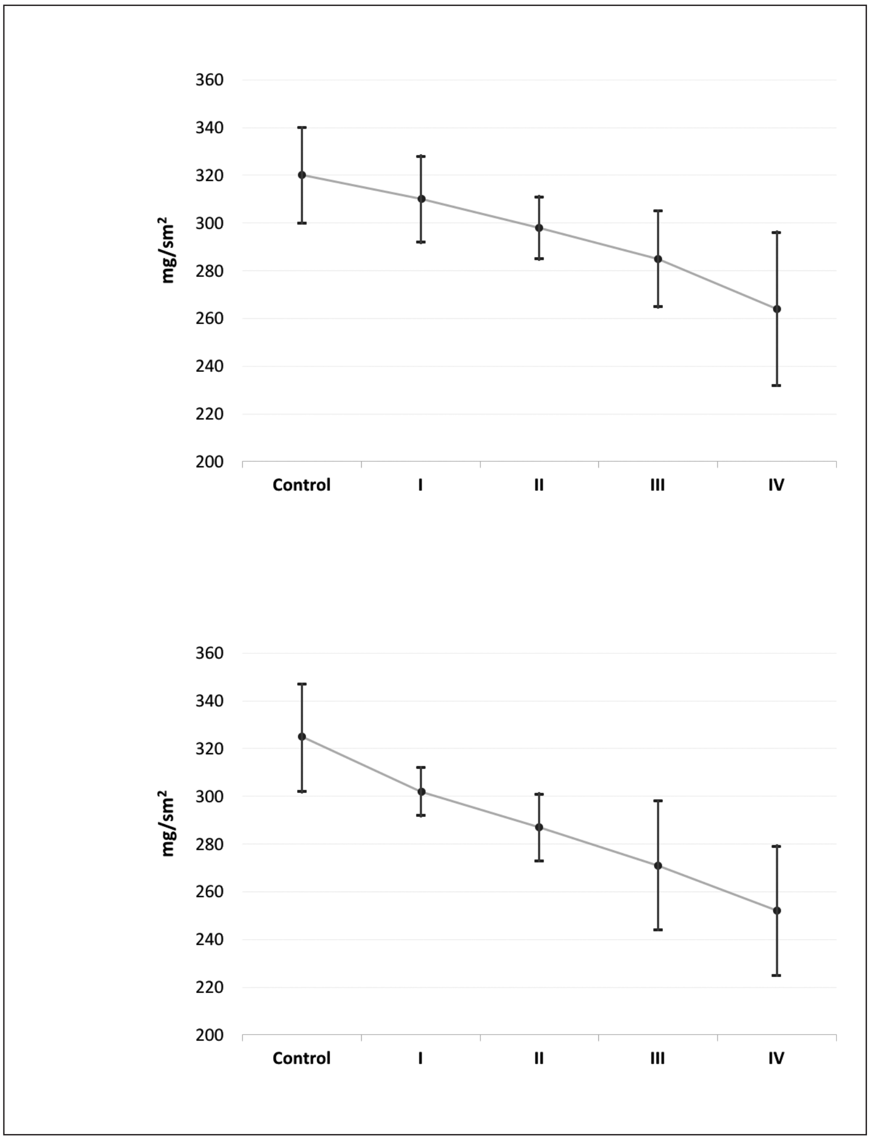

Fig. 1. Mineral density of bone tissue (mg/cm3) of lumbar vertabrae of experimental rats on the 28th and 56th days of the experiment

to a shaft of a vibration pump. Working frequency of the device was set with a remote control for modeling of vibration resonance processes of AFC-120 model. Amplitude of vibration equaled two mm. For experimental groups, the rate of vibration acceleration constituted: 0.05 g for group I; 0.13 g – for group II; 0.51 g and 1.15 g – for groups III and IV, respectively.

Six animals from each group were removed from the experiment on the 28th day of the experiment by rapid decapitation with simultaneous collection of blood and prior QCT scan of the lumbar spine. The rest of animals were kept in standard condition of the vivarium another 28 days without exposure to vibration. On the 56th day of the investigation, repeated assessment of bone tissue condition was performed on QCT of the lumbar region of the rats' spines, and the levels of free hydroxyproline and osteocalcin in rats' blood were measured [14-15]. The research was performed using analyzer Cobas 6000, Roche Diagnostics (Switzerland) and test system Immulite (Siemens AG, Germany). Immunochemical method with chemiluminescent detection was used. Investigation of mineral density of the bone tissue of trabecular layer in lumbar vertebrae (L_1 - L_6) was conducted by the method of computer tomography [16]. The examination was performed on computer tomographer Toshiba TSX-101 A, Aquilion 16. A standard sensor with section thickness 0.5 mm, 120.0 kV, 50 mA was applied for scanning. Processing of the data was performed using the programme IQ view-3D, 3D post-processing workstation, certification CE 0482 and FDA 510 (k), London, United Kingdom based on Windows 7 Professional SP 1, 32 bit (Microsoft, USA, 2009).

RESULTS

As a result of conducted computer tomography of rats' bone tissue, correlation between loss of mineral density and frequency of vibrations has been established. In particular, on the 28th day of the investigation, mineral density of lumbar vertebrae (L_1 – L_6) in control group was ranging from 311.90 ± 5.44 to 334.00 ± 8.08 mg/cm^3. The largest

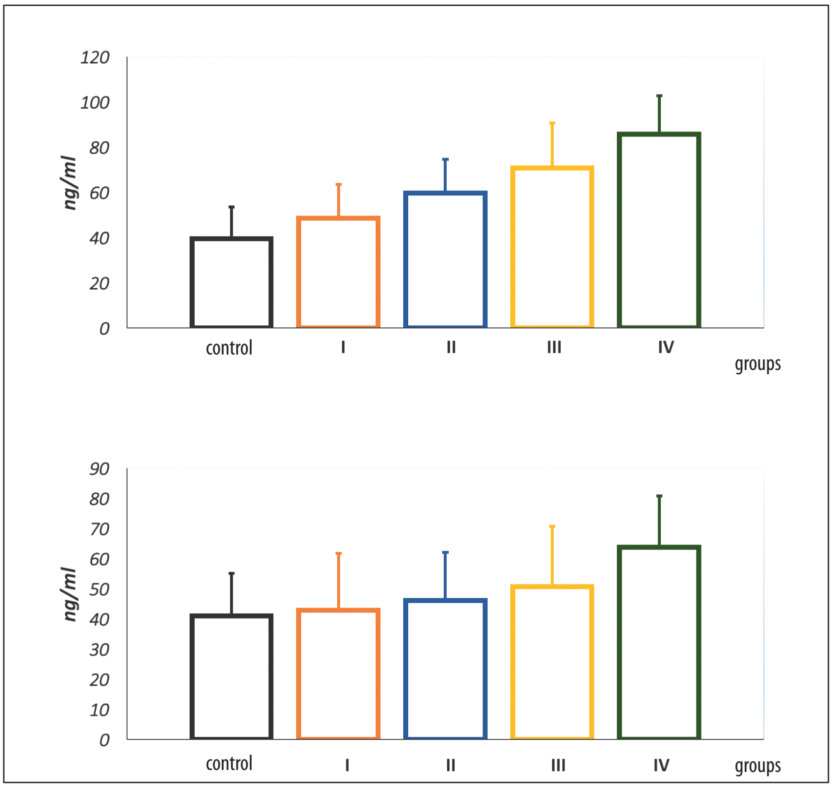

Fig. 2. Osteocalcin level (ng/ml) in the blood of experimental rats on the 28th (A) and 56th (B) days of the experiment.

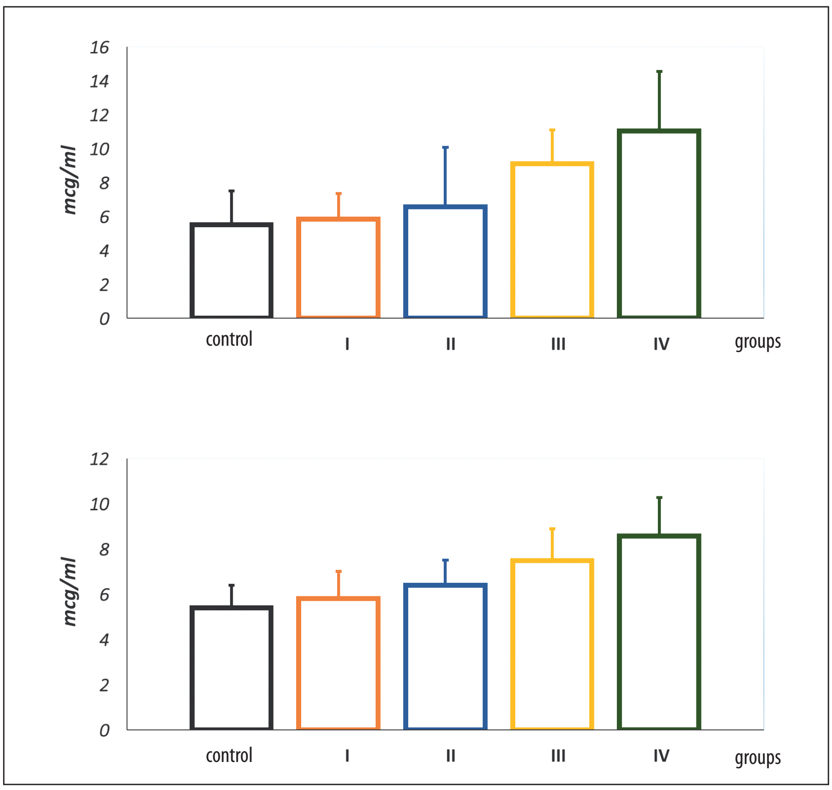

Fig. 3. Level of free hydroxyproline (mcg/ml) in the blood of experimental rats on the 28th and 56th days of the experiment

loss of bone mass of trabecular layer in the vertabrae was observed in III and IV groups of experimental rats, which decreased to ≤ 12 % ($p < 0.05$) and to 14 % ($p < 0.05$) and to 8 % ($p < 0.05$), respectively, compared with control group. In the first and the second groups, the density index decreased to value ≤ 4 % ($p > 0.05$) and to 8 % ($p < 0.05$), respectively, compared with control group. On the 26th day of the experiment, dynamics of bone tissue loss constituted in group I ≤ 10 % ($p < 0.05$), in group II – ≤ 12 % ($p < 0.05$), in group III – ≤ 17 % ($p < 0.05$) and in group IV – ≤ 22 % ($p < 0.05$) compared with control group (fig. 1).

Conducted blood analysis showed considerable differences in the levels of osteocalcin between control and experimental groups of rats. On the 28th day of the experiment the level of free osteocalcin in control group of animals constituted 39.52 ± 0.78 ng/ml. In experimental group I, the index equaled 48.55 ± 1.31 ng/ml, in groups II and III the indices constituted 59.60 ± 1.21 ng/ml and 70.80 ± 1.79 ng/ml, respectively. In group IV the index increased twice and constituted 85.75 ± 1.92 ng/ml ($p<0.05$). On the 56th day of the experiment, the dynamics of osteocalcin level was the following: in control group the index remained almost without changes and equalled 41.07 ± 0.62 ng/ml. In experimental group I, mean index was 42.82 ± 0.71 ng/ml ($p > 0.05$), in groups II, III and IV – 46.18 ± 0.70 ng/ml, 50.78 ± 1.19 ng/ml and 63.75 ± 0.95 ng/ml ($p < 0.05$), respectively (fig. 2.).

It should be mentioned that approximately 70-90% of osteocalcin synthesized by osteoblasts is present in bone matrix, and the rest enters the bloodstream. Thus, osteocalcin is considered the most specific protein of bone tissue. It is known that bone tissue consists of organic matrix and mineral phase, structural units of which are crystals of hydroxyapatite. Osteocalcin is usually found in mineralized tissue, it is a mediator in matrix mineralization and is characterised by high similarity to calcium. After release from osteoblasts, osteocalcin is deposited in the matrix of the bone tissue and is released into the blood, thus, this marker can indicate the rate of bone tissue remodelling [13, 14]. Rapid increase in osteocalcin level in rats' venous blood in experimental groups I, II, III and IV on the 28th day of the experiment indicates influence of medium- and high-frequency vibration on bone metabolism, increase in osteoblast activity as the response to acceleration of collagen catabolism, resulting in calcium loss and decrease in mineral mass of bone tissue. On the 56th day, metabolism of bone tissue gradually reduces to initial values. However, indices in groups III and IV remain at high levels, proving advantage of delaying loss of mineral bone component after cessation of vibrations.

On the 28th day of the experiment, the level of free hydroxyproline in control group of animals constituted 5.50 ± 0.20 mcg/ml. In experimental group I, the index constituted 5.84 ± 0.14, which is statistically insignificant difference ($p > 0.05$), in experimental group II indices increased by 19.3 %, in group III – by 65.3 %, and in group IV – twice, compared with control group, which constituted 6.54 ± 0.35 mcg/ml, 9.09 ± 0.19 mcg/ml and 11.04 ± 0.39 mcg/ml ($p<0.05$), respectively. These data indicate influence of medium- and high-frequency vibration on bone metabolism, namely, stimulation of catabolic phase of collagen metabolism, resulting in calcium loss and reduction of mineral density. On the 56th day of the experiment, dynamics of hydroxyproline level was the following: in control group the index remained almost without changes and constituted 5.41 ± 0.11 mcg/ml. In experimental group I, mean index was 5.80 ± 0.10 mcg/ml ($p > 0.05$), in group II it increased by 17.5 % compared with control, in group III – by 38 %, and in group IV – by 58.2 %, constituting 6.36 ± 0.11 mcg/ml, 7.48 ± 0.14 mcg/ml and 8.56 ± 0.16 mcg/ml ($p < 0.05$), respectively. The results of detecting the level of free hydroxyproline in blood of experimental rats on the 28th and 56th days after removal from the experiment are shown in fig. 3.

DISCUSSION

Bone is a *mechanosensitive* tissue, its condition is not only caused by genetic and immune factors, diet and lifestyle, but also by exogenous influences, such as whole body vibration [13]. As a result of the complex researches accomplishment, new information was obtained that significantly contributed to the existing concepts of the effect of general vibration oscillations on the structural and functional state of bone tissue. It is proved, that the general vibration with different frequency, amplitude and level of vibration acceleration with unequal force accelerates bone remodelling of the whole skeleton in rats.

Based on rat`s spine densitometry, we have found a decrease in the mineral density of the trabecular layer of the lumbar vertebrae under the impact of various vibration parameters. The inverse relationship between the impact of vibration frequency and the level of vibration acceleration and the density of bone tissue in the zones of interest of the skeleton of rats was traced. This shows that the cells of the bone tissue show great activity under the impact of high frequencies of vibration. Increasing vibration frequency from 15 to 75 Hz causes a stress on the body, which increases the activity of remodelling sites and is accompanied by accelerated loss of bone mass and the development of osteoporosis. Correlation between metabolism of bone tissue and its density and hydroxyproline concentration in the blood of experimental rats has been established in control and experimental groups of animals. In all experimental groups, there is correlation between the strength of vibration stimulus and the level of disturbance in bone tissue metabolism. In the process of collagen degradation, hydroxyproline is released into the bloodstream both as a free fraction and in polypeptide form, because it cannot be repeatedly used for synthesis of a new protein. Since half of its amount is found in the bones, where metabolism occurs more rapidly than in other tissues, its increase in the blood and urine reflects the process of bone resorption. Increase in the level of free hydroxyproline in 28 days of the experiment indicates impairment of dynamic balance between destruction and compensatory acceleration of collagen biosynthesis, which gradually decreases up to the 56th day.

The research presented physiological mechanisms of the impact of whole body vibration on the body and the adaptive response of the skeletal system to the stressor [3, 7]. In particular, the ability of vibration oscillation is not only to modulate the function of the precursors of osteoblasts and osteoclasts, but it also directly participates in the bone remodelling. It is important to take into account that with the same vibration acceleration and with the increasing of vibration frequency, the membrane deformation increases due to the increased peak velocity in each cycle. Continuous exposure of the body to vibration results in imbalance in RANKL/RANK/OPG system, differentiation and activation of osteoclasts with further increase in bone resorption [17, 18]. Thus, after cessation of exposure to vibration stimulus, imbalance of remodeling and further loss of bone mass is observed for a long time. Therefore, the obtained results enable to suggest that the rate of bone tissue metabolism rises with the increase in vibration frequency and the rate of vibration acceleration. It is accompanied by acceleration of the process of collagen catabolism and loss of bone mass, which further leads to appearance of osteoporosis even in remote period after cessation of influence of this stress factor.

CONCLUSIONS

It has been established that elevation of free hydroxyproline in the blood of experimental animals is observed with the increase in vibration frequency (from 15Hz to 75 Hz). After cessation of vibrations on the 56th day of the experiment, gradual decrease in hydroxyproline level is recorded, especially in experimental groups II, III and IV, and the results were statistically insignificant in experimental group I compared with control group. Decrease in mineral density of bone tissue in trabecular layer of lumbar vertebrae is observed, as well as reverse correlation between the influence of vibration frequency level and density of bone tissue. It has been revealed that with the increase in vibration acceleration level 0.51 g, the rate of bone tissue metabolism rises, processes of collagen catabolism and calcium loss are accelerated, resulting in appearance of osteoporosis in the future.

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