

Original Research

Metabolic and Structural Response of Bone to Whole-Body Vibration in Obesity and Sedentary Rat Models for Osteopenia

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Abstract

Introduction: Several studies indicate that obesity is associated with increased bone mass due to excessive mechanical stress. However, these data are controversial, and the risk of fractures in this group is high. This study aimed to investigate the effect of high-frequency whole-body vibration on the process of bone remodeling in rats with a sedentary lifestyle and obesity. **Material and Methods:** To assess bone metabolism in rat blood plasma, we determined the parathyroid hormone and calcitonin levels to quantify the loss of the bone mineral component, the x-ray diffraction method was used. **Results:** Using X-ray diffraction, we observed the increase of the crystalline phase volume fraction in rats with whole-body vibration (WBV), and the decrease of the mineral component in the first experimental group – rats with sedentary lifestyle and obesity. Besides, the first experimental group showed a higher concentration of the parathyroid hormone in the serum and lower calcitonin levels. It is worth noting that the experimental animals with WBV showed a decrease in fat mass and leptin concentrations in blood serum. **Conclusions:** The results indicate that obesity has a negative impact on mineral bone mass. Mechanical stimulation may serve as a promising alternative to medication therapy for osteopenia.

Keywords: Bone remodeling, bone mineral density, osteoporosis, X-ray diffraction.

Abbreviations: BMD – bone mineral density; HCD – High-calorie diet; LMC – Limited mobility condition, PTH – parathyroid hormone, RANKL/RANK/OPG – receptor activator of nuclear factor (NF)-κB-ligand / receptor activator of NF-κB / receptor osteoprotegerin; WBV – whole body vibration.

Introduction

Every year we witness an increase in the number of people with pathological disorders of the musculoskeletal system. Therefore, the study of the influence of various factors on bone metabolism is relevant. Sedentary lifestyle and obesity are among the factors that might influence bone mineral density. Calcium homeostasis is strictly regulated by several factors, such as the RANKL/RANK/OPG cytokine system, estrogens, vitamin D3, parathyroid hormone, and calcitonin. It also depends on one's digestive system absorption and

lifestyle. Since the bone is a mechanosensitive tissue, its condition depends both on the mentioned factors and exogenous influences, e.g., vibration oscillations. Several studies indicate that obesity is associated with increased bone mass due to excessive mechanical stress [1-9]. However, these data are controversial, and the risk of fractures in this group is higher [10-12]. Also, a significant number of people working in the industry are exposed to vibrations. Therefore, the number of patients with disorders of the musculoskeletal system grows. This leads to muscle hypertrophy, osteoarthritis, osteoporosis and calcination



of vertebrae, and the development of secondary radicular syndrome with trophic disorders of the extremities. Such changes mainly occur in two to five years of work with the constant exposure to vibration exceeding the maximum allowable levels. However, some literature sources point to the efficacy of moderate physical activity in increasing bone mass [13-15] despite such data. High-frequency whole-body mechanical oscillations can be used to prevent osteoporosis, increase bone mineral density, and reduce body fat percentage [15-22]. However, fracture risk factors depend on the mineral mass of its trabecular and cortical layers and its composite characteristics, i.e., bone micro-architectonics. Thus, to evaluate bone quality, we must consider the markers of bone remodeling, hormone panel, and bone micro-structure, which we determined an experiment using the x-ray diffraction method [23-24].

Our study aims to investigate the effect of high-frequency oscillations on the process of bone remodeling in rats with a sedentary lifestyle and obesity.

Materials and Methods

Animal model

The experimental study was performed on 54 Wistar male rats weighing 180-200 g, kept under the same vivarium conditions. All animal experiments were conducted in compliance with bioethical principles per 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), Law of Ukraine No. 3447-IV On the Protection of

Animals from Cruelty, General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001). The experimental rats were divided into three groups, 18 rats in each: the control group - standard vivarium conditions, the first experimental group - limited mobility condition + high-calorie diet (LMC+HCD), the second experimental group - LMC+HCD+WBV. The obesity condition was modeled through a high-calorie diet (C 11024, Research Diets, New Brunswick, NJ); the limited mobility condition was modeled using partition cages to restrict the rats' mobility. All experimental rats were weighed every two weeks. Vertical vibration oscillations were modeled using a 250 W vibrating table with the maximum pressure of 7 bar and 50 Hz frequency, $g = 0.3$ (Figure 1). After the 8th, the 16th and the 24th week, six animals from each group were removed from the experiment by decapitation under general intraperitoneal anesthesia at 0.3 g/kg.

Determining hormonal concentration

To assess bone metabolism in rat blood plasma, we determined the levels of parathyroid hormone and calcitonin using commercial EIA DRG-3645 PTH (Parathyroid hormone) Intact ELISA and EIA DRG-3648 Calcitonin ELISA (DRG International, Inc., USA). The study is based on the "sandwich" method (double-antibody method). Standards, controls, test samples and specific antibodies to the corresponding hormone labeled with biotin were added to the wells coated with PTH/CT antibodies. This forms the AB-PTH/CT-AB+Biotine complex. After the first incubation and washing phase, a chromogenic solution (tetramethylbenzidine) was added. The intensity of its color changes

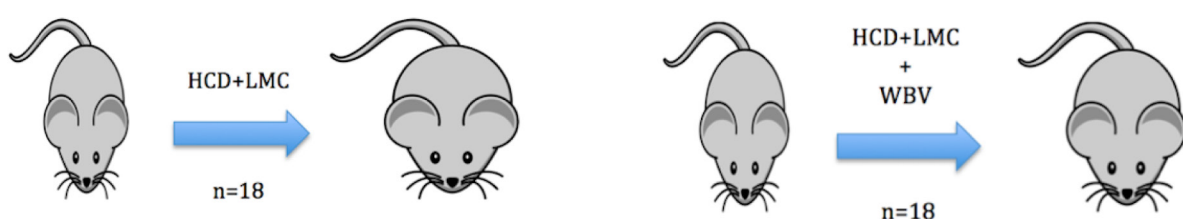


Figure 1: Experimental design.

in direct proportion to the concentration of the sample's specific antibodies. We measured the color intensity at a wavelength of 450 nm.

X-ray diffraction

To study the ultrastructure of the tibia bone mineral component, we used the X-ray diffraction analysis method. The femur was dried at 110°C in a drying cabinet. The X-ray diffraction spectra of the samples were obtained on an automated X-ray diffractometer in Cu K α radiation ($\lambda=1.5418 \text{ \AA}$), monochromatized by reflection from a plane (002) of a single pyrographic crystal, mounted on a diffracted beam [23-27]. We used the Bragg-Brentano focusing scheme ($\theta - 2\theta$). The diffraction patterns were recorded in the continuous movement mode of the detector with an angular velocity of 20/min, a constant value of the integration time $\tau = 1 \text{ s}$, x-ray tube voltage at $U = 26 \text{ kV}$, and anode current at $I = 15 \text{ mA}$.

Statistical analysis

Statistical analysis of the data was performed using StatSoft STATISTICA 8.0.360. In the STATISTICS package, the comparison of two average samples of normally distributed features (Student's t-criterion) was implemented in the Basic Statistics/Tables module. The t-test, independent, by variable submodule, was used for two different general summations.

Results

Rats' weight

The laboratory rats' weight in group I (HCD+LMC) increased to $194.63 \pm 6.1 \text{ g}$ in the 8th week, $309.45 \pm 6.39 \text{ g}$ in the 16th week and $340.82 \pm 8.62 \text{ g}$ in the 24th week, which indicates a statistically significant increase in weight while compared to the control group ($p = 0.014$). In the HCD+LMC+WBV group, the rats' weight increased to $198.3 \pm 6.61 \text{ g}$, $279.0 \pm 8.14 \text{ g}$, and $304.93 \pm 5.07 \text{ g}$ in the 8th, 16th, and 24th week, respectively ($p>0.05$), (Figure 2).

Leptin is the hormone involved in the regulation of body weight. While increasing weight by 10%, serum leptin levels may become more than 3 times higher. Therefore, to assess energy metabolism, it is advisable to determine its concentration. In the 8th week of the experiment, the leptin level in the control group was $5.25 \pm 0.42 \text{ ng/ml}$; in experimental group I, it amounted to $15.01 \pm 1.19 \text{ ng/ml}$ ($p = 0.000007$), and in experimental group II - $11.13 \pm 1.71 \text{ ng/ml}$ ($p = 0.004$). In the 16th week of the experiment, the dynamics of leptin levels were the following: in the control group, it remained nearly unchanged at $5.91 \pm 0.35 \text{ ng/ml}$, in experimental group I, the average level was at $21.01 \pm 1.95 \text{ ng/ml}$ ($p = 0.000008$), in experimental group II - $16.07 \pm 1.84 \text{ ng/ml}$ ($p = 0.00014$). In the 24th week of the experiment: the control group showed $4.93 \pm 0.25 \text{ ng/ml}$, experimental group I - $24.51 \pm 2.29 \text{ ng/ml}$ ($p = 0.000003$), and experimental

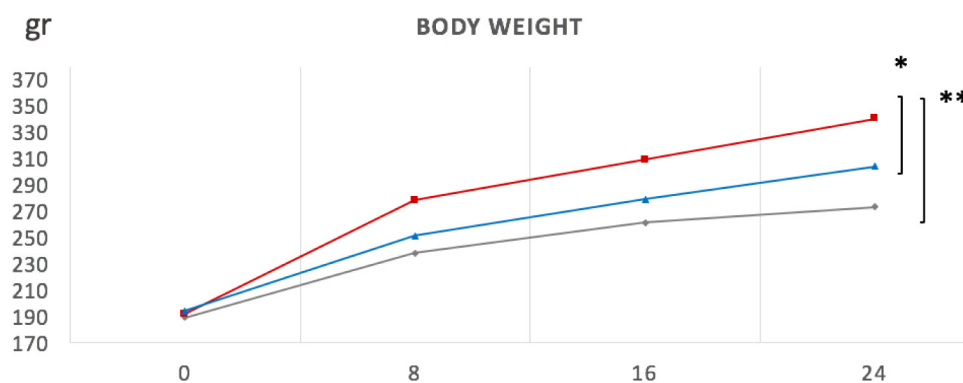


Figure 2: Body weight. * - groups do not differ statistically, $p > 0.05$; ** - groups differ statistically at $p = 0.014$. ■ control group; ■ limited mobility condition + high-calorie diet; ■ limited mobility condition + high-calorie diet + WBV

group II - 18.07 ± 1.67 ng/ml ($p = 0.000007$) (Figure 3A).

For the evaluation of bone tissue remodeling of the experimental rats, we

examined hormonal markers of bone condition, particularly parathyroid hormone (PTH) and calcitonin. The blood analysis showed significant differences in PTH levels between

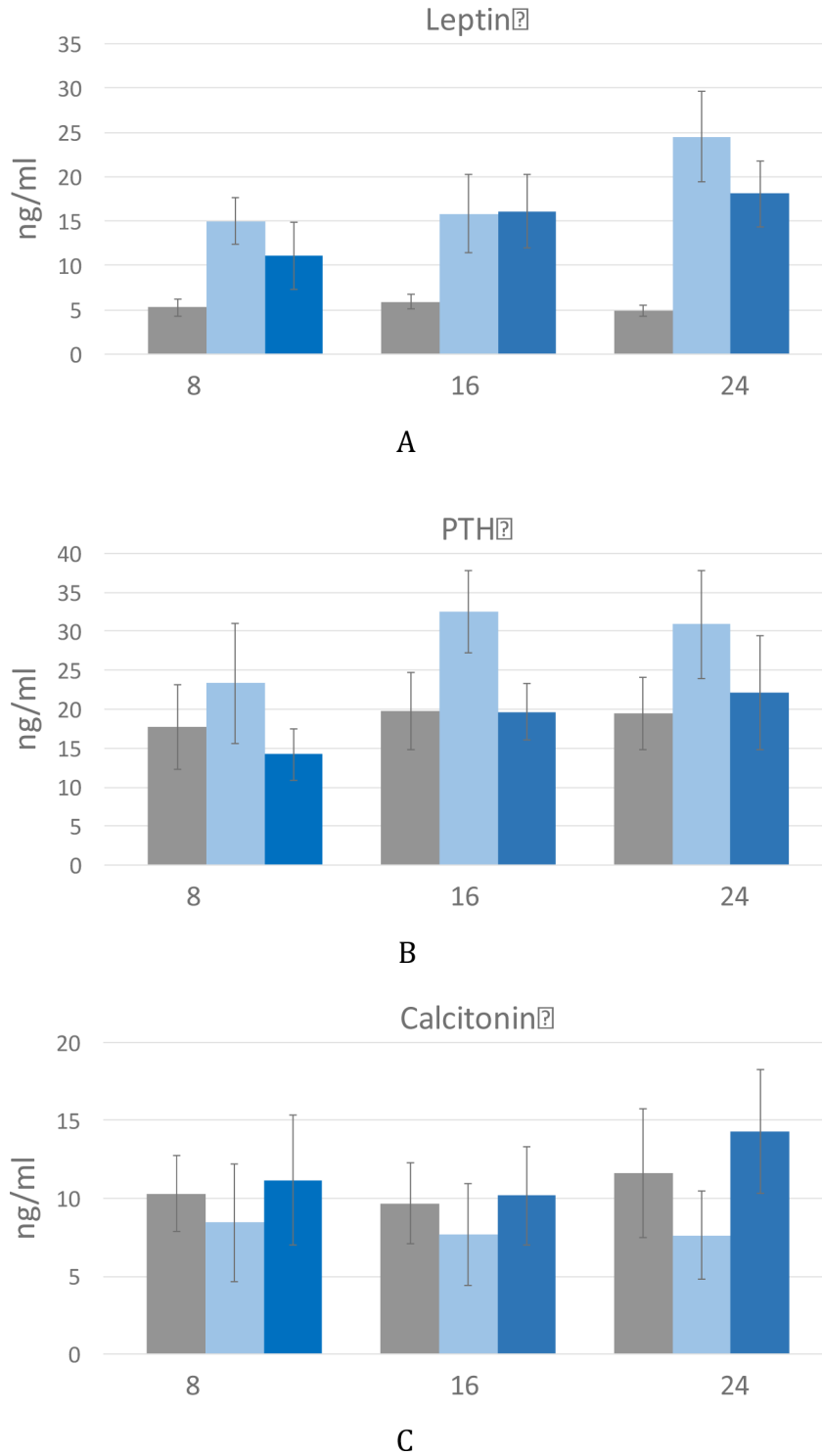


Figure 3: Cytokines levels and bone remodeling serum markers.

■ control group; ■ limited mobility condition + high-calorie diet; ■ limited mobility condition + high-calorie diet + WBV

(A - concentration of leptin, ng/ml; B - concentration of PTH, ng/ml; C - concentration of Calcitonin, ng/ml)

the control and experimental groups of rats (Figure 3B). In the 8th week of the experiment, the PHT concentration in the control group of animals was at 17.70 ± 2.42 pg/ml; in experimental group I, it was at 23.32 ± 3.45 pg/ml ($p=0.175$), and in experimental group II – 14.2 ± 1.5 pg/ml ($p>0.05$). In the 16th week of the experiment, there was the following dynamic of the parathyroid hormone level: in the control group, the figure remained almost unchanged and amounted to 19.72 ± 2.22 pg/ml; in experimental group I, it was at 32.5 ± 2.38 pg/ml ($p = 0.0015$), in experimental group II – 19.65 ± 1.62 pg/ml ($p>0.05$ for the control group and $p = 0.000633$ for the first experimental group). In the 24th week of the experiment, in the control group, the level was at 19.43 ± 2.05 pg/ml, in experimental group I – 30.92 ± 3.11 pg/ml ($p=0.007$), and in experimental group II at 22.08 ± 3.27 pg/ml ($p>0.05$).

In the 8th week of the experiment, the indicator of the rats' calcitonin level (Figure 3C) in the control group was at 10.3 ± 1.1 pg/ml, in experimental group I at 8.43 ± 1.7 pg/ml ($p>0.05$), in experimental group II – 11.18 ± 1.92 pg/ml ($p>0.05$). In the 6th week of the experiment, there were the following changes in the calcitonin level: in the control group, the figure remained practically unchanged at 9.67 ± 1.18 pg/ml, in experimental group I, the average level amounted to 7.68 ± 1.46 pg/mL ($p>0.05$), in experimental group II – 10.18 ± 1.4 pg/ml ($p>0.05$). In the 24th week of the experiment, in the control group, the figure was at 11.63 ± 1.86 pg/ml, in experimental group I – 7.62 ± 1.28 pg/ml ($p>0.05$), and in experimental group II – 14.3 ± 1.78 pg/ml ($p>0.05$).

Correlation analysis allowed us to establish the direct correlation ($p = 0.54$) between PTH and calcitonin in the 8th week of the experiment in the first group, the reverse correlation ($p = -0.77$) in the 16th week and the direct connection in the 24th week. In the 8th week of the experiment, the WBV groups showed a strong positive correlation ($p = 0.8857$), weak positive correlation $p = 0.25$ in the 16th week and direct positive correlation $p = 0.54$ in the 24th week of the experiment.

The diffraction patterns of the tibia series samples are shown in Figure 4 while compared to the theoretical diffraction pattern of the $\text{Ca}_{10}\text{P}_6\text{H}_2\text{O}_{26}$ chemical compound (hexagonal

syngony, space group P 63/m, unit cell parameters $a=9.42$ Å, $c=6.88$ Å). Significant erosion of the diffraction maxima of the $\text{Ca}_{10}\text{P}_6\text{H}_2\text{O}_{26}$ crystalline phase indicates a low degree of crystallinity of the compound due to the small size of the coherent scattering regions (the crystallite size does not exceed 10 nm). Also, a wide diffuse halo is observed in the diffraction patterns around the diffraction angle $2\theta \approx 21^\circ$, indicating the presence of an amorphous (disordered) phase represented by collagen fibers in the samples. In the series of samples, the highest content of the amorphous phase is observed in the samples of the first group in the 16th and 24th weeks of the experiment. The decrease in the intensity of the diffuse maximum of the samples obtained on the 8th day indicates a reduction of the amorphous phase contents. At the same time, on the 24th day, we can observe both the decrease of the diffuse maximum and the intensity of the crystalline phase maxima, which is particularly pronounced in the area of the most intense lines (211), (121), (112) and (300) of the $\text{Ca}_{10}\text{P}_6\text{H}_2\text{O}_{26}$ phase. To estimate the quantitative content of the amorphous and crystalline phases, we used the following ratios:

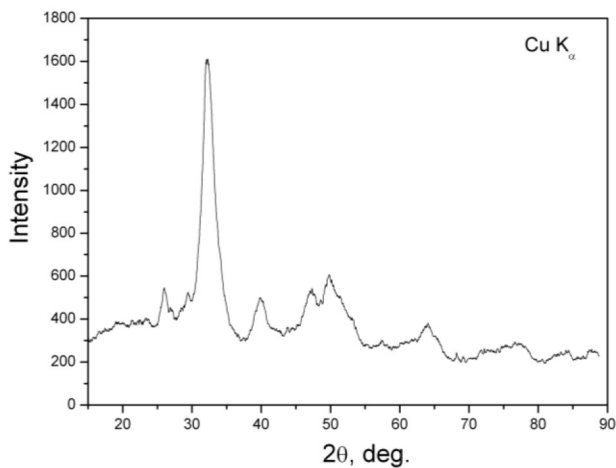
$$X_{am.} = \frac{I_{am}}{(I_{am} + I_{cr})}, X_{cr} = 1 - X_{am.}$$

(I_{am} – the maximum intensity from the amorphous phase, measured at $2\theta \approx 21.5^\circ$)

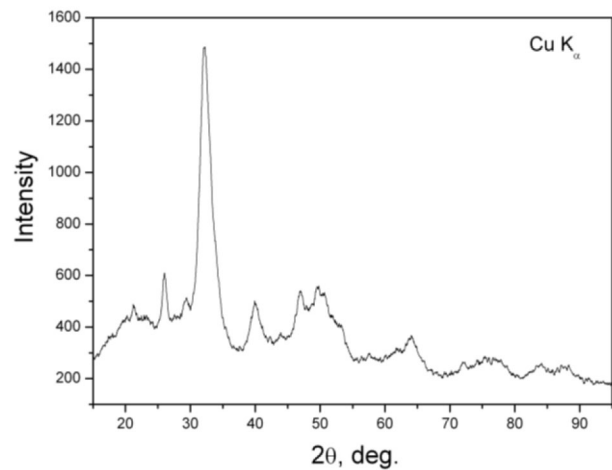
I_{cr} – the maximum intensity of the crystalline phase, measured at $2\theta \approx 32.1^\circ$, while taking into account the background scattering).

The calculations results are shown in Table 1. In the 8th wk. → 16th wk. → 24th wk. sequence, we can observe the increase of the crystalline phase volume fraction from 79% to 88/85% in the second experimental group and the decrease of the mineral component in the first experimental group (Figure 4 A/B/C).

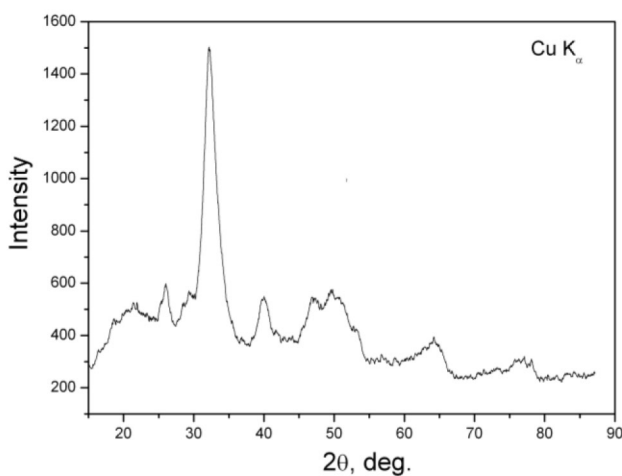
Figure 4D displays a significant increase in the intensity of crystalline phase lines in diffraction patterns. Also, in the samples of the 16th day of the experiment, there is a noticeable decrease in the intensity of the diffuse background from the amorphous phase. According to the results of the phase content estimation,



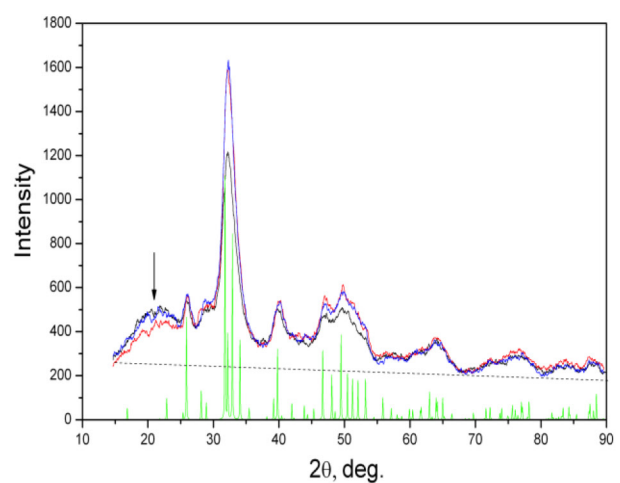
A



B



C



D

Figure 4. A, B, C: Diffraction patterns of the rat's femur in experimental group I (HCD+LMC) in the 8th, 16th and 24th week, respectively. To determine the integral intensity of the hydroxyapatite reflex, we chose the reflex of the 30-37° angular range since it is of the highest intensity. D – diffraction patterns of experimental group II samples (HCD+LMC+ WBV) in – 8th; – 16th ; – 24th week. The arrow indicates the position of the amorphous phase diffuse maximum, while the dashed line indicates the background scattering.

Table 1: Volume fractions of amorphous and crystalline phases of the samples.

Sample HCD+LMC	X_{am}	X_{cr}
8 th wk.	0.18	0.82
16 th wk.	0.2	0.80
24 th wk.	0.24	0.76
Sample HCD+LMC+WBV	X_{am}	X_{cr}
8 th wk.	0.21	0.79
16 th wk.	0.12	0.88
24 th wk.	0.15	0.85

the maximum increase in the crystalline phase content was observed in the WBV samples in the 16th and 24th weeks of the study.

Discussion

We used the presented model of obesity with a high-calorie diet to demonstrate that in the 8th week of the study, there already was a decrease of the mineral mass of trabecular and cortical layers of the rats' tibia, despite a significant increase in body weight of animals and increased mechanical stress. These structural changes

co-occur with an increasing level of leptin in serum and PTH. The mechanism of the mineral component loss is explained by the change in the correlation of RANKL/OPG expression levels of osteoblasts and the increased number of TRAP-positive osteoclasts. Such changes may be accompanied by increased activity of osteoclasts and reduced expression of anti-inflammatory cytokine IL-10. Obesity can enhance bone resorption through the regulation of proinflammatory cytokines, such as IL-6 and TNF- α , that stimulate the activity of the osteoclasts while regulating the RANKL/RANK/OPG path. This may further lead to chronic degenerative and inflammatory changes in the musculoskeletal system [28-31].

Obesity and a sedentary lifestyle might affect bone metabolism directly or indirectly through cytokines originating from adipocytes, particularly through leptin and adiponectin. This process is usually accompanied by a significant increase in serum leptin and a decrease in adiponectin [32]. Leptin's effect on the bone remains controversial. There are reports both on positive and negative effects. High concentrations of leptin in serum (as observed in our experimental animal model with obesity) may negatively affect bone metabolism. We have discovered that the increased level of leptin in serum is a negative regulator of bone mass [32, 33]. Moreover, of course, it should be noted that a high-fat diet, which often leads to obesity, prevents the absorption of calcium in the intestine. Free fatty acids can form calcium salt and contribute to low calcium absorption [29].

Nowadays, the WHO recommends moderate exercise as effective prevention of various diseases, including osteopenia. It decreases fat mass and increases bone mass (according to several experimental models with mice). High-frequency whole-body vibration is regarded as a means of mechanical influence that potentially improves the condition of the locomotor apparatus.

Besides cytokines, we conducted laboratory tests of the hormonal panel. Our research showed that in control (rats without obesity) and the HCD+LMC+WBV group, the mineral component amount was higher than in the HCD+LMC group. Besides, the first experimental

group showed a higher concentration of the parathyroid hormone in the serum (PTH) and lower calcitonin levels. The research results indicate that obesity has a negative impact on mineral bone mass, serum PTH, and calcitonin, partly explained in our conclusion. It is worth noting that the experimental animals showed decreased fat mass and the leptin concentration in blood serum.

We considered vertical whole-body vibration, as the means of mechanical stimulation, an alternative to pharmacological treatment of osteoporosis. Such stimulation induces the growth of the mineral component as a result of mechanical oscillations with micro-deformation of the bones. Previous studies presented by Rubin *et al.* have demonstrated an increase in the cortical and trabecular bone of turkeys subjected to vibration therapy [21, 22]. Some studies on animals and humans have proved positive effects of the vibration therapy on the structure and biomechanical properties of joints and muscles [14, 15].

Conclusion

Therefore, mechanical stimulation may serve as a promising alternative to medication therapy for osteopenia. While summarizing the data, our study provides a new understanding of the mechanisms of the mineral component loss in bone tissue in the case of obesity and sedentary lifestyle, while forecasting the future risks of osteoporotic fractures. The results of the experiment indicate that WBV is beneficial in preventing osteoporosis and bone mass loss.

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Ethical approval

Ethics Commission Report No. 10, 16.12.2019, Danylo Halytsky Lviv National Medical University.

Conflict of Interest

The authors declare no conflict of interest.

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