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CHANGES OF SELECTED BIOCHEMICAL PARAMETERS OF BLOOD, DENSYTOMETRIC AND STRENGTH BONES OF RATS UNDER LOW FREQUENCY VIBRATION

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The aim of the research is to analyze the changes of selected biochemical blood, densitometric parameters and bone strength of rats under exposure to low-frequency vibrations. Experiments were run on 30 Wistar rats randomly divided into three groups: the first group was first exposed to vibrations when rats were 20 days old: _20 – before puberty, the second group was subjected to vibrations from the 70-th day on: _70 – after reaching puberty, the third is the control group: _K. On the 145-th day the rats' _20 and _70 bones were subjected to biochemical examination, followed by densitometric and bone strength tests.

Key words: low-frequency vibrations, biochemical blood, densitometric parameters, bone strength tests.

1. Introduction

Few reports in literature on the subject suggest that short-time vibrations affect the blood biochemistry, and bones' densitometric features and resistance parameters. Vibrations might improve the blood circulation, partial pressure of oxygen, oxygen saturation of haemoglobin. Besides, the oxygen is better utilised by the tissues. These beneficial effects are attributable to the widening of the blood vessels, improved blood circulation (particularly in the micro-circulation systems) and improved hydrodynamic properties of blood. In the consequence, the risk of thrombus occurrence is reduced. Modifications of these parameters might bring about the normalisation of the membrane potential, improvements of metabolism, more efficient heat balance of cells. As a result, the patient's condition in generally improved and the immunity to infections is enhanced. Various control mechanisms might be applied to intensify the synthesis of proteins (improved regenerative action), to facilitate the removal of metabolism products (detoxification of the human body) and to stimulate the immune system.

It seems reasonable to suppose that vibrations restore the equilibrium of the upset energy processes, leading to regeneration of the whole body. It appears that vibrations might be employed in the treatment of broken bones, hard to-heal wounds, circulatory diseases and some psycho-somatic disorders. The relationship between exercising and the efficiency of metabolism is well-established. Research reveals that exercising stimulates the muscle development and enhances the functions of other organs as well as the immune system. As regards the modern lifestyle, most people, particularly those over 50, do not have enough exercise. Well chosen mechanical vibrations (with frequencies corresponding to the man's running) might help to make up for this deficiency. When subjected to vibrations, the reactions of circulatory system become the reflex movements, demonstrating the activation of the nervous system, particularly the vegetative system whilst muscles must be active to control vibrations. Vibration control is a most complex process, involving the interactions between afferent and efferent paths in the nervous system. The activity of the muscle system in response to vibrations might bring about the changes in the bioelectric activity of the brain, depending on the applied vibrations' parameters: frequency, amplitude and acceleration. Short-term application of low-frequency vibrations stimulates the muscle activity whilst the effects of long-term vibration exposure might be just the opposite [11].

Osteoporosis is a physiological or pathological phenomenon. After a tissue or an organ reaches its peak condition, further changes may be only for the worse (as shown by autogenesis). Women's bones begin to age when they are about 30 years old, the process is enhanced after menopause. In men it begins about 10 years later. Little exercise and excessive loading of muscles and bones are responsible for negative processes, such as rapid body ageing (only basic metabolic functions are maintained to supply the required energy) [1, 3, 7]. Muscle and bone response to proposed disturbances may slow up these retrogressive changes a little.

The relationship between physical exercise and the rate of metabolism has been well known for a long time. Research reveals that physical exercise stimulates muscle growth and strengthens other organs, as well as the immunological system [8–10, 12]. As far as lifestyle is concerned, modern people have decidedly too little exercise and hence they suffer from energy deficiency. A common feature of all diseases are cell malfunctions (or sometimes structural damage). Precisely controlled mechanical vibrations (with frequencies as those produced during running) might eliminate this deficiency. Vibrations may help to restore the equilibrium of disturbed energy processes and help the body to recover.

This method may be applied after bone breaking, in wound healing, in circulatory diseases and in some psychosomatic disorders. When exposed to vibrations, initial response of the circulatory system to vibrations is purely automatic, it is a result of activation of the central nervous system while the autonomic nervous system is also stimulated. Muscles actively absorb vibrations. Absorption of vibrations is a most complex process involving afferent and efferent nerve tracts. Muscle activities during vibrations may result in bioelectric activity, depending on vibrations parameters: frequency, amplitude and acceleration. Low-frequency short-term vibrations activate the muscles, while long lasting vibrations may hinder muscle action [11].

There is no adequate data showing how vibrations might affect biochemical parameters. Vibrations may improve blood circulation, partial pressure, hemoglobin saturation with oxygen and oxygen uptake in the tissues. These are effects of angiectasia, blood flow improvement (particularly micro-circulation) and improvement of dynamic properties of blood (reducing the risk of clotting). A change in those parameters may lead to normalization of membrane potential on the cell surface, metabolism and energy balance in cells may be improved, which in the end makes the body more fit and improves the overall resistance to illness. Protein synthesis (regeneration processes) and discharge of metabolism products (detoxification) is enhanced via several control mechanisms. At the same time immunity system is stimulated.

2. Methodology

The Authors advance the hypothesis that exposure to low-frequency vibrations may impact on selected blood, densitometric and bone strength parameters [4–6].

Experiments were run on 30 Wistar rats from the animal farm of the Jagiellonian University. During the tests the animals remained in standard cages and were fed with standard food GLM in standard dosages. They were given tap water to drink (with no limits) with an addition of vitamin formula (polfamix Z). The temperature in places where animals were kept was 21–23°C, humidity 55%. The light regime was automatically controlled (6,00–18,00 – day time, 18,00–6,00 night time). The animals were divided into three groups of equal size: the first group was first exposed to vibrations when rats were 20 days old (before puberty), the second group was subjected to vibrations from the 70-th day on (after reaching puberty), the third is the control group. It consists of a vibration-generating circuit and the control and analyzing circuit (Fig. 1).

Fig. 1. Experimental set-up. Vibration-generating circuit and control and analysis circuit.

The measuring stand was designed and prepared specially for the tests. During exposure the animals remained in a cage which was vibrated using the vibration generator VEB RTF Messelektronic type 11075 controlled via a function generator MX 2020 and a power amplifier ELMUZ. The analyzing circuit consisted of a SVAN 912 analyzer (charging input, with a pre-amplifier SV04) impedance 39 Ω/pF; integration time constant 4.6 s; 4 measurements sub-ranges 10 pC, 100 pC, 1 nC, 10 nC; measuring range 0.01 pC–25.9 nC (RMS); high-pass filter HP $= 0.6$ Hz with the conversion 6 dB/octave; low-pass filter 1 kHz with the conversion 24 dB/octave; natural noise level up to $1.7 \cdot 10^{-14}$C; sampling frequency 65536 Hz; 14-bit A/C converter; antialiasing filters; damping in the rejection band > 80 dB; stability of amplitude readouts ± 0.1 dB) and the magnetometer URSZULA 3 (8 measurement ranges: 100 pT, 300 pT, 1 nT, 3 nT, 10 nT, 100 nT, 300 nT; measurements and analyses were possible for three frequency bands: 50 Hz (pass band filter $Q = 20$); 1–20 Hz (Chebyshev's low pass filter of the 6-th order); 8–12 Hz (pass band filter $Q = 4$)).

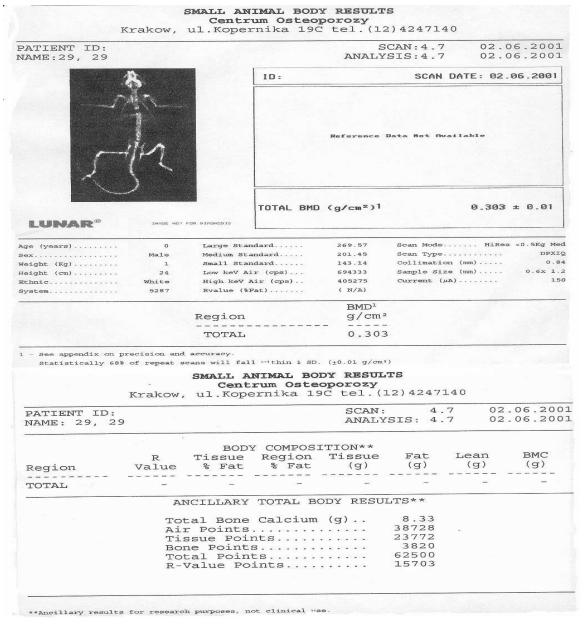
Fig. 2. EPOL 20 apparatus.

Rats were exposed to vibrations in the test laboratories of the Department of Animal Biopsychology of the Jagiellonian University. Vibrations applied during the first stages of the experiment had acceleration 1.22 m/s^2 and frequency 20 Hz. The exposure was repeated seven times, for 3 hours, at the same time of day. Induction of the magnetic field at the frequency 50 Hz was 140 nT, for the frequency range 1–20 Hz it would be as high as 270 nT. In the third stage (on 135-th day) rats were exposed to vibrations again. Vibrations with acceleration 4.20 m/s^2 and frequency 20 Hz were applied. The exposure was repeated 10 times, for 3 hours daily, at the same time of day. The induction of a magnetic field for the frequency 50 Hz was 120 nT, for the frequency range 1–20 Hz it would be 300 nT (test conditions were constantly monitored by the instruments in the control and analyzing circuit). On the 145-th day blood samples were collected and

the following biochemical tests were performed: determining calcium contents in blood plasma Ca, tests for hemoglobin, lactic acid, LDL and HDL cholesterol (tests were performed with the use of EPOL 20 apparatus (Fig. 2), densytometric with the use of LUNAR apparatus. Results are shown in Figs. 5–13.

EPOLL 20 is a novel, microprocessor-controlled high-precision colorimeter. Monochromatic radiation with quasi-double beam splitters ensure the high zero-point stability (monochromatic radiation is ensured by high-stability interference filters; twelve wavelengths are selectable, from the range 340–900 nm, the set of standard filters: 340, 405, 520, 540, 560, 630 nm; one-half transmission band < 8 nm; attenuation beyond the transmission band $> 10^5$; wavelength set accuracy ± 2 nm; absorbance range: 0–2500 j.A, measurement error: $\pm 1\%$ or 0.003 j.A over the whole range; absorbance zero point instability: < 0.005 j.A/hour and < 0.003 j.A/hour, 15 minutes after switching on).

The DXA method was applied to determine the bone mineral density with the use of the DPX-IQ LUNAR apparatus. Two most vital clinical sites of the rat skeleton: AP spine and femural were measured (the DPX-IQ is an X-ray densitometer used for measurements of bone density at all skeletal sites; it utilises a high performance detector technology, an advanced flatbed scanner and is equipped with a radiation source X (fixed RTG lamp potential: 38/70 KeV, stability $\pm 0.05\%$, double energy beam 2×36 KeV collimated to the radius 0.01 m in diameter; the measurements are fast (in five seconds) and at a low radiation dose (up to 10 mR), an automatic calibration system brings the reproductibility error down to 0.52% whilst the BMD measurement accuracy is less than 1%). Densitometric measurement data are shown in Fig. 3.



SMALL ANIMAL BODY RESULTS
Centrum Osteoporozy
Krakow, ul.Kopernika 19C tel.(12)4247140

PATIENT ID:
NAME:29, 29

SCAN:4.7 02.06.2001
ANALYSIS:4.7 02.06.2001

ID: SCAN DATE: 02.06.2001

Reference Data Not Available

TOTAL BMD (g/cm²)[1] 0.303 ± 0.01

LUNAR®

Age (years)	0	Large Standard	269.57	Scan Mode	Hires +0.5Eq Med
Sex	Male	Medium Standard	201.45	Scan Type	DPXIQ
Weight (Kg)	1	Small Standard	143.14	Collimation (mm)	0.04
Height (cm)	24	Low keV Air (cps)	694333	Sample Size (mm)	0.6x 1.2
Ethnic	White	High keV Air (cps)	405275	Current (uA)	150
System	9287	R-value (%Fat)	(N/A)		

Region	BMD[1] g/cm²
TOTAL	0.303

1 - See appendix on precision and accuracy.
Statistically 68% of repeat scans will fall within 1 SD. (±0.01 g/cm²)

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BODY COMPOSITION**

Region	R Value	Tissue % Fat	Region % Fat	Tissue (g)	Fat (g)	Lean (g)	BMC (g)
TOTAL	-	-	-	-	-	-	-

ANCILLARY TOTAL BODY RESULTS**

Total Bone Calcium (g)	8.33
Air Points	38728
Tissue Points	23772
Bone Points	3820
Total Points	62500
R-Value Points	15703

**Ancillary results for research purposes, not clinical use.

Fig. 3. A result of densitometric tests.

The mechanical testing of bones was performed in three-point bending on a testing machine Instron 4502 at a loading span $L = 22$ mm and a loading rate $v_t = 2$ mm/min, air humidity 50%, temperature 23°C (minimal tensile/compression load 0.04 N (+/-0.2 mN), maximal tensile/compression load 10 kN, loading rate 0.05–1000 mm/min, maximal nominal load-to-rate ratio: 10 kN at the rate up to 500 mm/min and 4 kN at 500–1000 mm/min, span return speed automatically adjusted to the maximal possible standard rate available in manual-control operation: large – 1000 mm/min, small – initially 50 mm/min, reaching up to 1000 mm/min within 5 sec, steady-state span displacement stability: 0.1%, displacement range: 1000 mm; machine flexibility: nominal axial rigidity – 50 kN (span in the central position), guiding screws: chromium-plated 37 mm in diameter, with pre-loaded spherical double nuts and tapered bearings; main drive: a dc magneto-electric motor with a low-inertia printed-circuit armature and an integrated tachometer providing the feedback control of velocity, guiding: four chromium-plated rods passing through a mobile span. Applications: tensile tests, compression tests, bending test, shear strength tests, fatigue endurance tests; power: 500 VA main unit, 250 VA – console, 250 VA – connector (total 1250 VA), weight: 220 kg (main unit) $+30$ kg (micro-processor control unit) $+8$ kg (console), dimensions: base 737×745 mm, total height 1795 mm).

Fig. 4. INSTRON 4502 tester – general view.

Bending tests were digitally controlled utilising the SERIES IX Automated Materials Testing System 1.02 C software (Instron Corporation). The bending curve was obtained for each tested specimen. Statistical analysis was then applied to the selected dependent variables: load at yield – F [kN], displacement at yield – s [mm], stress at yield σ_g [MPA], strain at yield ε [–], Young modulus – E [MPa], energy to yield point –

EYP [J], energy to break point – *EBP* [J] (Fig. 14–20). Strength parameters are derived from standards formulas.

The ratio $\Delta F/\Delta s$ is equal to the tangent of an inclination angle between the steepest linear section of the bending curve and the displacement axis. Energy-related parameters (*EYP*, *EBP*) are obtained by integrating the areas beneath the bending curve.

3. Results of experiments

Statistical analyses of biochemical test results (Fig. 5–10), as well as densitometric, (Fig. 11–13) and bone strength and endurance test data (Fig. 14–20) were performed to check whether those variables should follow the normal distribution (Shapiro-Wilk tests). Furthermore, the descriptive statistics are provided. Statistical inferring was conducted for the significance level $p < 0.05$. The T-Student test was applied supported by the Cochron-Cox procedure for independent tests [2].

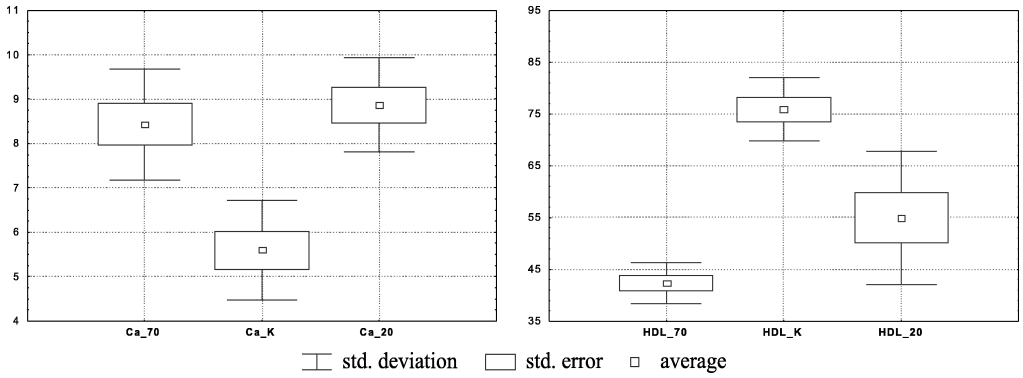


Fig. 5. The effects of vibration exposure on Ca level [mg/dl].

Fig. 6. The effects of vibrations on HDL cholesterol level [mg/dl].

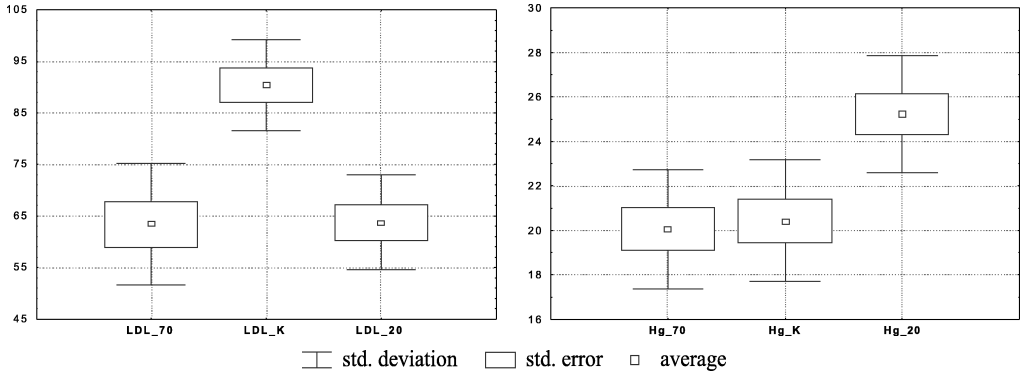


Fig. 7. The effects of vibrations on LDL cholesterol level [mg/dl].

Fig. 8. The effects of vibrations on Hemoglobin level Hb [g%].

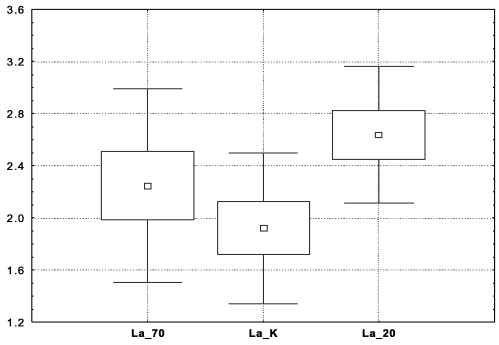


Fig. 9. The effects of vibrations on lactic acid level La [mmol/l].

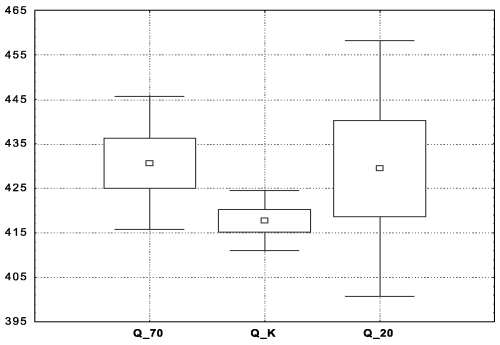
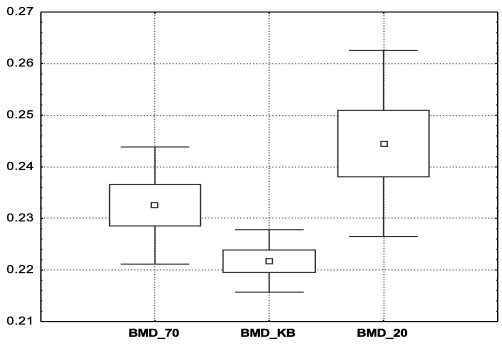


Fig. 10. The effects of vibrations on body mass [g].



Fig, 11. The effects of vibrations to BMD bone [g/cm^2].

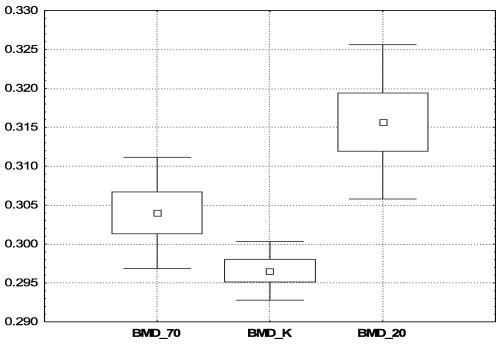


Fig. 12. The effects of vibrations to BMD rats [g/cm^2].

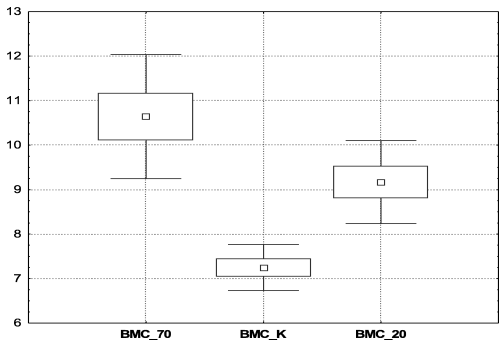


Fig. 13. The effects of vibrations to BMC [g].

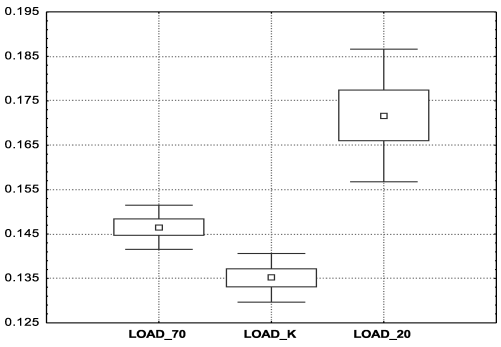


Fig. 14. The effects of vibrations on load at yield – F [kN].

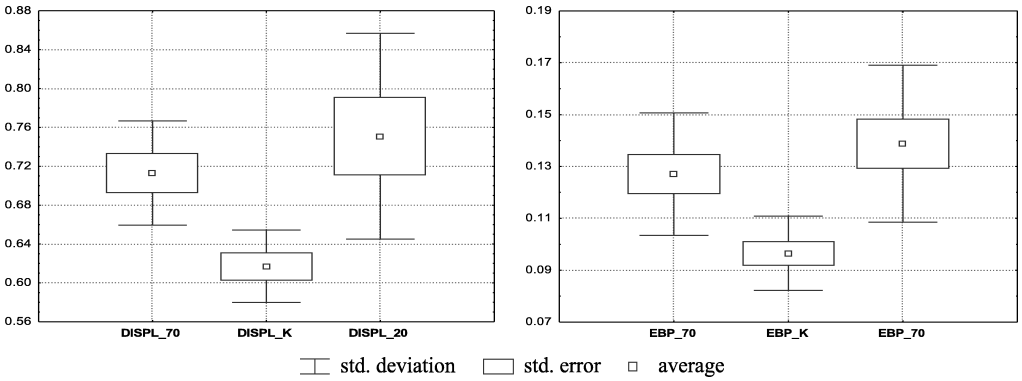


Fig. 15. The effects of vibrations on displacement at yield – s [mm].

Fig. 16. The effects of vibrations on energy to break point – *EBP* [J].

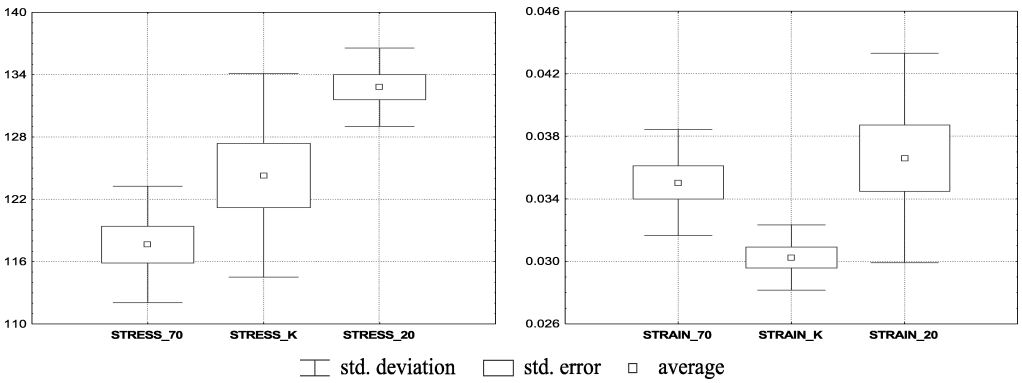


Fig. 17. The effects of vibrations on Stress at yield – σ_g [MPa].

Fig. 18. The effects of vibrations on strain at yield – ε [–].

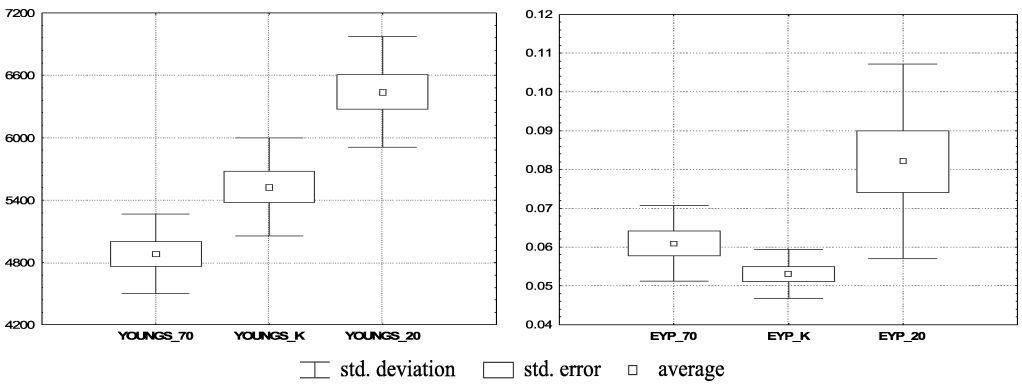


Fig. 19. The effects of vibrations on Young's modulus – E [MPa].

Fig. 20. The effects of vibrations on energy to yield point – *EYP* [J].

4. Conclusion

The results of biochemical tests and strenght bones lead us to the following conclusions:

- The Ca contents in blood plasma, reaching up the value 8.87 mg/dl $\pm$ 1.06 for exposed rats _20 and 8.43 mg/dl $\pm$ 1.25 for the group _70 is higher while compared to the value obtained in the control group: 5.60 mg/dl $\pm$ 12.86,
- The decrease in HDL cholesterol level in exposed rats is statistically significant. The value for _20 was 54.83 mg/dl $\pm$ 12.86, for the group _70 it would be 42.34 mg/dl $\pm$ 3.92, while compared to the control group value: 75.91 mg/dl $\pm$ 6.09.
- The decrease in LDL cholesterol level in exposed rats is statistically significant. The value for _20 was 63.73 mg/dl $\pm$ 9.20, for the group _70 it would be 63.44 mg/dl $\pm$ 11.82, while compared to the control group value: 90.37 mg/dl $\pm$ 8.80.
- The results of tests for hemoglobin Hb, lactic acid La and body mass proved to be statistically insignificant due to large standard deviation.
- A statistically significant increase in the BMD in groups exposed to vibrations in relation to the control group, the investigation is for whole rat body conducted and after obtaining a thigh bones.
- A statistically significant increase in the BMD in groups exposed to vibrations.
- A statistically significant increase in the breaking force in groups exposed to vibrations in relation to the control group, increase of bone deflection for the group _70. These might be indicative of the strengthening of the bone structure and as regards the bone deflection, it is suggestive of enhanced bone flexibility and reduced fragility.
- Bones' bending strength is greater in the group _20 while compared to _70. That might be suggestive of positive effects of vibrations in the pre-puberty periods.
- Deformation understood as elongation of extreme tensioned fibres is larger in groups exposed to vibrations than in the control group (statistically significant for the group _70). It might imply that bone loading proceeds over a wider displacement range without any breaking, with a better safety margin. The interpretation of this parameter stems from the close relationship between the elongation of external bone layers and the deflection values.
- Young modulus assumes larger values in the group _20 while compared to the control group. In the group _70 the reverse is true-the values of Young modulus are lower than in the control group. This parameter is actually a material constant and as such is not associated with strength. It appears that the larger the Young modulus, the faster the load increase with progressing deformation during the early phase of bending.
- Energy-related parameters *EYP* and *EBP* have statistically higher values in the groups exposed to vibrations. In other words, more energy has to be supplied, and hence more work is to be performed, to break a bone of an animal exposed to low-frequency vibrations than of one from a control group.

- The analysis of strength parameters of bones leads us to the conclusion that exposure to low-frequency vibrations has beneficial effects on bone endurance. The most favourable strength parameters were reported in the group of animals exposed to vibrations already in the pre-puberty period.

The analysis of results obtained for the exposed groups reveals that:

- animals exposed to vibrations have a larger body mass in comparison to the control group,
- training at an early age group _20 leads to an increase in the body mass in relation to the "older" group _70.

Acknowledgment

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References

[1] TRĘBACZ H., *Właściwości mechaniczne kończyn udowych dorosłych szczurów po unieruchomieniu kończyny*, Materiały konferencyjne Bionika, Biomechanika i Robotyka w Inżynierii Biomedycznej, Zakopane – Kraków 2000.

[2] OKABA W., *Metody Statystyki Matematycznej i Metodyka Doświadczalnictwa*, PWN, Warszawa 1980.

[3] CZAJKOWSKA E., SKIERSKA B., WYSOKIŃSKA J., SZWDYN A., GAJEWSKI K., *Morfometryczne parametry kości szczurów poddanych wysiłkowi fizycznemu w różnym wieku*, Wychowanie Fizyczne i Sport, 1, 79–90 (1995).

[4] DAMIJAN Z., RUMIŃSKI M., *Wpływ drgań niskoczęstotliwościowych na parametry wytrzymałościowe kości szczurów*, Czasopismo Techniczne Politechniki Krakowskiej, Z1-M/2004, 35–43 (2004).

[5] DAMIJAN Z., *Changes of selected biochemical parameters blood and strength bones of rats under low frequency vibration*, Molecular and Qantum Acoustics, **25**, 69–78, Gliwice 2004.

[6] DAMIJAN Z., KASPRZAK C., PANUSZKA R., *Influence of low-frequency vibration on changes of biochemical parameters of living rats*, Journal of the Acoustical Society of America, **115**, 2443, 2004.

[7] FROST H. M., *The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents*, Bone Miner., 2, 73–85 (1987).

[8] FROST H. M., *Bone "mass" and the "mechanostat": A proposal*, Anat. Rec., 219, 1–9 (1987).

[9] FROST H. M., *Skeletal structural adaptations to mechanical usage (SATMU): The hayline cartilage modeling problem*, Anat. Rec., 226, 423–432 (1990).

[10] IWAMOTO J., TAKEDA T., ICHIMURA S., *Effect of exercise on tibial and lumbar vertebral bone mass in mature osteopenic rats: bone histomorphometry study*, J. Orthop Sci., **3**, 5, 257–263, 1998.

[11] ENGEL Z., *Ochrona środowiska przed drganiami i hałasem*, PWN, Warszawa 1993.

[12] PAWLIKOWSKI M., *Mineralizacja kości*, AGH, 1998.