Evaluation of the bone tissue mechanical parameters after induced alimentary Cu-deficiency followed by supplementary injection of Cu nanoparticles in rats

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Abstract. The paper studies the evaluation of mechanical properties of bone tissue with Cu-deficiency in the body. The studies have been conducted on the bones of rats subjected to a complete Cu-deficient diet and the Cu-deficient diet, followed by rehabilitation diet. Within the frames of research testing on bending of animals’ femoral bones was conducted, the scheme for flexure of the axes “move-force” midsection was drawn. The Young’s modulus, ultimate normal and tangential stress limits were defined. The animals under research were divided into three groups: a) with Cu-deficiency, b) Cu-deficiency within 5 weeks with the following rehabilitation, c) Cu-deficiency within 8 weeks with the following rehabilitation. Depending on time and Cu-deficiency in the body the analysis of the obtained mechanical characteristics was performed. The effect of Cu-deficiency on bone tissues’ rigidity and strength was detected. It was also shown that Cu-deficiency within 5 weeks and further rehabilitation the mechanic properties of the bone tissues are restored and the hardening analogue takes place. The recovery does not take place at the Cu-deficiency within 8 weeks, what the authors connect with the tissue texture transformation.

1. Introduction
It is known that copper is involved in maintaining adaptive mechanisms of the human body [1]. This is due to the active participation in the processes of antioxidant defense. The main factors governing the copper absorption are the concentration of copper in the diet and the organism’s supply of these microelement. With a high copper content in the diet, absorption is reduced and endogenous losses increase. Copper absorption processes are competitively interconnected with zinc, cadmium, and iron. An imbalance in the copper metabolism leads to several diseases.

More than half of the copper content in the body is contained in the musculoskeletal system. Copper impact on the bone metabolism appears as participating in the synthesis of the conjunctive tissue of bone and copper deficiency leads to disruption of bone mineralization. At present people with copper deficiency are proven to suffer from osteoporosis [2, 3]. Osteoporosis is a metabolic bone disease characterized by low bone mineral density and increased risk of low-energy fractures [4-7].

The current stage of nanotechnology development allows stimulating of the living organisms with nanomaterials. That is based on the nanoparticles physical-chemical characteristics and related effects, such as the ability to penetrate into the various body organs and tissues and stimulate metabolic processes in biotic doses [8-10]. In this study the results of defining bone tissue mechanical characteristics according to copper deficiency and its various timing recovery (introduction of nanoparticles of copper in the body) in the body are shown.
2. Materials and Methods

We used Wistar rats, which were divided into those subjected to the Cu-deficient diet (Diet) only, Diet and a Cu-restorative injections (supplementation - supp.), as well as a control group (see table 1). Laboratory animals in Groups 2 and 3 were intramuscularly administered with aqueous solutions of copper nanoparticles at a dose of 2.0 mg / kg body weight (supp.). Nanoscale copper particles with a spherical shape (103 ± 2 nm) were used in the experiment. Animal studies have been approved by the local ethics committee Kazan State Medical University, Russia.

To make analysis convenient the objects under research were divided into three groups: 1 group was subjected only to diet I; 2 group was subjected during 5 weeks to diet I, followed by the recovery diet II; 3 group was subjected during 8 weeks to diet I, followed by the recovery diet II. In several charts below the shorthand " Diet"/" Supp." will be used, i.e. 5/4 means that it was "diet" for 5 week and "supplementation" for 5 week.

Table 1. The table of diets of rats under research.

| Group | Diet (weeks) | Supp. (weeks) | Total (weeks) | Number of animals | Max Weight (gramm) | Male/Female (number) |
|-------|--------------|---------------|---------------|-------------------|-------------------|---------------------|
| 1     | 4            | 0             | 6             | 7                 | 139               | 3/4                 |
|       | 5            | 0             | 7             | 7                 | 146               | 4/3                 |
|       | 6            | 0             | 8             | 7                 | 135               | 3/4                 |
|       | 8            | 0             | 10            | 7                 | 235               | 3/4                 |
|       | 5            | 4             | 11            | 7                 | 202               | 4/3                 |
|       | 5            | 6             | 13            | 7                 | 260               | 3/4                 |
|       | 5            | 8             | 15            | 7                 | 391               | 3/4                 |
|       | 5            | 10            | 17            | 7                 | 407               | 4/3                 |
|       | 8            | 2             | 12            | 7                 | 195               | 3/4                 |
|       | 8            | 4             | 14            | 7                 | 213               | 4/3                 |
|       | 8            | 6             | 16            | 7                 | 260               | 3/4                 |
| Control group | 6            | 10            | 185           | 5/5               |

In the experimental study femoral bones of rats were taken, weighed, their density estimated, geometrical parameters measured, that followed by a bending test. For the tests in bending experimental equipment was designed and assembled, its circuit is shown in figure 1: extracted bone with the proximal part (position 1) was placed in the glasses using Wood's alloy (position 2), after which the centering of retaining ring was made (position 3), which was attached to the upper press traverse (position 5), the scales were set on a rigid support (position 4) which was attached to the lower fixed traverse (position 5). While testing the upper traverse was moved with velocity 0.2 mm/min and through the retaining ring (position 3) this move was transmitted to the bone (position 1), measurements of the applied force, movement and time being made at that. After the samples’ deformation the bone walls measurement was performed.

The average length of the studied samples between the bindings of the proximal sections is 12 mm, diameter is 2 mm, thickness is 0.5 mm. figure 2 shows a typical diagram obtained in bend tests (characteristic values for strength and movements are given).

Plot OA is that of proportionality, at this section the function is close to a linear one; at this stage of loading the bone undergoes elastic deformation; section AB is that of nonlinearity (it is not long); at this stage there occurs crushing of the bone beams, similar to plastic deformation [11]; BC is the appearance of the first crack, is characterized by the fall of the applied force; CD is a crack growth (depending on samples the number of such sections might be up to 3); DE is the appearance of a large crack and subsequent destruction.
To determine the Young's modulus and the modulus of the ultimate stress the beam pattern for the ring section was applied; the boundary conditions, the load value and measurements are shown in figure 3. In this case, the conditions of load are as follows:

$$\sigma_{\text{max}} = \left| \frac{M_{\text{max}}}{W} \right| \leq [\sigma],$$  \hfill (1)

where $\sigma_{\text{max}}$ - maximum normal load in the section, $M_{\text{max}}$ - maximum moment, $W$ - the moment of resistance at the bend, $[\sigma]$ - admitted normal load for material.

![Figure 1. Scheme of the testing stand.](image)

For the given scheme of load the movements are as follows.

$$y_{\text{max}} = \frac{Fl^3}{48EJ},$$  \hfill (2)

where $F$ - force, $L$ – the sample length, $E$ – the Young modulus, $J$ – the section inertia moment.

For the linear section of the diagram (section OA in figure 2), applying the formula (1), (2), we obtain:

$$E = \frac{Fl^3}{48Jy},$$  \hfill (3)

To estimate the admitted normal and tangential stresses we use the formula:

$$[\sigma] = \frac{1}{2W} \left| F_{\text{max}} \right|,$$  \hfill (4)

$$[\tau] = \frac{2}{J \cdot d} \left| S_x^{*} \right|,$$  \hfill (5)

where $S_x^{*}$ is a static moment of the intercepted section relative to Ox.
Figure 2. The characteristic diagram at the bending test: OA is the section of elastic deformation, AB is the section of nonlinearity, B is cracking formation, CD is the development of a crack, D is destruction.

The calculation of $[\sigma]$ - admitted normal stresses and $[\tau]$ - admitted tangential stresses for material (in point B, figure 2) was performed along with evaluation of a crack growth at various Cu-deficiencies (section CD in figure 2).

Figure 3. Calculation scheme. F - force (N), L - length of bone, R - radius of bone, h - thickness of bone

3. Results
The obtained results were subjected to statistical analysis, and then used for calculating the Young's modulus, the ultimate tangential and normal stresses. For the convenience of analysis at the graphs the calculated mechanical parameters are divided into groups, the line indicates similar characteristics for the control group, the calculated values of which do not contradict researches in the field [12-14].
Figure 4. The average Young's modulus, horizontal line – control group

Figure 5. The average admitted tangential stresses, horizontal line – control group

Figure 6. The average admitted normal stresses, horizontal line – control group

**Group 1:** with the Cu deficiency term increase in bone tissue the stiffness decreases (decrease in Young's modulus, see figure 4), the ultimate values of the normal and tangential stresses are also reduced with increasing Cu deficiency term (see figures 5 and 6), (hardness, shear strength, bending and stretching decrease).
Group 2: with increasing recovery period bone tissue stiffness tends to the average stiffness of the control group (see figure 4), the ultimate value of the tangential stresses is also in the range of shear stresses of the control group, at the maximum term of recovery increases to 10%. The value of the ultimate normal stresses increases with the growth of Cu nanoparticles recovery period up to 20% (see figures 5 and 6), (stiffness recovers, the shear strength slightly increases and tensile strength still more increases).

Group 3: with the recovery term increase the bone tissue stiffness increases sharply compared to that of the control group (see figure 4), the value of the limiting shear stresses also increases compared to the value of the limiting shear stresses in the control group, at a maximum recovery term it increases by 30%. The value of the ultimate normal stress decreases sharply with increasing Cu nanoparticles recovery term, up to 40% (see figures 5 and 6) the stiffness and shear strength greatly increases, tensile strength is reduced.

4. Conclusion
The study has assessed the effect of Cu deficient diets followed by supplementary introduction of Cu nanoparticles on the mechanical characteristics of bone tissue in rats. It was found that copper deficiency leads to a drastic reduction in the stiffness of bone tissue and strength. In case of copper deficiency in the body within 5 weeks and subsequent supplementary recovery stiffness of the bone tissue is restored, the ultimate shear stresses increase to 10% of normal, allowable normal stresses increase by 20%, thus the hardening of bone happens. In case of 8 weeks copper deficiency and subsequent supplementary recovery the bone tissue stiffness is greatly increased (up to 40%), the allowable tangential stresses are also greatly increased (up to 30%), but the normal allowable stresses drop (to 40%), which may be explained by the irreversible bone tissue restructuring [15, 16].

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