

# Comparative features of bioelements content in blood, liver and bone tissues in a rat model of crush-syndrome

Inna Krynytska<sup>1</sup>, Ivan Smachylo<sup>2</sup>, Sergii Grabchak<sup>3</sup>, Mariya Marushchak<sup>4</sup>

## ABSTRACT

### Objective

Crush syndrome (CS) remains a life-threatening condition. This research aims to investigate the changes of the macro- and microelement content in blood, liver and bone tissue in a rat model of CS.

### Methods

Studies were conducted on 40 nonlinear mature white male rats. The left hind limb of the rat was subjected to the mechanical pressure for 4 hours. The compressed area was 4 cm<sup>2</sup> with a compressive force of 4.25 kg/cm<sup>2</sup>. Determination of total calcium, magnesium and inorganic phosphate contents in blood serum was performed on a semi-automatic biochemical analyser Humalyzer 2000 (Human, Germany) using standard reagent kits. The content of calcium, magnesium, copper and zinc in the liver and bone tissue was determined by atomic absorption spectrometry on a Selmi C-115 M spectrophotometer.

### Results

It was found that serum content of calcium decreased on the 1st day of observation (by 10.1%), but increased on the 14th day (by 23.0%); the magnesium content progressively decreased during postcompression period, and the content of inorganic phosphate increased on the 14th day of observation by 36.6% ( $p < 0.05$ ) exceeded control data. The content of calcium in the liver tissue started to increase on the 3rd day of observation, and on 14th day by 25.9% ( $p < 0.05$ ) exceeded the control data. Magnesium content in the liver tissue was progressively decreasing during all days of observation. At the same time the content of zinc and copper in the liver tissue gradually increased during postcompression period. The content of calcium, magnesium, zinc and copper in the bone tissue significantly changed from the 3rd day of the postcompression period, in particular the content of bioelements vs. control group progressively decreased by 30.6%, 42.7%, 43.9% and 30.1% respectively on the 14th day of observation. **Conclusion** : The postcompression period in a rat model of CS is characterized by the pronounced imbalance of macro- and microelements content in blood, liver and bone tissues, which is important for the regulation of metabolic processes. These findings warrant further studies and can be used for developing new treatments that are efficient for dysmacro- and dysmicroelementosis that develop in case of experimental CS.

### Keywords:

crush syndrome; rats; macroelement; microelement; blood; liver; bone.

## INTRODUCTION

Crush syndrome (CS) remains a life-threatening condition. In recent decades, there has been a growing trend around the world of military conflicts, emergencies, natural and man-made disasters, accompanied by an increase of the number of victims<sup>1,2,3</sup>. According to X. Bosch *et al.*, CS develops in approximately 2–5% of all earthquake victims, more than 50% of traumatic rhabdomyolysis victims, and 10.5% of beaten victims<sup>4</sup>.

Crush syndrome is characterized by severe course, the development of systemic

1. Inna Krynytska, Department of Functional and Laboratory Diagnostics, I Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
2. Ivan Smachylo, L.Ya. Kovalchuk Department of Surgery No.1, Urology, Minimally Invasive Surgery, I Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
3. Sergii Grabchak, L.Ya. Kovalchuk Department of Surgery No.1, Urology, Minimally Invasive Surgery, I Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
4. Mariya Marushchak, Department of Functional and Laboratory Diagnostics, I Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

## Correspondence

Inna Krynytska, Prof, MD, PhD, Dsci, I. Horbachevsky Ternopil National Medical University, Functional and Laboratory Diagnostics Department, Maydan Voli, 1, 46001, Ternopil, Ukraine.  
Email: [krynytska@tdmu.edu.ua](mailto:krynytska@tdmu.edu.ua)

inflammatory response syndrome and disseminated intravascular coagulation, which lead to multiple organ failure<sup>5</sup>. Despite the active introduction of the latest medical technologies, a significant reduction in mortality from CS, which in its severe forms reaches 85-90%, even in specialized hospitals has not yet been achieved<sup>6</sup>. Conducting clinical trials of CS is difficult, due to the variety of injuries in victims and the difficulty of systematizing the obtained data<sup>7</sup>. In addition, providing medical care to a large number of victims in the face of a shortage of forces and resources also complicates research. All this determines the important role of the experiment in the study of CS<sup>8</sup>.

The biochemical basis of CS is endogenous intoxication by the products of ischemia and tissue reperfusion<sup>9</sup>. Metabolic disorders in compressed tissues, toxic products formed in the focus of compression, lead to the development of endotoxemia with subsequent generalization of the process with damage to vital organs, especially the liver. Endogenous intoxication is accompanied by a complex of metabolic disorders, among which one of the markers is an imbalance of the activity of the antioxidant system and the level of free radical oxidation<sup>10,11,12,13</sup>. Excess of toxic metabolites, formed during EI, can adversely affect the course of remodelling in bone tissue, which is a dynamic structure that is constantly updated and is controlled by many systemic and local factors<sup>14</sup>. Toxins, in addition to directly affecting bone tissue cells, can disrupt the mechanisms of regulation and metabolic support of this process. At the same time, metabolic processes in bone tissue and the balance of bone remodelling are largely determined by the content of macro- and microelements. It should be noted that for the normal metabolism is important not a single trace element, but a complex of bioelements and their balance<sup>15,16,17,18</sup>.

Therefore, the aim of our research was to investigate the changes of the macro- and microelement content in blood, liver and bone tissue in a rat model of CS.

## MATERIALS AND METHODS

### Animals

The experimental studies were conducted on 40 nonlinear mature white male rats weighing 180 - 200 g, which were distributed into the following five groups: the control group and four experimental groups (1, 3, 7 and 14 days of observation, respectively) with 8 animals for every group. The selected durations of

the study were consistent with the generally accepted periods of crush syndrome development: early period (1 to 3 days), intermediate period (3 to 7 days) and late (restorative) period (7 to 21 days).

### Crush syndrome modelling

The experiments were carried out under deep anaesthesia using intraperitoneal administration of ketamine hydrochloride (at 100 mg/kg of body weight). The left hind limb of the rat was subjected to the mechanical pressure for 4 hours, using an apparatus designed for this purpose at the Department of Functional and Laboratory Diagnostics of the I. Horbachevsky Ternopil National Medical University. The compressed area was 4 cm<sup>2</sup> with a compressive force of 4.25 kg/cm<sup>2</sup>. The integrity of large vessels and bony structures of the lower extremity was preserved. Thus, the crush syndrome of moderate degree was modelled in animals<sup>19</sup>.

### Bioelements evaluation

Blood serum was obtained by whole blood centrifugation. Determination of serum total calcium, magnesium and inorganic phosphate content was performed on a semi-automatic biochemical analyser Humalyzer 2000 (Human, Germany) using standard reagent kits. The results were expressed in mmol/l.

The content of calcium, magnesium, copper and zinc in the liver and bone (femur) tissues was determined by atomic absorption spectrometry on a Selmi C-115 M spectrophotometer. Analytical parameters were chosen according to the literature data: pressure 0.4 kg/cm<sup>3</sup> and 20 mm of water column; flame temperature 2250° C, wavelength: Ca – 495.5 nm, Mg – 285.2 nm, Zn – 213.9 nm, Cu – 324,7 nm. After determining the content of the element in the solution, the mass of the sample was introduced and the content of the element in 1 gram of study tissue was obtained. The results were expressed in mg/g of ash.

### Statistical analysis

Statistical processing of digital data was performed using Excel software (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). The Kolmogorov–Smirnov test was used to determine the normality of data distribution. The analysis of the study results was carried out using non-parametric statistical methods, the choice of which was based on the not normal distribution of the values. Quantitative characteristics were presented in the form of median (Me) and quartiles – lower and upper (Lq; Uq). Comparison of the two quantitative characteristics

was performed using the Mann-Whitney U test. Comparison of three or more groups on a quantitative basis was carried out using the Kruskal-Wallis test.

## ETHICAL CLEARANCE

All manipulations with experimental animals were carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The experimental design and protocol were approved by the Bioethics Committee of I. Horbachevsky

Ternopil National Medical University (protocol No. 61 of November 13, 2020).

## RESULTS

Blood serum total calcium content on the 1st day of the postcompression period significantly decreased by 10.1%, on the 3rd and 7th days - practically did not differ from control values and on the 14th day of observation was significantly higher by 23.0 % vs. controls (table 1).

**Table 1.** Changes of the macroelement content in blood serum of rats with crush-syndrome model (Me [Q25-Q75])

Bioelement	Group of rats/Day of postcompression period				
	Control, n=8	1st day, n=8	3rd day, n=8	7th day, n=8	14th day, n=8
Calcium, mmol/l	2.38 [2.32; 2.41]	2.14 [2.08; 2.19] $p_1 < 0.05$	2.27 [2.23; 2.30] $p_1 > 0.05$ $p_2 > 0.05$	2.40 [2.36; 2.46] $p_1 > 0.05$ $p_3 < 0.05$	2.63 [2.61; 2.65] $p_1 < 0.05$ $p_4 > 0.05$
Magnesium, mmol/l	0.73 [0.69; 0.75]	0.71 [0.69; 0.73] $p_1 > 0.05$	0.70 [0.69; 0.72] $p_1 > 0.05$ $p_2 > 0.05$	0.62 [0.60; 0.64] $p_1 < 0.05$ $p_3 < 0.05$	0.53 [0.51; 0.55] $p_1 < 0.05$ $p_4 < 0.05$
Inorganic phosphate, mmol/l	1.34 [1.29; 1.37]	1.45 [1.41; 1.47] $p_1 < 0.05$	1.50 [1.47; 1.54] $p_1 < 0.05$ $p_2 > 0.05$	1.83 [1.60; 1.95] $p_1 < 0.05$ $p_3 < 0.05$	1.83 [1.79; 1.85] $p_1 < 0.05$ $p_4 > 0.05$

Note.  $p_1$  – changes are significant in relation to the indices of control animals;  $p_2$  – significance of changes between the group on the first day of observation and rats on the third day of observation;  $p_3$  – significance of changes between the group on the third day of observation and rats on the seventh day of observation;  $p_4$  – significance of changes between the group on the seventh day of observation and rats on the fourteenth day of observation.

At the same time, serum magnesium content practically did not change in the early postcompression period (during the first three days), while after 7 and 14 days it significantly decreased, respectively, by 15.8% and 25.4% vs. control values. The level of serum inorganic phosphate increased on the 1st day of observation by 7.8%, continued to increase on the 3rd (by 11.6%) and 7th day (by 36.6%) of the postcompression period and remained significantly high by the 14th day vs. controls.

In the liver tissue, the calcium content on the 1st day of the postcompression period also practically did not change, and on the 3rd day of the experiment was increased by 18.2% and remained probably high up to 14th day (by 25.9% ( $p < 0.05$ )) exceeded the control data) (Table 2).

At the same time, the magnesium content significantly decreased already on the 1st day of the postcompression

period by 19.2%, fluctuating within these limits until the 7th day, and then again decreased by 106.7% compared to the data of the control group. It should be noted that on 14th day of the postcompression period, the magnesium content was significantly lower than the data on the 1st, 3rd and 7th days of observation. The content of zinc in the liver tissue gradually increased from the 1st day (by 12.8%) to the 14th day (by 34.0%) of the observation vs. control. The same trend was observed for the dynamics of changes of the content of copper in the liver tissue, in particular, this index did not change on the 1st day of the experiment, but increased on the 3rd day of the experiment (by 25.0% ( $p < 0.05$ )), and after 14 days its content by 55.0% significantly exceeded the control values.

The calcium content in the bone tissue on the 1st day of the crush-syndrome postcompression period did

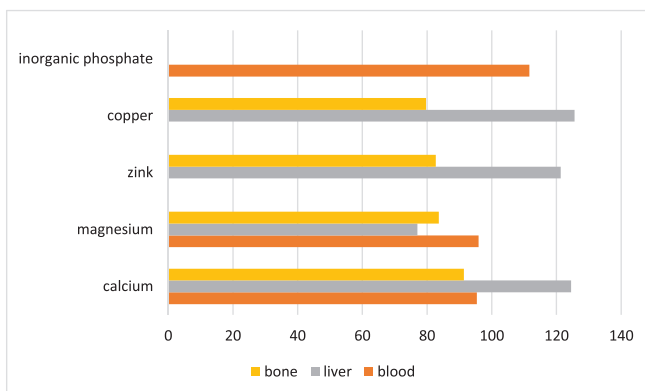
**Table 2.** Changes of the macro- and microelement content in the liver and bone tissue of rats with crush-syndrome model (Me [Q25 – Q75])

Bioelement	Group of rats/Day of postcompression period				
	Control, n=8	1st day, n=8	3rd day, n=8	7th day, n=8	14th day, n=8
<b>Liver</b>					
Calcium, mg/g of ash	0.27 [0.23;0.31]	0.29 [0.28;0.31] $p_1>0.05$	0.33 [0.32;0.36] $p_1<0.05$ $p_2>0.05$	0.35 [0.33;0.35] $p_1<0.05$ $p_3>0.05$	0.34 [0.32;0.38] $p_1<0.05$ $p_4>0.05$
Magnesium, mg/g of ash	0.31 [0.25;0.39]	0.26 [0.24;0.28] $p_1<0.05$	0.24 [0.21;0.24] $p_1<0.05$ $p_2>0.05$	0.23 [0.22;0.25] $p_1<0.05$ $p_3>0.05$	0.15 [0.14;0.16] $p_1<0.05$ $p_4<0.05$
Zinc, mg/g of ash	0.47 [0.45;0.51]	0.53 [0.51;0.54] $p_1<0.05$	0.57 [0.56;0.58] $p_1<0.05$ $p_2<0.05$	0.59 [0.55;0.60] $p_1<0.05$ $p_3>0.05$	0.63 [0.62;0.65] $p_1<0.05$ $p_4<0.05$
Copper, mg/g of ash	0.20 [0.18;0.22]	0.22 [0.21;0.23] $p_1>0.05$	0.25 [0.24;0.25] $p_1<0.05$ $p_2>0.05$	0.25 [0.24;0.26] $p_1<0.05$ $p_3>0.05$	0.31 [0.28;0.31] $p_1<0.05$ $p_4<0.05$
<b>Femur</b>					
Calcium, mg/g of ash	289.03 [283.59; 300.60]	282.71 [277.05; 293.43] $p_1>0.05$	264.18 [259.54; 266.96] $p_1<0.05$ $p_2<0.05$	211.93 [209.23; 217.57] $p_1<0.05$ $p_3<0.05$	200.58 [199.41; 211.54] $p_1<0.05$ $p_4>0.05$
Magnesium, mg/g of ash	35.60 [33.60; 36.90]	32.42 [31.60; 33.01] $p_1>0.05$	29.77 [29.05; 30.24] $p_1<0.05$ $p_2>0.05$	23.98 [23.08; 24.73] $p_1<0.05$ $p_3<0.05$	18.59 [17.51; 19.11] $p_1<0.05$ $p_4<0.05$
Zinc, mg/g of ash	0.41 [0.39;0.45]	0.40 [0.37;0.42] $p_1>0.05$	0.34 [0.32;0.35] $p_1<0.05$ $p_2<0.05$	0.27 [0.24;0.29] $p_1<0.05$ $p_3<0.05$	0.23 [0.21;0.27] $p_1<0.05$ $p_4>0.05$
Copper, mg/g of ash	17.65 [17.08; 18.20]	17.13 [16.82; 17.24] $p_1>0.05$	14.08 [13.50; 14.25] $p_1<0.05$ $p_2<0.05$	12.59 [12.03; 13.59] $p_1<0.05$ $p_3<0.05$	12.33 [12.13; 12.68] $p_1<0.05$ $p_4>0.05$

Note.  $p_1$  – changes are significant in relation to the indices of control animals;  $p_2$  – significance of changes between the group on the first day of observation and rats on the third day of observation;  $p_3$  – significance of changes between the group on the third day of observation and rats on the seventh day of observation;  $p_4$  – significance of changes between the group on the seventh day of observation and rats on the fourteenth day of observation.

not change significantly (Table 2). Subsequently, this macroelement decreased on the 3rd day of observation by 8.6%, continued to decrease to 7th day (by 26.7%) and remained significantly low until 14th day (was lower by 30.6% compared to controls). The same trend was observed for the dynamics of changes of the magnesium content in the bone tissue, in particular, this macroelement progressively decreased: on the 3rd day of observation – by 16.4%, on the 7th day – by 32.7% and on the 14th day – by 42.7% vs. control group. The content of zinc in the bone tissue gradually decreased from the 3rd day (by 17.3%) to the 7th day (by 33.3%) and remained significantly low on the 14th day of observation (by 43.9% was lower vs. controls). The same trend was observed for the dynamics of changes of the content of copper in the bone tissue, in particular, this microelement significantly decreased on the 3rd (by 20.3%) and 7th (by 28.7%) days of observation and remained decreased on 14th day (by 30.1% vs. control group).

It is important not only to determine the quantitative changes in the content of macro- and microelements in various tissues, but also to compare the direction of these changes in different terms of the postcompression period of CS. So, on the 1st day of observation, the macro- and microelement composition of the liver tissue underwent the most pronounced changes (fig. 1),



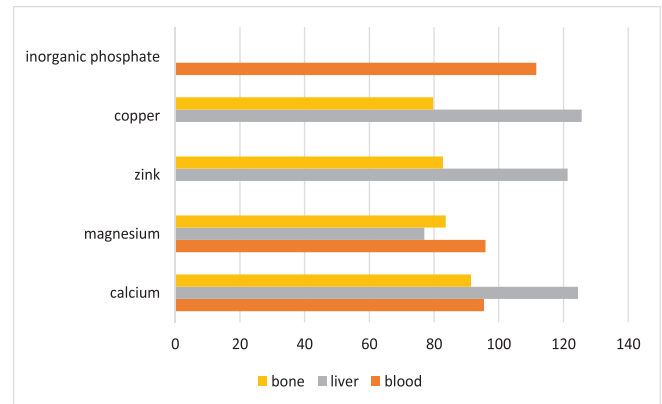
**Figure 1.** Changes in macro- and microelements content in a rat model of crush-syndrome on

1st day of the postcompression period, %.

which is probably associated with the development of endogenous intoxication. At the same time, hypocalcemia and hyperphosphatemia were found in the blood serum of rats.

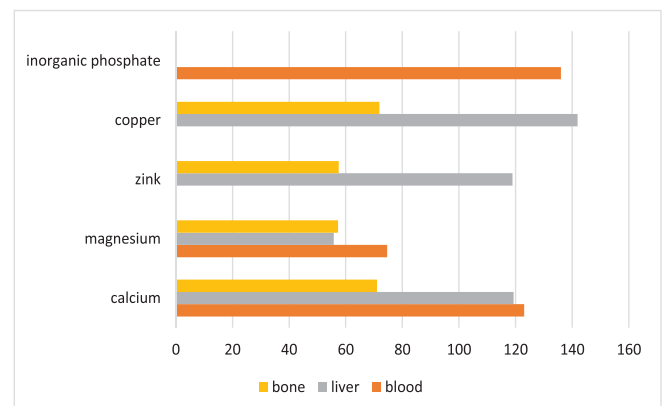
On the 3rd day of observation a significant imbalance of

macro- and microelements composition was observed in the liver and bone tissues. So, in the liver, against the background of an increase in calcium, zinc and copper content, the content of magnesium decreased. It should be noted that magnesium content also was decreased in bone tissue and blood serum. Unlike unidirectional changes in magnesium content, other studied parameters change in opposite directions, in particular, calcium, zinc and copper content in the bone tissue decreased, while their content in the liver tissue increased (fig. 2).



**Figure 2.** Changes in macro- and microelements content in a rat model of crush-syndrome on 3rd day of the postcompression period, %.

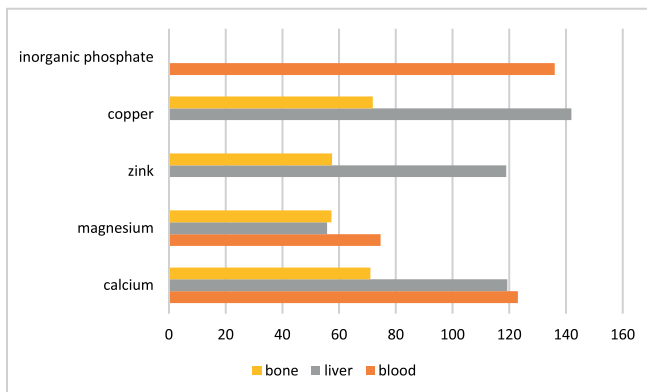
On the 7th day of observation against the background of the preserved dynamics in macro- and microelements changes in the liver tissue, the content of calcium, magnesium, zinc and copper continued to progressively decrease in the bone tissue (fig. 3).



**Figure 3.** Changes in macro- and microelements content in a rat model of crush-syndrome on 7th day of the postcompression period, %.

This trend of changes was not observed in the blood serum, where hyperphosphatemia was found, and the calcium content significantly did not differ from the control group. It should be noted a unidirectional decrease in the magnesium content in the studied tissues of rats with modelled CS.

On the 14th day of observation the lowest values of magnesium content in the liver and bone tissues of rats with modelled CS were reflected by a decrease in its content in blood serum, however, the severity of the changes was less (fig. 4).



**Figure 4.** Changes in macro- and microelements content in a rat model of crush-syndrome on 14th day of the postcompression period, %.

The content of calcium, zinc and copper on the 14th day of observation were highest in the liver tissue and lowest in the bone tissue.

## DISCUSSION

The pathogenesis of CS combines various mechanisms, including ischemia, endotoxicosis and inflammation, which cause multiorgan changes<sup>20</sup>. In our opinion, the imbalance of macro- and microelements is an equally important trigger for the development of pathobiochemical reactions that occur in case of CS. In the present study we found that in the blood serum of rats with experimental CS calcium content decreases on the 1st day of the postcompression period, but increases on the 14th day; the magnesium content progressively decreases during all days of observation, and the content of inorganic phosphate gradually increases, and on the 14th day of the postcompression period by 36.6% ( $p < 0.05$ ) exceeds the control data. The content

of calcium, magnesium, zinc and copper in the bone tissue significantly changes from the 3rd day of the postcompression period, in particular the content of bioelements vs. control group progressively decreases by 30.6%, 42.7%, 43.9% and 30.1% respectively on the 14th day of observation.

Under physiological conditions, the skeleton contains 99% of calcium, 87% – phosphorus, 58% – magnesium of the total content in the body, while the mineral part of the bones is in constant contact with the surrounding tissue fluid<sup>21,22</sup>. The release of various organic acids (eg, lactic, uric acid) from destroyed muscle cells in case of CS leads to metabolic acidosis, which further exacerbates hyperkalemia and causes redistribution of bioelements in blood and body tissues, including bone tissue<sup>23</sup>.

We found multidirectional changes of calcium and magnesium contents in blood serum – hypercalcemia and hypomagnesemia. It is known that magnesium deficiency is accompanied by an increase of oxidative stress with a simultaneous weakening of antioxidant protection<sup>24</sup>, which is confirmed by the results of our previous study<sup>25</sup>. One of the probable mechanisms of such a multidirectional dynamic of changes of these two trace elements is the development of inflammation, which develops during CS.

As for inorganic phosphate, a significant increase of its content in blood serum was found in rats with experimental CS. During the destruction of muscle cells, inorganic and organic phosphorus components are dissolved, and a large amount of inorganic phosphorus is released into the blood plasma, which leads to hyperphosphatemia<sup>26</sup>. Hyperphosphatemia causes the deposition of calcium phosphate on damaged muscle cells and other tissues<sup>27</sup>. In addition, suspending the renal  $1\alpha$ -hydroxylase enzyme, which is responsible for producing the active form of vitamin D, leads to early hypocalcaemia, which is usually asymptomatic. After complete cell necrosis, calcium, first entering the cytoplasm of muscle cells, is released back into the plasma. This, in combination with secondary hyperparathyroidism, which develops as a result of early hypocalcaemia and high levels of vitamin D (produced in large numbers by glomerular cells), leads to late hypercalcemia<sup>26</sup>.

For zinc and copper, the content of these trace elements in the femur gradually decreased during all days of observation and on the 14th day of the postcompression

period was 56.1% and 69.9% of the control data. Zinc plays an important role in bone metabolism, which makes it an important component of the calcified matrix. Excessively low concentrations of zinc in bone tissue are the cause of gradual loss of bone mass<sup>28,29</sup>. Copper refers to bioelements, the deficiency of which leads to significant violations of metabolic processes, in particular in bones and connective tissue. The highest content of copper is found in young osteons, which promotes the synthesis of collagen and elastin and mineralization of bone tissue<sup>30</sup>. Copper provides bone tissue strength by influencing collagen metabolism through prolyl- and lysyl hydroxidase<sup>31</sup>.

Such changes of zinc and copper may be related to the expression of zinc-, copper-containing superoxide dismutases and metallothioneins. Excessive intake of copper increases the concentration of free metal forms in body tissues, which leads to the activation of the formation of reactive hydroxyl radicals, which are toxic to cell membranes, leading to their destruction<sup>32</sup>. Copper leads to the formation of structural changes in internal organs, including the liver, by activating tissue processes of lipid peroxidation<sup>32</sup>.

Thus, the established bioelement imbalance in the studied tissues of rats with modelled CS disrupts cellular

metabolism, deepens oxidative stress, thereby activating other systemic processes, leading to progression of CS and deepening of multiorgan damages<sup>34,35</sup>.

## CONCLUSIONS

The postcompression period in a rat model of CS is characterized by the pronounced imbalance of macro- and microelements content in blood, liver and bone tissues, which is important for the regulation of metabolic processes. These findings warrant further studies and can be used for developing new treatments that are efficient for dysmacro- and dysmicroelementosis that develop in case of experimental CS.

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## AUTHORS' CONTRIBUTION

IK, MM have given substantial contributions to the conception and the design of the manuscript. IS, SG, IK took part in the acquisition, analysis, and interpretation of the data. All authors have participated in drafting the manuscript, MM revised it critically. All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

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