

# Effect of Motor Activity Restriction on Copper Content in Rats

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In rats subjected to increasing restriction of motor activity starting from day 21 days of life, copper content was evaluated by electron paramagnetic resonance (EPR) spectroscopy by analyzing the levels of copper-containing paramagnetic complexes in the heart and liver tissues. Copper content was assessed by the intensity of the characteristic EPR signal from the Cu<sup>2+</sup>-diethyldithiocarbamate complex (Cu<sup>2+</sup>-(DETC)<sub>2</sub>). It was found that after restriction of motor activity for 30 days, copper content in the heart and liver tissues increased by 2.2 and 2.9 times, respectively.

**Key Words:** *copper; heart; liver; motor activity restriction*

Physical and motor activities constitute a biological necessity for human health and life. Modern life is characterized by low physical activity at work and at home due to automation and mechanization of professional working activities, longer bed rest, sedentary lifestyle, *etc.* The natural motor activity of the modern children is changing. Compared to preschool children, school children spend more time in static positions. Every year children spend more and more free time with entertainment gadgets, at the TV screen or computer monitor. The problem of changes in physiological functions and mechanisms of their development in a growing organism under conditions of reduced motor activity seems relevant.

Copper is an important trace element for normal functioning of the human body. Copper is involved in cell respiration, iron metabolism, neurotransmitter production, pigment formation, connective tissue biosynthesis, hormonal regulation, and immune disorders. Copper is a constituent of many essential enzymes such as ceruloplasmin, cytochrome oxidase, tyrosinase, ascorbinase, amine oxidase, *etc.* [1,2]. Copper par-

ticipates in the antioxidant defense system being as cofactor of the enzyme superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion radicals [2-6]. SOD is the most important component of the antioxidant system. The importance of balanced copper homeostasis and its safe transport is supported by the existence of a number of copper-associated diseases. Researchers point to high prevalence of copper-dependent conditions and diseases, especially among individuals with genetic diseases of the nervous system, chronic inflammatory diseases, anemias, musculoskeletal diseases, *etc.* Adequate copper supply leads to improvement of fibrinolytic activity of the blood and reduces the risk of cardiovascular diseases [7,8]. Given the vital role of copper, precise regulation of its content is crucial for the homeostasis maintenance in living organisms.

Our aim was to study the intensity of copper content in the heart and liver tissues of growing rats after motor activity restriction.

## MATERIALS AND METHODS

The study was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes) and recommen-

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dations of the Local Bioethical Committee of Kazan Federal University (Protocol No. 39 of December 22, 2022). The study involved rats of both sexes that were divided into two groups: control group rats ( $n=10$ ) kept in standard cage under conditions of unrestricted motor activity and rats subjected to increasing restriction of motor activity (RMA group;  $n=10$ ) over 30 days.

For modeling increasing RMA, the animals were placed into individual pen-case-like cages for 30 days starting from the age of 21 days. This protocol was described in detail in our previous work [9]. A distinguishing feature of this hypokinesia model (with horizontal body position) is a diminished effect of the immobilization stress, which is achieved via gradual increase in the exposure to RMA conditions. After 30 days of RMA, the rats reached the age of 51 days. The control group matched the experimental group in age. According to published reports, 51 days of age in rats corresponds to the pubertal period of development, accompanied by pronounced changes in the endocrine system, which has an active influence on the regulation of cardiac activity [10,11].

Copper content in the heart and liver specimens was measured by electron paramagnetic resonance (EPR) spectroscopy with spin capture method developed in Institute of Chemical Physics of the Russian Academy of Sciences. The details of the experiment and measuring technique were described in detail in our previous work [12,13]. Spin trap components were administered to animals intraperitoneally anesthetized with 25% solution of urethane (800 mg/kg body weight). All the drugs were diluted in saline. For the formation of spin traps, DETC-Na solution was injected intraperitoneally in a dose of 500 mg/kg (in 2 ml water per 300 g of body weight). The spin trap interacts with copper yielding a stable radical  $\text{Cu}^{2+}\text{-(DETC)}_2$ . In 40 min after injection of the spin trap components, the rats were fixed on an operating table, opened, and the heart and liver were extracted in total and quickly dried. The myocardium and liver parenchyma were mechanically minced and frozen in liquid nitrogen in special disposable syringes for measurements. The prepared samples cut to fit the measurement cell were weighted immediately before spectroscopy; the weight of the samples was about 100 mg.

Changes in the spectra of biological samples were recorded using a Bruker X-band (9.5320 GHz) EMX/plus spectrometer. The sample in a finger-shaped Dewar vessel (Bruker) was placed in a cell of a double resonator (model ER 4105DR) and the reference sample was placed in another cell of the same resonator. During measurements, each sample was placed under the same conditions as the reference sample, which allowed quantification and direct comparison of the

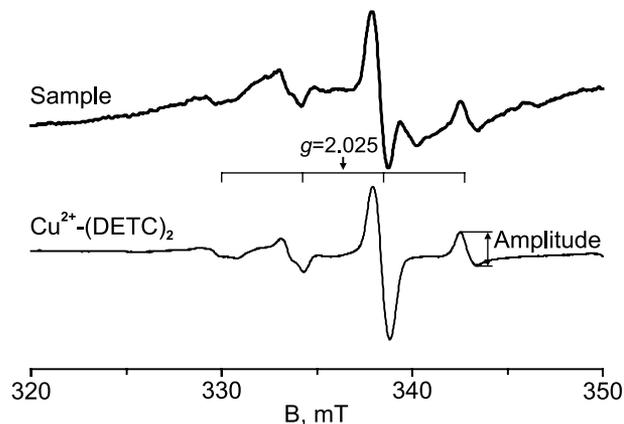
intensity of the samples under study. The measurements were conducted at a magnetic field modulation frequency of 100 kHz, modulation amplitude of 2 G, microwave radiation power of 2 mW, time constant of 327 msec, and temperature of 77 K. Throughout the experiments, the modulation amplitude, gain, and microwave power settings were carefully selected to ensure that overmodulation and saturation of the electron paramagnetic resonance signal were avoided. The EPR spectra amplitudes were normalized by the weight of the sample and by the amplitude of the standard sample signal.

Statistical analysis was performed using SigmaPlot 11.0 software. During data processing, the samples were checked for normality (Shapiro–Wilk test) and equality of variance. Significance of the results was assessed using the Student's  $t$  test and the Mann–Whitney  $U$  test. The results were presented as the mean and standard error of the mean ( $M\pm SEM$ ). The differences were considered significant at  $p<0.05$ .

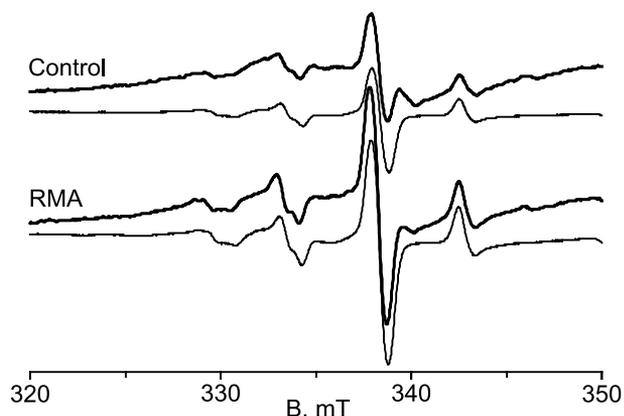
## RESULTS

Copper content in the heart and liver of rats after 30-day RMA was determined by EPR spectroscopy. The EPR spectrum of the sample from the heart of a control group rat and the reference spectrum of the  $\text{Cu}^{2+}\text{-(DETC)}_2$  complex are shown in Figure 1. The spectrum contains a characteristic signal of this complex, its amplitude was calculated during the analysis of the spectra of heart and liver tissue samples. The signal intensity from the complex is directly proportional to the copper content in the sample.

Figure 2 shows the obtained EPR spectra of the heart tissues of a control rat and a RMA group rat. Figure 3 shows the summarized intensity data of the integrated  $\text{Cu}^{2+}\text{-(DETC)}_2$  signal in the spectra of the



**Fig. 1.** EPR spectrum of the tissue from control rat heart (sample) and reference spectrum of  $\text{Cu}^{2+}\text{-(DETC)}_2$  complex with amplitude equal to the contribution to the sample spectrum. The spectra were obtained at 77 K.



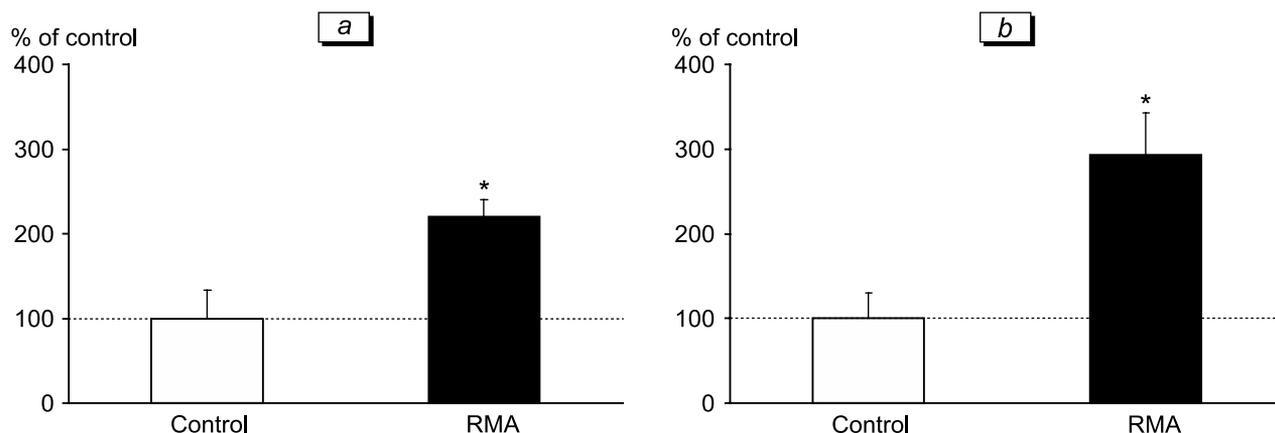
**Fig. 2.** EPR spectra of heart tissue of a control rat and a rat subjected to 30-day RMA. Thin lines are reference spectra of the  $\text{Cu}^{2+}$ -(DETC)<sub>2</sub> complex with amplitudes equal to the contribution to the spectrum of the corresponding sample. The spectra were obtained at 77 K.

heart and liver samples. RAM for 30 days led to a significant increase in copper content in the heart and liver by ~2.2 and 2.93 times, respectively ( $p < 0.05$ ).

Chronic excess of copper in tissues leads to growth arrest, hemolysis, decreased hemoglobin content, and degradation of the liver, kidney, and brain tissues. Copper homeostasis in the body is accompanied by rapid binding of copper by organic molecules. In cells, copper ions prevailed in the form of complex compounds with bioactive substances (nucleic acids, amino acids, biogenic amines, and hormones or are incorporated into proteins). Most of the alimentary copper enters the liver, where it binds to the serum proteins  $\alpha$ -globulins (ceruloplasmin). Low levels of ceruloplasmin lead to accumulation of “free” copper (not bound with ceruloplasmin). The question remains open which copper form is bound by spin trap (“free” or “bound” to the carrier protein). The main toxic effect of copper on cell metabolism is produced by the “free” fraction.

Serum copper and ceruloplasmin levels are elevated in various inflammatory processes, myocardial infarctions, and liver diseases. This fact is supported by the increased incidence of cardiovascular disease in individuals with elevated blood copper levels. Hypokinesia of different durations was found to increase ceruloplasmin content [14]. The maximum increase was found on day 7 of hypokinesia, which according to the researchers indicates the presence of compensated stress response during hypokinesia. At later terms (days 14, 21, and 28), the ceruloplasmin content slightly decreased but remained above the control, reflecting certain stages of metabolic changes in RMA [14]. Copper accumulation initiates oxidative stress, impaired production of cytochrome-c oxidase and SOD-1, and accumulation of proinflammatory cytokines (IL-1 $\alpha$ , IL-6, IL-12, etc.) [15]. Any experimental way of RMA modeling includes a stressor component. According to previous reports, 30-day hypokinesia increased the synthesis and secretion of proinflammatory cytokines. Proinflammatory cytokines IL-1, IL-6, and TNF $\alpha$  promote the synthesis of ceruloplasmin; increased plasma concentrations of ceruloplasmin under conditions of hypokinesia provides antioxidant defense of the body [16].

Our results show that increasing restriction of motor activity leads to elevation of copper content in organs. Congenital or acquired disorders of copper metabolism cause the development of multiple defects. It has been previously shown that 16-day hypokinesia in animals induced liver enlargement with signs of venous plethora, hypoxia, glycogen loss, dystrophy, and foci of necrosis [17]. RMA led to changes in the functioning of the cardiovascular system: a decrease in heart weight, weakening of the heart muscle, degenerative changes, etc. Disturbance in the heart rhythm and heart pain at rest are indications for measuring the serum level of copper. Unfortunately, copper deficiency or excess in various diseases are rarely studied,



**Fig. 3.** Relative copper content in the heart (a) and liver (b) of rats after 30-day RMA. \* $p < 0.05$ .

which reduces the effectiveness of prevention and treatment of many diseases.

The increase in copper content in RMA allows us to hypothesize a close relationship between its level in the body and the mode of motor activity. Published data indicating significant changes in the cardiovascular system, internal organs, blood flow system, and oxygen supply of the organism against the background of RMA also suggest that some of these changes are caused by an increase in the copper content in the key tissues of the body.

EPR spectroscopy was carried out in the Zavoisky Physical-Technical Institute, Federal Research Center Kazan Scientific Center, Russian Academy of Sciences as a part of the State Assignment project.

**Conflict of interest.** The authors have no conflicts of interest to declare.

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