UDC 577.112+616.15+616-092.9+663.85 **https://doi.org/10.15407/biotech18.02.079**

BLOOD METALLOPROTEINS IN RATS UNDER CONDITIONS OF ENERGY DRINK CONSUMPTION

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Received 2025/03/17
Revised 2025/03/22
Accepted 2025/04/30

The study *aimed* to evaluate the effect of energy drinks on the level of Cu, Fe, ceruloplasmin activity and transferrin saturation in the blood serum of rats.

Materials and Methods. The study was conducted on white male Wistar rats divided into two groups: control and experimental. The experimental group received the energy drink Burn for 30 days. Blood samples were taken under anesthesia by decapitation on the 1st day after the drink was discontinued. The content of Cu and Fe was determined by atomic absorption spectrophotometry. Ceruloplasmin activity and transferrin saturation were studied by the method of Babenko G.O.

Results. Against the background of energy drink consumption in the experimental group, a decrease in the level of Cu by 43% and Fe by 67% was observed compared to the control. Transferrin saturation decreased by 42% and ceruloplasmin activity by 40%. The data obtained indicate that the consumption of an energy drink causes complex changes in the metabolism of Cu and Fe, which is reflected in a decrease in the level of these trace elements in the blood and the activity of metalloenzymes, in particular ceruloplasmin and transferrin, which can lead to the development of oxidative stress and impaired functioning of both individual organs and the body as a whole.

Conclusions. The results obtained indicate the development of dysmicroelementosis in the context of energy drink consumption, which can have adverse effects on the functioning of red blood cells, body systems, and tissues, an increase in the level of endogenous intoxication, and impaired tissue respiration.

Keywords: energy drink, rats, blood, Cu, Fe, ceruloplasmin, transferrin saturation.

Recent decades have seen a significant increase in the popularity of energy drinks, which are actively consumed by people of all ages to improve performance and concentration and reduce fatigue. The main active ingredients of such beverages are caffeine, taurine, B vitamins, and other stimulants that affect metabolic processes in the body. However, the effect of energy drinks on mineral metabolism, in particular on the level of vital trace elements and metalloenzymes, remains insufficiently studied.

Copper and Iron are among the key trace elements involved in critical biochemical processes. Iron is necessary for the synthesis of hemoglobin, myoglobin, and a number of enzymes in the respiratory chain that ensure cellular respiration and oxygen transportation [2]. At the same time, Copper plays the role of a cofactor of many enzymes, including ceruloplasmin, superoxide dismutase, and cytochrome oxidase, which are involved in redox reactions and antioxidant protection of cells.

The balance of these trace elements in the blood is maintained by specialized transport proteins. Ceruloplasmin is the main protein that transports Cu and is involved in the regulation of the oxidation of Fe to its trivalent form (Fe^{3+}), which is necessary for binding to transferrin, the main transport protein of Fe. Disruption of this balance can lead to changes in the absorption, transport, and deposition of trace elements, which, in turn, can have negative consequences for metabolic processes.

Citation: Partsei, Kh. Y., Artysh, M. B., Ersteniuk, H. M., Tokaryk, H. V., Maksymchuk, T. P. (2025). Blood metalloproteins in rats under conditions of energy drink consumption. *Biotechnologia Acta*, 18(2), 79–81. https://doi.org/10.15407/biotech18.02.079

This study aimed to evaluate the effect of energy drinks on the level of Cu, Fe, ceruloplasmin activity and transferrin saturation in the blood serum of rats.

Materials and Methods. The experimental study was conducted on white male Wistar rats weighing 150–220 g, which were kept in the vivarium of IFNMU. All manipulations complied with modern ethical and legal requirements for animal experiments. In particular, the study was in accordance with the norms of the European Convention for the Humane Treatment of Laboratory Animals (Strasbourg, 1986), the Order for the Improvement of Experimental Work with Animals, and the General Principles of Animal Experiments adopted at the First National Congress on Bioethics (Kyiv, 2001). In addition, all procedures complied with the provisions of the Law of Ukraine "On Protection of Animals from Cruelty" (2010).

Animals were kept in groups of five in standard laboratory conditions with controlled light, temperature, a balanced diet, and unlimited access to water. The rats were divided into two groups: the control group - healthy animals receiving a standard diet and water; and the experimental group - animals that consumed the energy drink for 30 days, after which blood was taken on the first day after the experiment was terminated. The Burn energy drink was administered orally through drinkers to rats daily for 30 days. The animals were housed in separate cages while receiving the drink. The dosage was calculated taking into account body weight (per 1 kg) and species characteristics of the rats.

Blood was taken under anesthesia (intramuscular injection of sodium thiopental, 60 mg/kg) by decapitation on the 1st day after the cessation of the drink. The level of ceruloplasmin and transferrin saturation in the blood serum was estimated according to the method of Babenko G.O. [1]. The principle of the method is based on determining the reserve capacity of blood serum for ron binding. To do this, a solution containing a precisely specified amount of Iron is added to the serum. When transferrin is completely saturated with Iron, excess iron ions precipitate an equivalent amount of this excess iron on serum protein, which will cause a certain degree of serum turbidity. The intensity of the turbidity, determined using a photoelectrocolorimeter, gives an idea of the degree of transferrin saturation with iron in the test serum. The principle of the method for determining ceruloplasmin activity is based on the oxidation of p-phenylenediamine with the participation of CP. The enzymatic reaction was stopped by the addition of sodium chloride. The test samples are colorimetrically analyzed using a photoelectrocolorimeter against a control sample under a 530 mm green light filter. The determination of the content of Cu and Fe in the blood of animals was carried out by atomic absorption spectrophotometry using a SHIMADZU AA-7000 spectrophotometer [6]. STATISTICA 7 software was used for statistical processing of the results using Student's t-test.

Results and Discussion. The trace elements Cu and Fe perform essential biological functions; in particular, they are involved in the processes of metabolism and antioxidant defense. Copper plays a significant role in ensuring oxidative reactions and is also part of key enzymes that regulate the antioxidant balance [4]. Our results indicate a gradual decrease in the content of copper in the blood of experimental animals under the influence of the energy drink by 43% compared to the control (Fig. 1). Ferrous, which is a necessary element for oxygen transport and the functioning of a number of enzyme systems, has also changed. The content of Fe in the blood decreased in experimental animals by 67% compared to control values. Such dynamics may indicate an imbalance in the iron transport system, which is likely due to changes in the structure of proteins responsible for its regulation.

One of the key regulators of iron metabolism is transferrin, which ensures the binding and transfer of Fe in the body. Under the influence of the energy drink, its saturation in the experimental group of animals decreased by 42% compared to the control animals (Fig. 2). Such a change may contribute to an increase in the proportion of total iron, which is a catalyst for the formation of reactive oxygen species and increases oxidative stress [3]. A decrease

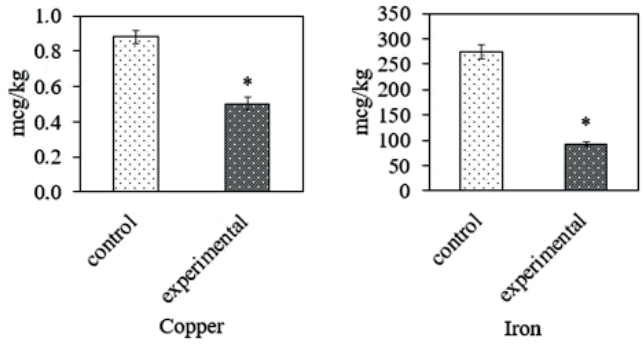

Fig. 1. **Effect of energy drink on the content of Cu and Fe in the blood serum of rats**
* — $p < 0.001$ — reliability compared to the control group

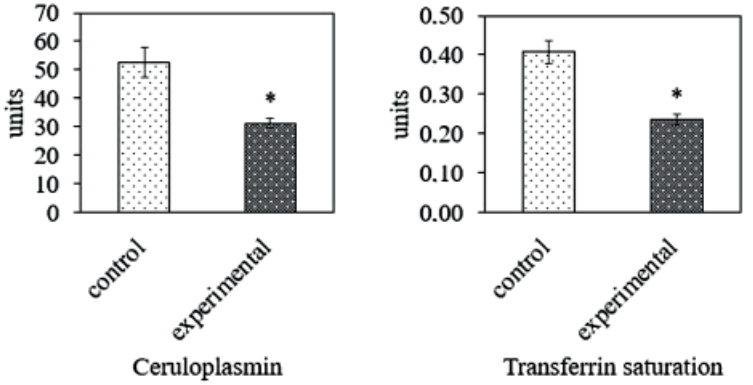

Fig. 2. **Effect of energy drink on ceruloplasmin activity and transferrin saturation in the blood serum of rats**
* — $p < 0.001$ — reliability compared to the control group

in ceruloplasmin activity, which is also involved in the regulation of iron metabolism and antioxidant defense, confirms the negative impact of energy drinks on the balance of trace elements. Already on the 1st day after the end of the drink administration, this indicator decreased by 40% compared to the control. It is known that ceruloplasmin deficiency can lead to the accumulation of free-form Fe ions, which puts an additional burden on the antioxidant system and increases the risk of oxidative cell damage.

The data obtained indicate that the consumption of an energy drink causes complex changes in the metabolism of Cu and Fe, which is reflected in a decrease in the level of these trace elements in the blood and the activity of metalloenzymes, in particular ceruloplasmin and transferrin, which can lead to the development of oxidative stress[5] and impaired functioning of both individual organs and the body as a whole.

Conclusion. The results obtained indicate the development of dysmicroelementosis in the context of energy drink consumption, which can have adverse effects on the functioning of red blood cells, body systems, and tissues, an increase in the level of endogenous intoxication, and impaired tissue respiration. The findings emphasize the potential risks of energy drink consumption and the need for further research to assess their impact on homeostasis and metabolic processes in the body.

Authors` contribution

Partsei Kh. Y. — experimental study, collection of material, analysis of the data obtained, preparation of theses for publication; Artysh M.B. — participated in the collection of material; Ersteniuk H. M. advising on the formation of the purpose and objectives of the study; Tokaryk H.V. — participated in the editing of the abstracts; Maksymchuk T.P. — participated in the formulation of conclusions.

Funding source

The work is a fragment of interdepartmental research work "Scientific substantiation and improvement of diagnosis and treatment of endocrinopathies based on the study of priority etiopathogenetic factors and comorbidities" (2019–2024, State registration number 0120U0105103) without special funding.

REFERENCES

1. Guranych, S. P., Voronych-Semchenko, N. M., Guranych, T. V. (2017). Prooxidant-antioxidant status of dental pulp and oral mucosa of rats with experimental iodine deficiency and insulin resistance. *Ukr. J. Medic., Boil. Ta Sport*., 2(4), 16–20. (In Ukrainian).
2. Gkouvatsos, K., Papanikolaou G, Pantopoulos K. (2012). Regulation of iron transport and the role of transferrin. *Biochim. Biophys. Acta*., 1820(3), 188–202. https://doi.org//10.1016/j.bbagen.2011.10.013.
3. Khan, F., Ahmad, N., Ahmed, S. (2021). Role of ceruloplasmin in oxidative stress and energy drink-induced toxicity. *Oxid Med. Cell Longev*., 6634792. https://doi.org//10.1155/20/663479
4. Lewandowski, Ł., Kepinska, M., Milnerowicz, H. (2018). Inhibition of copper-zinc superoxide dismutase activity by selected environmental xenobiotics. *Environmental Toxicology and Pharmacology. Elsevier BV*., 58, 105–13. URL: http://dx.doi.org/10.1016/j.etap.2017.12.022.
5. Maury, P. K., Kumar, P., Chandra, P. (2015). Biomarkers of oxidative stress in erythrocytes as a function of human age. *World Journal of Methodology. Baishideng Publishing Group Inc.,* 5(4), 216. URL: http://dx.doi.org/10.5662/wjm.v5.i4.216.
6. Shkurashivska, S., Ersteniuk, H. (2019). The effect of adrenaline on the mineral and trace element status in rats. *Open Life Sciences. Walter de Gruyter GmbH*., 14, 158–64. URL: http://dx.doi.org/10.1515/biol-2019-0018.