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Diagnostics of elemental and molecular changes in organs in obese rats subjected to transcranial Direct Current Stimulation (tDCS) using X-ray and infrared spectroscopic techniques.

Abstract

Obesity is one of the diseases that is currently classified as an epidemic. Increasingly, it affects not only adults, but also adolescents and children. Obesity is a multifunctional and multi-organ disease, contributing to the development of type 2 diabetes, cardiovascular diseases, neurodegenerative diseases, cancers, etc. In obesity, as in the case of the development of most diseases, it is extremely important to identify as soon as possible the deviations that appear at the initial stage of disease. Due to the biologically complex mechanism of obesity development, currently does not exist safe and effective treatment for this disease. One of the proposed therapeutic methods for obesity is the transcranial direct current stimulation (tDCS), which is a non-invasive technique involving the use of low electrical currents to stimulate or inhibit the activity of the selected brain area. Since this technique is still in the experimental stage, there is a need to evaluate its effectiveness and potential side effects before the introduction of the tDCS for medical applications. For this purpose, experiments using Wistar rats were proposed. Studies were aimed at understanding the biochemical response of the body: (1) at the early stage of obesity induced by a high-calorie diet (comparison of non-obese and obese groups), (2) under the influence of tDCS stimulation (comparison of obese groups without stimulation, with sham stimulation, as well as with two variants of stimulation anodal and cathodal) and (3) using different tDCS stimulation protocols (comparison of two groups with anodal stimulation, with the different stimulation period, with the different stimulation frequency and with the different stimulation current). The research included diagnostics in the field of elemental composition and structures of lipids and proteins in brain areas directly and indirectly involved in the regulation of appetite as well as the in organs/tissues that are usually affected in obesity i.e. adipose tissue, liver, kidney, heart and muscle tissue.

The two methods of the X-ray fluorescence analysis (XRF) were used to assess elemental changes, i.e. X-ray fluorescence analysis induced by synchrotron radiation (SRXRF) – examination of brain tissue, and X-ray fluorescence analysis with total X-ray reflection (TXRF) – other tissues. Verification of structural changes in lipids and proteins was carried out using Fourier Transform Infrared Spectroscopy (FTIR). The analytical techniques proposed for the implementation of the topic of this doctoral thesis, under the applied experimental conditions, showed full suitability for the determination of a number of elements and infrared absorption bands relevant from the point of view of research issues. In particular, the surface masses of Na, Mg, Cl, K, Ca, P, S, Fe, Cu, Zn in the examined areas of brain tissues and the concentrations of K, Ca, Fe, Cu, Zn, Se, Br, Rb, Sr were determined in selected organs/tissues, and in addition, molecular parameters relating to e.g. the lipid saturation/unsaturation, the triglyceride levels and the proportion of protein secondary structure forms. In addition, the involvement of advanced data mining methods in the experimental data analysis allowed interesting observations to be identified in terms of the elemental and molecular parameters studied. It was found that the most pronounced changes in the elemental composition and the structure of proteins and lipids in the early stage of

obesity induced by a high-calorie diet are observed in the brain, liver and adipose tissue. In the brain, however, the greatest elemental changes and, to a lesser extent, changes in molecular parameters in obesity occur in areas directly related to appetite regulation. In addition, due to the conducted experiments, Rb was indicated as a potential marker that could be a factor describing the early stage of obesity. Regarding the tDCS stimulation, it was found that the elemental and the macromolecular changes were present both in the brain tissue and in the examined organs. In the brain, as in obesity, the most changes accompanying tDCS stimulation were observed in the hunger and satiety centers. With regard to other organs and tissues, the greatest effect of tDCS stimulation on the biochemical changes was observed in the liver and adipose tissue. In addition, it was found that as a result of tDCS stimulation in the liver and adipose tissues, there is a tendency to reverse the obesity-induced changes in the level of elements and molecular parameters. It was also shown that the polarization of the flowing currents in the tDCS stimulation slightly affects the intensity of biochemical changes in the brain, while these changes are much more influenced by the value of the applied current intensity as well as the frequency of stimulation.

The present doctoral thesis showed that the use of a combination of SRXRF, TXRF and FTIR techniques, supported by modern data mining methods, is an effective tool for comprehensive observation of elemental and macromolecular changes involved in complex processes accompanying the development of obesity, as well as those occurring in organs and tissues under the influence of tDCS stimulation.