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CARBOHYDRATE METABOLISM IN BLOOD AND LIVER OF RATS WITH EXPERIMENTAL DIABETES AND ITS CORRECTION BY ZINC CITRATE

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The influence on zinc citrate in a quantity 20 and 50 mg Zn/kg of body weight on the indexes of the carbohydrate metabolism in rats' blood and liver during the streptozotocin-induced diabetes was investigated. Experiments were conducted on white laboratory rats which were divided into four groups: I — the control group, II, III, IV — research groups. An experimental diabetes mellitus (EDM) was induced in all animals of research groups by intraperitoneal injection of Streptozotocin in 45 mg/kg body weight dosage, after 24-hour fasting. Rats from the group II with the EDM recieved only basic ration, while zinc citrate solution was added to the ration of animals from groups III and IV for four weeks in 20 and 50 mg Zn/kg body weight dosage.

It was determined during the research that blood levels of glucose, glycosylated hemoglobin, lactate and the activity of lactate dehydrogenase in erythrocytes and liver tissue increased but the blood level of pyrivate and the activity of glucose-6-phosphate dehydrogenase in erythrocytes and liver tissue decreased. The normalization of these indexes occurred under the condition of zinc citrate addition to the rats' diet in 20 and 50 mg Zn/kg body weight dosage. This shows the possitive effect of Zinc on rats' carbohydrate metabolism in a state of experimentally-induced diabetes. The recieved results may indicate on the possibility of the prevention of this disease in animals and people with the administration of zinc citrate.

Keywords: RATS, CARBOHYDRATE METABOLISM, ZINC CITRATE, EXPERIMENTAL DIABETES MELLITUS

ВУГЛЕВОДНИЙ ОБМІН У КРОВІ ТА ПЕЧІНЦІ ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНИМ ЦУКРОВИМ ДІАБЕТОМ ТА ЙОГО КОРЕКЦІЯ ЦИТРАТОМ ЦИНКУ

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Досліджено вплив цитрату цинку в кількостях 20 і 50 мг Zn/кг маси тіла на показники вуглеводного обміну в крові та печінці щурів зі стрептозотоцин-індукованим діабетом. Експерименти були проведені на білих лабораторних щурах, розділених на чотири групи: I група — контрольна, II, III і IV — дослідні. У тварин усіх дослідних груп на тлі 24-годинного голодування було викликано експериментальний цукровий діабет (ЕЦД) шляхом внутрішньоочеревинного введення стрептозотоцину з розрахунку 45 мг/кг маси тіла. Щурі II групи з ЕЦД споживали виключно основний раціон, а тваринам III і IV груп з ЕЦД до основного раціону додавали розчин цитрату цинку в кількостях 20 і 50 мг Zn/кг маси тіла.

У результаті проведених досліджень було встановлено, що у крові щурів II групи з ЕЦД зростав вміст глюкози, глікозильованого гемоглобіну, лактату та активність лактатдегідрогенази (в еритроцитах та тканині печінки), однак знижувався вміст пірувату й активність глюкозо-6-фосфатдегідрогенази (в еритроцитах та тканині печінки). За умови додавання до раціону тварин цитрату цинку в кількостях 20 і 50 мг/кг маси тіла відбувалася нормалізація цих показників, що засвідчувало позитивний вплив цинку на вуглеводний обмін у щурів з ЕЦД. Отримані результати вказують на можливість профілактики цього захворювання у тварин і людей шляхом застосування цитрату цинку.

Ключові слова: ЩУРИ, ВУГЛЕВОДНИЙ ОБМІН, ЦИТРАТ ЦИНКУ, ЕКСПЕРИМЕНТАЛЬНИЙ ЦУКРОВИЙ ДІАБЕТ

УГЛЕВОДНЫЙ ОБМЕН В КРОВИ И ПЕЧЕНИ КРЫС С ЭКСПЕРИМЕНТАЛЬНЫМ САХАРНЫМ ДИАБЕТОМ И ЕГО КОРРЕКЦИЯ ЦИТРАТОМ ЦИНКА

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Исследовали влияние цитрата цинка в количествах 20 и 50 мг Zn/кг массы тела на показатели углеводного обмена в крови и печени крыс из стрептозотоцин-индуцированным диабетом. Эксперименты были проведены на белых лабораторных крысах, разделенных на четыре группы: I группа — контрольная, II, III и IV — опытные. У животных всех опытных групп на фоне 24-часового голодания был вызван экспериментальный сахарный диабет (ЭСД) путем внутрибрюшинного введения стрептозотоцина из расчета 45 мг/кг массы тела. Крысы II группы с ЭСД употребляли исключительно основной рацион, а животным III и IV групп с ЭСД к основному рациону добавляли раствор цитрата цинка в количествах 20 и 50 мг Zn/кг массы тела.

В результате проведенных исследований было установлено, что в крови крыс II группы с ЭСД повышалось содержание глюкозы, гликозилированного гемоглобина, лактата и активность лактатдегидрогеназы (в эритроцитах и ткани печени), однако снижалось содержание пирувата и активность глюкозо-6-фосфатдегидрогеназы (в эритроцитах и ткани печени). При добавлении в рацион животных цитрата цинка в количествах 20 и 50 мг/кг массы тела происходила стабилизация этих показателей, что свидетельствовало о положительном влиянии цинка на углеводный обмен у крыс с ЭСД. Полученные результаты могут свидетельствовать о возможности профилактики этого заболевания у животных и людей путем применения цитрата цинка.

Ключевые слова: КРЫСЫ, УГЛЕВОДНЫЙ ОБМЕН, ЦИТРАТ ЦИНКА, ЭКСПЕРИМЕНТАЛЬНЫЙ САХАРНЫЙ ДИАБЕТ

Diabetes mellitus is a heterogeneous group of diseases that occur as a result of an absolute or relative insulin deficiency and have one symptom in common — hyperglycemia. The importance of the diabetes problem is conditioned by a substantial prevalence on this disease, as well as that it is a cause of difficult concominant diseases and complications, early disablement and morbidity. Nowadays it is established that Zinc deficiency is one of the potential factors of diabetes mellitus [4, 5, 13]. Zn deficiency can aggravate cytokine-induced autoimmune damage of pancreatic islet cells. Zn plays an important role in biosynthesis and insulin storage as well as in conformational integrity of an insulin hexamer; it also affects the ability of pancreatic islet cells to produce insulin [4, 8, 17]. Some of diabetes complications may be related to the increase of intracellular oxydants and free radicals as a result of intracellular Zn and Zn-dependent antioxidant enzymes decreasement [12]. Zn impacts on both diabetes type 1 and 2 development. But still the mechanism of Zn action in the treatment and prevention of the diabetes and its complications is yet unclear [3]. That is why the aim of our research was to establish the role of Zn in the correction of carbohydrate metabolism in rats' blood and liver during the experimental diabetes.

Materials and methods

The research was implemented during 2015–2016 on white laboratory rats which were held in an Institute of Animal Biology NAAS vivarium. It was competed in accordance to rules and international recomendations, approved by European Convention on vertebrate protection, used for experimental and scientific purposes and to ethical expertise of biological researches that was carried out in the Institute of Animal Biology NAAS (protocol № 56 from 8.06.2016). Animals with body weight from 150 to 170 g were divided into four groups, each consisted of 7 animals: I — the control group, II, III, IV — research groups. An experimental diabetes mellitus (EDM) was induced in all animals from the research groups by intraperitoneal injection of Streptozotocin

(*Sigma*, USA) in 45 mg/kg body weight dosage after 24-hour fasting. Hyperglycemia was detected using portable glucose meter (*Gamma-M*) by measuring sugar level in the blood taken from the tail vein. Rats from the group II with the EDM received basic ration only while zinc citrate solution was added for four weeks to the ration of animals from the groups III and IV in 20 and 50 mg Zn/kg body weight dosage. The zinc citrate solution was synthesised by a nanotechnology method [15]. The animals were withdrawn from the experiment by means of decapitation under light ether anesthesia.

As a material for our research we used rats' blood and liver tissue. Level of glycosylated hemoglobin (HbA1c) in whole blood was measured with the help of colorimetry [19], which is based on acid hydrolysis of keto-amine bond in a presence of oxaloacetic acid. As a result of this reaction 5-oxymethylfurfurol is produced, which in the reaction with 2-thiobarbituric acid (TBA) creates a coloured comlex compound. Its intensity is measured with the help of the spectrophotometer at 443 nm wave lenght.

The estimation of plasma glucose level with the help of glucose oxidase method is based on its ability to acetify in the presence of glucose oxidase to gluconic acid and hydrogen peroxide. The last one, under the influence of peroxidase, in the reaction with phenol and aminophenazone produces coloured chinonimine, which is estimated photometrically. Measurements were implemented using standart kits, made by *LACHEMA* (Czech Republic) on a biochemical analyser *Humalyzer-2000*.

The activity of carbohydrate metabolism enzymes was estimated in erythrocyte lysates and liver homogenates: lactate dehydrogenase (LDH; EC 1.1.1.27) and glucose-6-phosphate dehydrogenase (G-6-PDH; EC 1.1.1.49)

Enzyme activity was estimated with a spectrophotometric method based on a use of conjugate oxidizing systems or reduced nicotinamide coenzymes [18].

Spectrophotometric measurements were implemented on a spectrophotometer SF-26 at *t* +37 °C. The intake at 340 nm was estimated at time interval 3 min. A minimolar coefficient of extinction $NADH+H^+$ i $NADHPH+H^+$ (*Acros Organics*, Belgium) (6.22 at 340 nm) was used. Estimation of enzyme activity was implemented in 0.05 M tris-HCl buffer, that contained 5×10^{-4} M EDTA, pH 7.5. Total volume of the reaction mixture was 3 ml.

The recieved digital data was statistically procecced using a computer program *Statistica*. Student's test was used to determine significant difference between averages.

Results and discussion

As a result of our research a credible increase of glucose level in three times in rats' plasma was established in animals from the group II with EDM compared to its level in animals from the control group. Glucose plasma level in rats from the groups III and IV that received zinc citrate solution in quantity 20 and 50 mg Zn/kg of body weight credibly increased in 2.7 and 2 times respectively, compared to its level in animals from the group I. But a credible decrease on 14 % of this index was observed in rats' blood from the group IV compared to animals from the group II (*fig. 1*).

Glycated hemoglobin is the main evaluation criteria of diabetes compensation and it represents the degree of carbohydrate metabolism disorder. Glycation of hemoglobin in patients with diabetes is an irreversible process; it does not depend on insulin presence and reflects the degree of diabetes compensation for the past 90 days. Elevated level of glycated hemoglobin is one of the early indexes of carbohydrate metabolism disorder.

Blood level of glycated hemoglobin (HbAIc) increased credibly on 20 % in rats from the group II compared to the group I (*fig. 2*) which indicates the increase of hemoglobin percentage that is irreversibly connected with glucose molecules and possible dysfunction of oxygen transportation by blood [17]. The EDM in animals from the group II in a state of elevated blood glucose and glycated hemoglobin levels and probably under the condition of increased active forms of Oxygen leads to the activation of polyol pathway of glucose oxydation which results in a development of cell damage in a condition of elevated lipid peroxydation products.

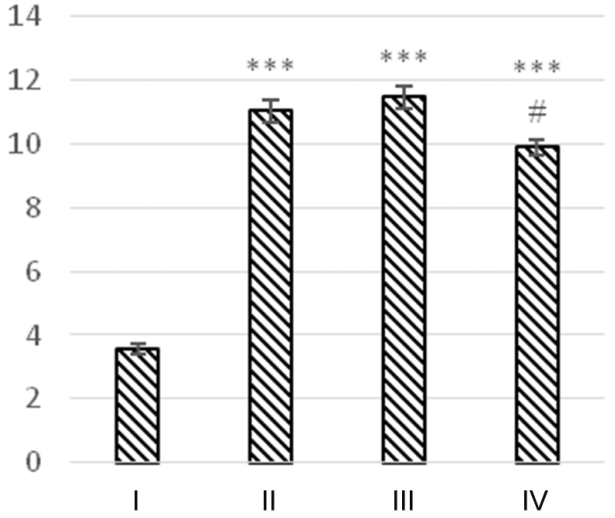

Fig. 1. Rats' glucose plasma level, mmol/l

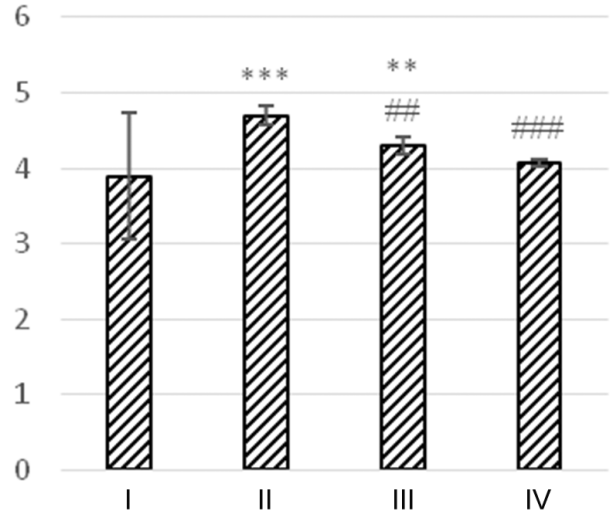

Fig. 2. Glycated hemoglobin level, %

Note: here and forward * —P<0.05, ** — P<0.01, *** — P<0.001 significance of indexes in groups II, III, IV compared to the group I; # — P<0.05, ## — P<0.01, ### — P<0.001 significance of indexes in groups III, IV compared to the group II; group I — the control group, group II — EDM; group III — EDM+20 mg Zn/kg of body weight; group IV — EDM+50 mg Zn/kg of body weight

A credible increase of HbAIc level was observed in animals from the group III (on 10 %) compared to this index in animals from the group I. Comparing HbAIc levels in animals from the groups III and IV in relation to the group II we detected their credible decrease on 9 and 13 %, respectively. The received data matches with the data in literature, which states that zinc addition to the patients' ration with type 2 diabetes influences positively on the glucose control, that appears as the decrease of HbA1c [2].

Carbohydrate metabolism that provides energy and necessary carbohydrate compounds for the formation of other organic substances occurs at a presence of lactate dehydrogenase and glucose-6-phosphate dehydrogenase. The activity of LDH indicates a glycolitic pathway of glucose transformation. We established a credible increase of LDH activity in erythrocytes (on 29 %) and liver tissue (on 76 %) in animals with EDM from the group II compared to the level of this enzyme in animals from the group I (*fig. 3a, 3b*).

The activation of LDH in erythrocytes (where anaerobic metabolism prevails) indicates a mobilisation of energetic resources, needed for a maximum ATP production that is necessary for intracellular processes, cations transport through the membrane and maintanance of its integity [1].

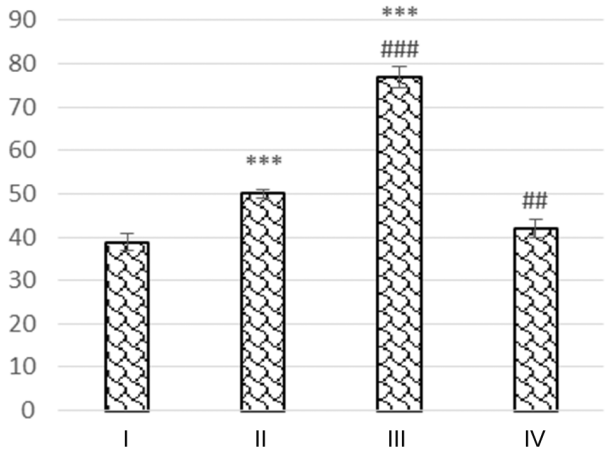

Fig. 3a. Activity of lactate dehydrogenase in erythrocytes (mcmol/min mg of protein)

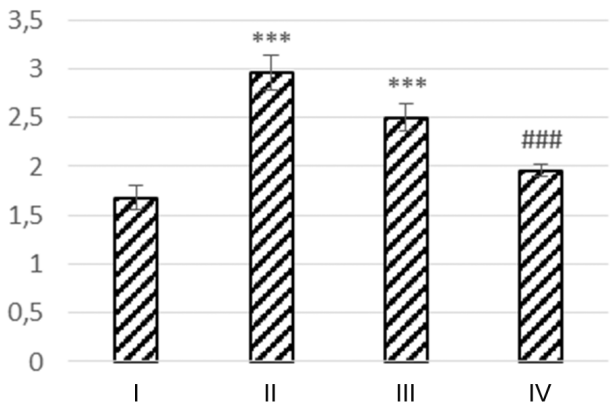

Fig. 3b. Activity of lactate dehydrogenase in liver tissue (mmol/min mg of protein)

The increase of LDH in liver tissue may indicate an intensification of anaerobic glucose katabolism, which is more intense in this tissue compared to anothers. This can be explained by the fact that metabolic processes that need major energetical resources are more active in liver. Beside that the major role of liver in carbohydrate metabolism consists in that it provides the consistent blood glucose concentration.

In our research the credible increase of LDH activity in animals' erythrocytes (in 2 times) and liver tissue (in 1.5 times) from the group III compared to the control group was established. But there was a credible decrease of LDH activity in rats' erythrocytes and liver tissue from the group II compared to the group IV on 16 % and 34 %, respectively. Received data indicates the direct connection between zinc content in rats' ration and LDH activity. Probably it can be explained by the fact that LDH is a Zn-containing enzyme and is mainly sited in cytosole [23].

The ratio of lactate to pyrivate is the index of degree of cell metabolism disorders [5]. Blood level of pyrivate decreased credibly on 21 %, while lactate level credibly increased on 65 % in rats from the group II compared to the group I (*fig. 4, 5*). This preditermines the offset of equilibrium between lactate and pyrivate towards to the decrease of pyrivate concentration. Since the pyrivate is an acetyl-CoA precursor (a primary substrate in TCA cycle) its decrease will cause a decrease of total substrate flow in this cycle.

The lactate level was credibly decreased on 16 % and 44 % in animals from the groups III and IV in a state of Zn citrate addition compared to the group II, while pyrivate level was increased on 16 % in animals from the group IV. This may indicate the normalization of metabolic processes in rats' blood under the Zn influence. The accumulated pyrivate is used for glucose regeneration in a pathway of its conversion to oxaloacetate. Besides that pyrivate may be transformed into alanine.

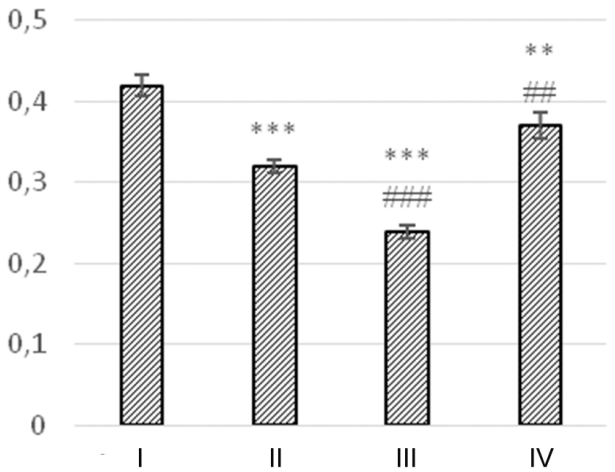

Fig. 4. Rats' blood pyrivate level, mM

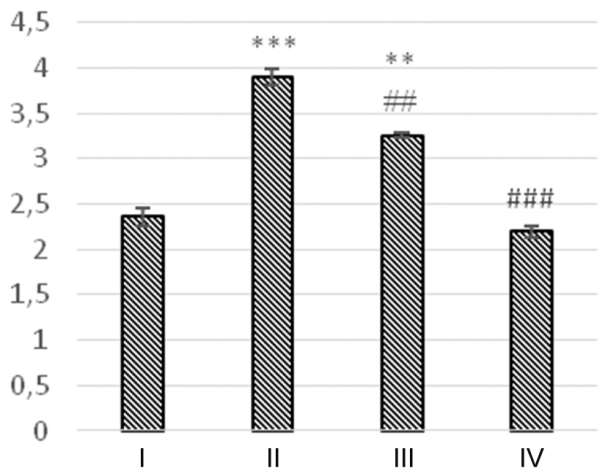

Fig. 5 Rats' blood lactate level, mM

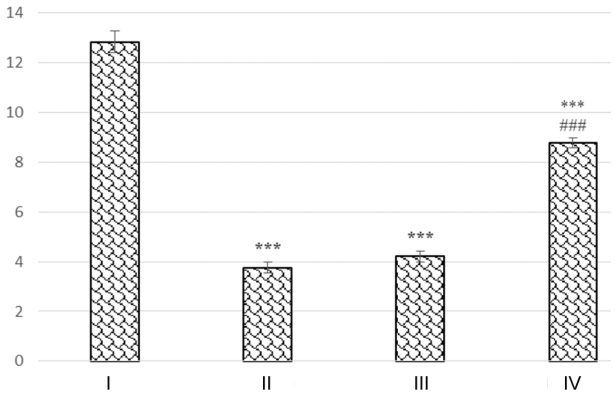

Fig. 6a. The activity of G-6-PDH in erythrocytes, nmol/min•mg of protein

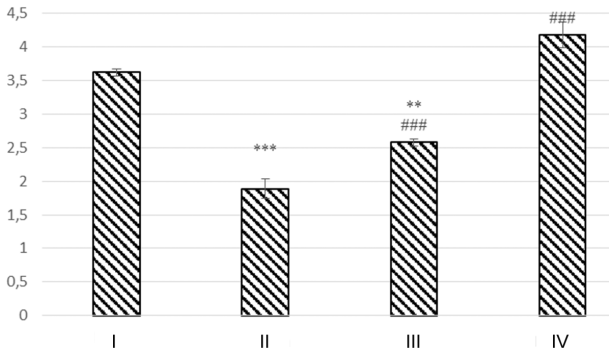

Fig. 6b. The activity of G-6-PDH in liver, nmol/min•mg of protein

Glucose-6-phosphate dehydrogenase activity indicates the intensity of glucose transformation in pentose phosphate pathway. We established a credible decrease of G-6-PDH activity in erythrocytes (on 71 %) and liver tissue (on 48 %) compared to the level of this enzyme in animals from the group I (*fig. 6a, 6b*). High glucose level in animals from the group II inhibits G-6-PDH and therefore leads to the decrease of intracellular NADPH level and the increase of oxidative stress. But it is established that the oxidative stress can cause the G-6-PDH expression [21, 22].

In our research a credible decrease of G-6-PDH in erythrocytes (on 67 %) and liver tissue (on 29 %) of animals from the group III was established, while a decrease of this enzyme's activity in erythrocytes (on 32 %) and its increase — in liver tissue (on 15 %) were observed compared to the control group I.

Besides that a credible increase of G-6-PDH activity was established in liver of rats from the group III (in 1.4 times) and the group IV (in 2.2 times), as well as in erythrocytes in rats from the group IV (in 2.3 times) compared to the activity of this enzyme in animals from the group II. The received results indicate the activation of glucose transformation in pentose phosphate pathway, while the intensity of this process depends directly on zinc level in rats' ration.

Conclusion

It was determined during the research that blood levels of glucose, glycosylated hemoglobin, lactate and the activity of lactate dehydrogenase (in erythrocytes and liver tissue) increased but the level of pyrivate and the glucose-6-phosphate dehydrogenase activity (in erythrocytes and liver tissue) decreased. Normalization of these indexes occured under the condition of zinc citrate addition in a quantity of 20 and 50 mg Zn/kg body weight to the rats' diet. This shows the positive effect of Zinc on rats' carbohydrate metabolism in a state of experimentally-induced diabetes. The received results may indicate on the possibility of the prevention of this disease in animals and people with the administration of zinc citrate.

Perspectives for further research. We plan to investigate the carbohydrate metabolism indexes under the influence of other elements, such as magnesium, vanadium and chromium, and to elaborate base for the development of biologically active supplements for diabetes prevention.

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