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ADENINE NUCLEOTIDE CONTENT AND ACTIVITY OF AMP CATABOLISM ENZYMES IN THE KIDNEY OF RATS FED ON DIETS WITH DIFFERENT PROTEIN AND SUCROSE CONTENT

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Background. Excessive consumption of sucrose or protein deficiency in the diet can induce metabolic disorders in the kidney, whose functioning requires significant ATP energy expenditure. The study investigated the levels of the purine nucleotides ATP, ADP, AMP, and the activity of the enzymes F_oF_1-ATPase, 5′-nucleotidase, and AMP deaminase in the kidneys of rats exposed to different levels of protein and sucrose in their diet.

Materials and Methods. The research was conducted on white non-linear rats, which were kept under different dietary regimens for a period of 4 weeks. Quantitative evaluation of the ATP, ADP, and AMP content was performed by thin-layer chromatography on Silufol sheets. F_oF_1-ATPase activity was determined by the accumulation of P_i. 5′-nucleotidase activity was measured based on the amount of inorganic phosphorus released in AMP hydrolysis. AMP deaminase activity was determined by the accumulation of ammonia.

Results and Discussion. Research results revealed that in the mitochondria of the animals' kidneys under conditions of low-protein diet, a significant reduction in AMP content was observed compared to the control, while ATP and ADP content remained unchanged. Simultaneously, the activities of 5′-nucleotidase, AMP deaminase, and F_oF_1-ATPase in the kidneys of animals on a low-protein diet were maintained at control levels. However, in rats maintained on a low-protein/high-sucrose diet, depletion of all adenine nucleotides is observed against an increase in the hydrolytic activity of F_oF_1-ATPase, AMP deaminase, and 5′-nucleotidase activities. The 5′-nucleotidase activity in

animals of this group reaches maximum values in comparison with the control, indicating an enhanced AMP degradation mediated by 5′-nucleotidase in the conditions of low-protein/high-sucrose diet consumption.

Conclusion. Excessive sucrose consumption in the context of dietary protein deficiency is accompanied by a depletion of the adenine nucleotides pool in the mitochondrial fraction and a significantly increased activity of purine catabolism enzymes in the cytosolic fraction of rat kidneys. This may result in an imbalance in the energy supply of renal cells. The obtained results open up prospects for developing a strategy for correcting energy metabolism disorders in the conditions of nutritional imbalance.

Keywords: nutrients, kidney, F_oF_1-ATPase, 5′-nucleotidase, AMP deaminase

INTRODUCTION

The mechanisms of energy balance disruption that lead to changes in the course of essential physiological processes in the kidney under sucrose and dietary protein imbalance are still poorly understood. It is known that the production of an adequate amount of ATP in the kidneys is a necessary condition to support cellular anabolism, physiological processes such as reabsorption and the regulation of water-salt and acid-base balance (Srivastava *et al.*, 2020). The correlation between kidney function and the state of the energy supply system indicates that alterations in tubule cell metabolism contribute to chronic kidney disease, diabetic kidney disease, and fibrosis activation (Kang *et al.*, 2015).

Previous studies have demonstrated that a high-sucrose diet induces histological changes in kidney tissues (Sánchez-Solís *et al.*, 2018), may exacerbate proteinuria (de Souza *et al.*, 2021), and increases the risk of non-alcoholic fatty liver disease and type 2 diabetes (García-Arroyo *et al.*, 2021). Defects in oxidative phosphorylation that may occur with excessive sucrose consumption can lead to increased oxidative stress, loss of membrane potential, and decreased ATP content, all of which are associated with the pathogenesis of kidney diseases (Ruiz-Ramírez *et al.*, 2020). It has been shown that the dysfunction of kidney mitochondria, in particular, disruption of the energy production processes, leads to disturbances of the reabsorption and filtration capacity of the kidneys, which underlies the pathogenesis of different nephropathies (Hallan & Sharma, 2016). Lower protein intake decreases kidney mass and glomerular filtration capacity and leads to a greater kidney tubular structural injury and inflammation (Fotheringham *et al.*, 2021; Kopylchuk & Voloshchuk, 2023).

The impact of low-protein/high-sucrose diet remains unexplored, although such a diet is quite common. This is important for understanding the pathogenetic mechanisms of kidney dysfunction under different feeding regimens.

Therefore, investigating the relationship between the nutritional composition of the diet and the cellular energy status of kidney cells is crucial. The ratio and content of ATP, ADP, and AMP in cells depend on energy needs and expenditures, where AMP and ADP act as positive effectors stimulating catabolic processes. 5′-nucleotidase (EC 3.1.3.5) and AMP deaminase (EC 3.5.4.6) enable metabolic transformations of purine nucleotides and control the levels of specific intracellular modulators: AMP, adenosine, and inosine (Tapbergenov *et al.*, 2022). The purpose of this study was to assess the levels of ATP, ADP, AMP, and the activities of AMP deaminase, 5′-nucleotidase, and F_oF_1-ATPase in rat kidneys subjected to different dietary protein and sucrose concentrations.

MATERIALS AND METHODS

Experimental animals and protocols. The section "Experimental animals and protocols" was prepared by analogy with our previous publication (Voloshchuk & Kopylchuk, 2021). In the study, 10–12-week-old white nonlinear rats (n = 36) weighing 130–140 g were used. The animals were separated into solitary plastic cages with *ad libitum* access to water. The animals were housed at a controlled temperature of 20 °C ± 1 °C with a 12-hour light-dark cycle. The animals were monitored daily, and weighed three times a week. All manipulations were conducted according to the general ethical principles of experiments on animals adopted by the Sixth National Congress of Ukraine on Bioethics, in accordance with international bioethical norms and approved by the bioethics commission of the Educational and Scientific Institute of the Biology, Chemistry and Biological Resources of Yuriy Fedkovych Chernivtsi National University (protocol No 2 of 24.12.2021).

Rats were divided into four groups of 9 animals in each group as follows: C – control diet, animals receiving full-value semi-synthetic ration; LPR – animals receiving low-protein ration; HSR – animals receiving high-sucrose ration; LPR/HSR – animals receiving low-protein high-sucrose ration.

The animals of group C received a standard ration containing 14% of protein (casein), 10% of fat, and 76% of carbohydrates, balanced by all the essential nutrients. The animals of the LPR group received isoenergetic ration containing 4.7% of protein, 10% of fat, and 85.3% of carbohydrates. The animals of the HSR group received a high-sucrose diet containing 40% of sucrose and balanced by all other essential nutrients. The animals were maintained on the corresponding diet during four weeks.

The control diet (C) was the AIN-93 diet (Reeves *et al*., 1993), all ingredients are presented in **Table**.

Composition of the experimental diets (g/kg diet)

Ingredient	Diet			
	Control	LPR	HSR	LPR/HSR
Cornstarch, g/kg	620.7	714.1	320.7	414.1
Casein, g/kg	140	46.6	140	46.6
Sugar, g/kg	100	100	400	400
Fiber (cellulose microfiber), g/kg	50	50	50	50
Mineral mix, g/kg [1]	35	35	35	35
Vitamin mix, g/kg [1]	10	10	10	10
L-cystine, g/kg	1.8	1.8	1.8	1.8
Choline bitartrate, g/kg	2.5	2.5	2.5	2.5
Soy oil, g/kg	40	40	40	40

Note: [1] Mineral and vitamin mix – based on the AIN-93G vitamin and mineral mixes.
LPR – animals receiving a low-protein ration; HSR – animals receiving a high-sucrose ration; LPR/HSR – animals receiving a low-protein high-sucrose ration

Preparation of mitochondria. Mitochondrial fraction of the kidney was separated by differential centrifugation (Heraeus Biofuge, Germany) in the following buffer medium: 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl; pH 7.4 at 0–3 °C (Itoh *et al*., 2002). The protein content in the mitochondrial fraction of the kidney was measured by Bradford method (Kruger, 2002).

ATP, ADP, and AMP content. Quantitative evaluation of the ATP, ADP, and AMP content was performed by thin-layer chromatography on Silufol sheets (Zarubina *et al*., 1982). Free nucleotides were extracted from the mitochondrial fraction with 0.8 M $HClO_4$ for 30 min at 0–4 °C. Protein-free perchlorate extracts were obtained by centrifugation for 15 min at 1500 g. Supernatants were neutralized with K_2CO_3 to pH 7.0 and centrifuged again under the same conditions, upon which the supernatant aliquots were loaded onto chromatographic sheets. After the separation of adenine nucleotides in the mobile phase consisting of dioxane, isopropanol, water, and ammonia (4:2:4:1), they were quantitatively determined by direct spectrometry. Nucleotide spots were detected in UV light and eluted from the sheets with 0.1 M HCl for 20 min. The eluate absorption was measured at 260 nm.

Enzyme assays:

F_oF_1-ATPase activity was determined by the accumulation of P_i. The activity was determined in the incubation solution containing 400 µmol of Tris-HCl (pH 7.4), 5 µmol of ATP disodium salt, 7.5 µmol of $MgSO_4$, $1 \cdot 10^{-2}$ µmol of 2,4-dinitrophenol, 7.5 µmol of $CaCl_2$, 120 µmol of NaCl, 20 µmol of KCl. The reaction was initiated by the addition of 50 µL of a mitochondria suspension containing 1 mg of protein, incubated for 15 min at 37 °C. The contents of P_i were determined colorimetrically (Gabibov, 1986).

5′-nucleotidase activity was determined by the accumulation of inorganic phosphorus released during the hydrolysis of AMP and expressed in P_i µmol per 1 min per 1 mg protein (Kopylchuk *et al*., 2009).

AMP deaminase activity was determined using colorimetric method described by Giusti and Galanti (Giusti, Galanti, 1984). Enzyme activity was expressed in µmol of ammonia per 1 min per 1 mg protein.

Statistical analysis. Statistical analysis of the results was carried out using Excel software. Characteristics of the study group were expressed as Mean±Standart Deviation for normal distribution. The differences among experimental groups were detected by ANOVA. A significance level of $P \leq 0.05$ was considered indicative of a statistically significant difference.

RESULTS AND DISCUSSION

According to the results of the study, in the mitochondria of the kidney of rats kept on a low-protein diet no changes in the content of ATP and ADP were found (**Fig. 1–2**). However, there was a decrease in AMP level in the mitochondria of the kidney of rats maintained on the low-protein diet by 2 times compared with the control (**Fig. 3**). AMP acts as an allosteric activator of AMP-activated protein kinase (АМФК, EC 2.7.11.31) –

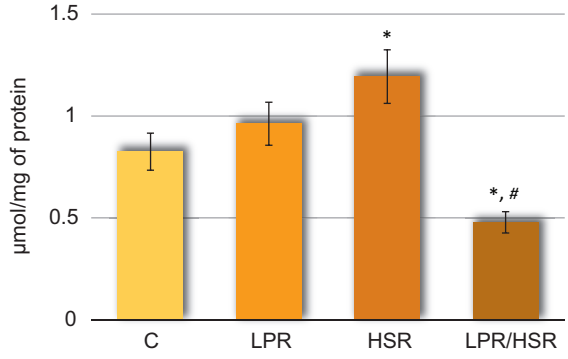

Fig. 1. The ATP content in the rat kidney mitochondrial fraction under different protein and sucrose content in a diet
Note (here and forwards): C – animals receiving complete semi-synthetic ration; LPR – animals receiving a low-protein ration; HSR – animals receiving a high-sucrose diet; LPR/HSR – animals receiving a low-protein high-sucrose diet. * – significantly different from the control, $P \leq 0.05$; # – significantly different from group HSR, $P \leq 0.05$

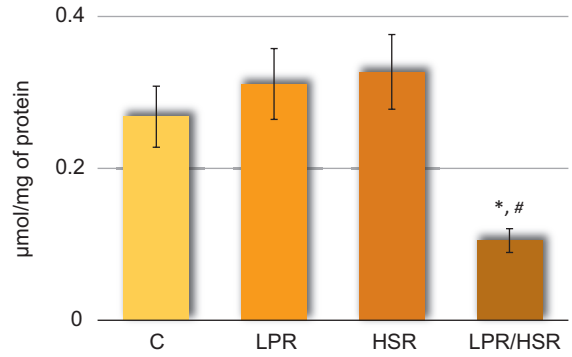

Fig. 2. The ADP content in the rat kidney mitochondrial fraction under different protein and sucrose content in a diet

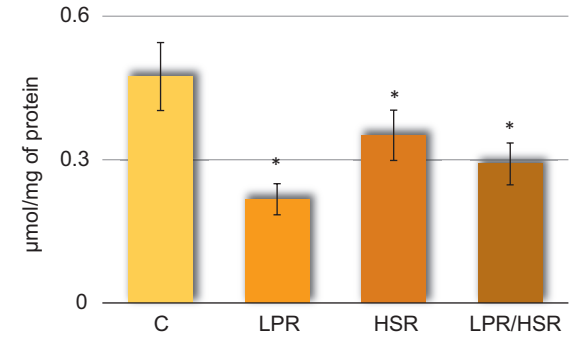

Fig. 3. The AMP content in the rat kidney mitochondrial fraction under different protein and sucrose content in a diet

a central regulator of energy homeostasis, controlling the intensification of catabolic processes to generate energy during states of energy deficiency and, conversely, the inhibition of cellular anabolism (Kim *et al*., 2016). Therefore, the consequence of the observed reduction in AMP content under the investigated conditions will likely be a decrease in the activity of AMP-activated protein kinase. Under conditions of exogenous protein deficit, such changes could potentially be considered a protective mechanism, as the reduction in AMPK activity may lead to a slowing of protein catabolism and an enhancement of protein synthesis through an increased mTORC1 activity (He *et al*., 2017).

Simultaneously, in the mitochondria of kidneys of the rats subjected to a high-sucrose diet, an increase in ATP content was recorded (**Fig. 1**), concomitant with a decrease in AMP content (**Fig. 3**). Presumably, under the investigated conditions, the increase in ATP concentration is associated with enhanced glucose influx from the bloodstream to the kidneys. This is due to the increased ATP demand for glucose reabsorption in the proximal tubules of the kidneys. Excessive influx of exogenous glucose leads to an increase in the number of energy-dependent transporters, such as glucose transporter (GLUT) and sodium-glucose co-transporter (SGLT), on the cell membrane surface. These proteins are responsible for the reabsorption of glucose from the primary filtrate and its subsequent release into the bloodstream (Wilding, 2014).

In the mitochondria of the kidneys of animals receiving a low-protein/high-sucrose diet, depletion of the pool of all adenosine nucleotides was observed (**Fig. 1–3**). Putatively, the consequence of such a pronounced reduction in the content of adenosine nucleotides will be a disruption of all energy-dependent processes in the kidneys, including the maintenance of water balance, reabsorption, regulation of acid-base homeostasis, and the synthesis of biologically active substances (Naber & Purohit, 2021).

It is known that the maintenance of the adenosine nucleotide pool occurs through a balance among three fundamental processes: *de novo* nucleotide synthesis, their resynthesis, and catabolism to end products. The resynthesis of ATP from ADP and inorganic phosphate utilizing the energy from the electrochemical gradient across the inner mitochondrial membrane is catalyzed by F_oF_1-ATPase. However, under conditions of energy process imbalance, disruption of membrane potential is observed, leading to the switch of F_oF_1-ATPase activity from synthase to hydrolase (Mise *et al*., 2020).

According to the obtained results, in the mitochondria of the kidney of rats kept on a low-protein diet no changes in the hydrolase activity of F_oF_1-ATPase were found (**Fig. 4**). However, in the mitochondrial fraction of rat kidneys subjected to a high-sucrose diet, a twofold increase in hydrolase activity was recorded compared to the control indicators. The most pronounced activation of F_oF_1-ATPase was characteristic of animals that consumed a low-protein/high-sucrose diet. On the one hand, the observed changes indicate a disruption of the membrane potential (Rieger *et al*., 2021), and therefore, ATP hydrolysis under the investigated conditions is considered as one of the possible ways to maintain the H^+ concentration. On the other hand, the observed increase in the hydrolase activity of F_oF_1-ATPase may be regarded as one of the reasons for the observed decrease in ATP content in kidney mitochondria under the investigated conditions.

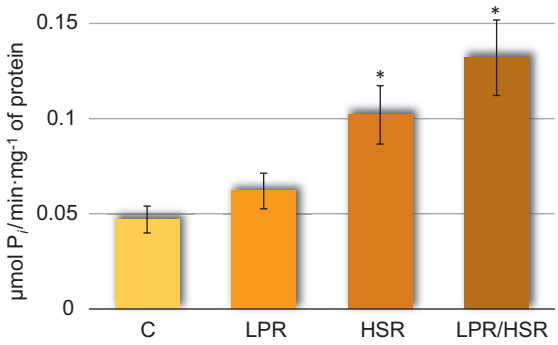

Fig. 4. Activity of F_oF_1-ATPase in the rat kidney mitochondrial fraction under different protein and sucrose content in a diet

It has been demonstrated that under conditions of prolonged hyperglycemia, the increased hydrolysis of ATP may be associated with a decrease in proton potential, leading to the activation of reactive oxygen species (ROS) production and the development of oxidative stress (Alcántar-Fernández *et al*., 2019).

Additionally, the observed decrease in the content of adenine nucleotides in the kidneys under a low-protein/high-sucrose diet conditions may be attributed to changes in the activity of key enzymes involved in adenine nucleotides catabolism – AMP deaminase and 5′-nucleotidase. These enzymes catalyze the transformation of AMP into various products: AMP deaminase catalyzes the deamination of AMP to IMP with the release of free ammonia, and 5′-nucleotidase catalyzes the dephosphorylation of AMP to adenosine (Zabielska *et al*., 2015).

According to the obtained results, in the cytosolic kidney fraction of rats kept on a low-protein diet no changes in the activity of AMP deaminase and 5′-nucleotidase were found (**Fig. 5–6**).

In the kidneys of animals on a high-sucrose diet, a significant increase in both AMP deaminase and 5′-nucleotidase activities was observed: AMP deaminase activity increased by almost 3 times compared to the indicators of the control group of rats (**Fig. 5**), and 5′-nucleotidase activity approximately doubled (**Fig. 6**).

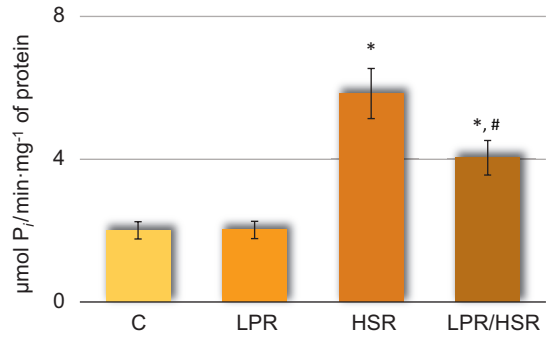

Fig. 5. Activity of AMP deaminase in the cytosolic kidney fraction of rats under different protein and sucrose content in a diet

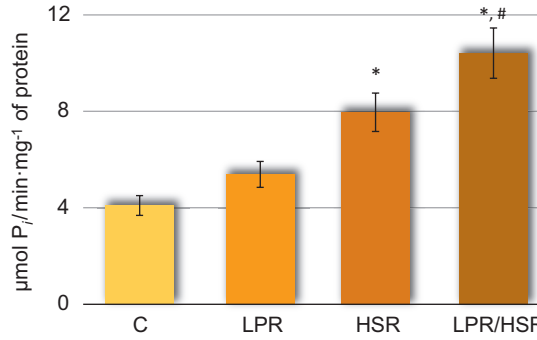

Fig. 6. Activity of 5′-nucleotidase in the cytosolic kidney fraction of rats under different protein and sucrose content in a diet

Considering that adenosine is the product of the 5′-nucleotidase reaction and is an important signaling molecule, the increase in 5′-nucleotidase activity can be regarded as a compensatory response aimed at increasing the concentration of adenosine. It has been shown (Shen *et al*., 2018) that the binding of adenosine to A2B receptors on the surface of epithelial cells in the nephron tubules activates the PI3K/Akt signaling pathway. This, in turn, ensures the activation of intracellular protective mechanisms against apoptosis induced under conditions of hyperglycemia. In particular, the established role of adenosine in signaling the inhibition of caspase-1 activity, which prevents the initiation of the cell apoptosis program, has been demonstrated (Tian *et al*., 2019).

In addition, the increase in AMP deaminase activity under the investigated conditions may also play a crucial regulatory role, as the reaction product – IMP – is further transformed into inosine (Guo *et al*., 2021). Inosine serves as a regulator of the expression of several proteins, including superoxide dismutase (SOD), sirtuin (SIRT), IkB-α, and inducible nitric oxide synthase (iNOS). Inosine plays a critical role in the activation of antioxidant defenses and intracellular signal transmission. Moreover, the accumulation of intracellular inosine inhibits the synthesis of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), thus preventing the development of the inflammatory process in the kidney under conditions of hyperglycemia. In turn, the increase in AMP deaminase and 5′-nucleotidase activities can be considered as one of the reasons for the observed decrease in AMP content (**Fig. 3**).

It should be noted that in the cytosolic fraction of the kidneys of animals consuming a low-protein/high-sucrose diet, both enzymatic activities examined exceed the control values. However, the 5′-nucleotidase activity in animals of this experimental group reaches its maximum values (**Fig. 6**). Thus, under the conditions of a low-protein/high-

sucrose diet, a shift in AMP degradation involving 5′-nucleotidase to form adenosine is observed in the cytosolic fraction of the kidney. Presumably, such a shift has a significant regulatory effect because adenosine, the product of 5′-nucleotidase activity, is considered an extracellular signaling molecule involved in various biochemical processes that support tissue homeostasis and repair. Specifically, adenosine prevents the loss of megalin and cubilin – receptor proteins responsible for up to 97% of albumin reabsorption in the proximal tubules of the kidney under conditions of proteinuria associated with hyperglycemia (Tian *et al*., 2019).

It is known that hyperglycemia leads to the disruption of the permeability of the kidney filtration barrier, resulting in the development of proteinuria (Gómez-Sámano *et al*., 2018). The preservation of megalin and cubilin allows the reabsorption of filtered proteins and amino acids across the damaged filtration barrier, preventing their loss in conditions of exogenous protein deficiency. Additionally, the binding of adenosine to A2A receptors on podocyte membranes leads to the regeneration of the integrity of the filtration barrier by enhancing the synthesis of cytoskeletal components, preventing the progression of albuminuria (Pandey *et al*., 2019). There are also studies confirming the role of adenosine in initiating processes aimed at alleviating the consequences of hyperglycemia: adenosine contributes to the reduction of local inflammation caused by increased glucose in the lumen of renal tubules, vasodilation, which is necessary for improving filtration pressure in affected kidneys, and when binding to A1A receptors, it promotes the reduction of symptoms of reperfusion ischemic damage (Yap & Lee, 2012).

Thus, under the conditions of animals consuming low-protein diet, the levels of ATP and ADP do not change significantly, but the level of AMP decreases, while the activity of F_oF_1-ATPase, AMP deaminase and 5′-nucleotidase remain at control levels. The results obtained suggest that under conditions of protein deficiency in the diet, *de novo* formation of AMP is likely to be disrupted, as amino acids are required for its synthesis. After four weeks of consumption of a high-sucrose diet, an increase in ATP content, maintenance of ADP content at control levels, and a decrease in AMP content are observed in the rat kidney mitochondria, indicating a redistribution among adenine nucleotides.

The identified changes are probably aimed at increasing the energy reserve of kidney cells in response to the increased demand for ATP necessary for glucose reabsorption in the proximal tubules of the kidney under conditions of excessive glucose intake. Furthermore, the decrease in AMP content correlates with an increase in the activity of enzymes involved in its catabolism – AMP deaminase and 5′-nucleotidase. The most pronounced changes in the studied parameters were observed in the LPR/HSR group of animals. They showed a decrease in the levels of all adenine nucleotides and a simultaneous increase in the activity of F_oF_1-ATPase, AMP deaminase and 5′-nucleotidase, which presumably indicates a disruption of the compensatory abilities of kidney cells in conditions of protein deficiency and excess sucrose in the diet. The obtained results open up the prospects for further research on possible mechanisms of disruption in energy supply processes in kidney cells under short-term consumption of a low-protein/high-sucrose diet.

CONCLUSION

The results obtained indicate that excessive sucrose consumption in the context of dietary protein deficiency leads to a depletion of the mitochondrial adenine nucleotide pool and a significant increase in the activity of enzymes involved in purine catabolism

in the cytosolic fraction of rat kidneys. These alterations may induce metabolic changes in renal cells, potentially resulting in disruptions in cellular energy supply and functional activity of kidneys.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors did not receive any funding for this research and declare no conflicting interests.

Human Rights: this article does not contain any experiments with human objects.

Animal Rights: all international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [H.P.; O.M.]; methodology, [O.M.]; investigation, [H.P.; O.M.]; resources, [H.P.; O.M.]; data curation, [H.P.; O.M.]; writing – original draft preparation, [O.M.]; writing – review and editing, [H.P.; O.M.]; visualization, [O.M.]; supervision, [H.P.]; project administration, [H.P.; O.M.]; funding acquisition, [–].

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ВМІСТ АДЕНІЛОВИХ НУКЛЕОТИДІВ І АКТИВНІСТЬ ЕНЗИМІВ КАТАБОЛІЗМУ АМФ У НИРКАХ ЩУРІВ ЗА УМОВ РІЗНОЇ ЗАБЕЗПЕЧЕНОСТІ РАЦІОНУ ПРОТЕЇНОМ І САХАРОЗОЮ

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Вступ. Надмірне споживання сахарози або нестача протеїну в раціоні можуть індукувати метаболічні порушення у нирках, функціонування яких потребує значних затрат енергії АТФ. У роботі досліджено вміст пуринових нуклеотидів АТФ, АДФ, АМФ і активність ензимів F_oF_1-АТФ-ази, 5′-нуклеотидази, АМР-дезамінази у нирках щурів за умов різної забезпеченості раціону протеїном та сахарозою.

Матеріали та методи. Дослідження проводили на білих нелінійних щурах, яких утримували протягом 4 тижнів за різних режимів харчування. Вміст АТФ, АДФ і АМФ визначали методом тонкошарової хроматографії на силуфолових пластинках. Активність F_oF_1-АТФ-ази визначали за накопиченнням $Ф_н$. Активність 5′-нуклеотидази визначали за накопиченням неорганічного фосфату, що вивільняється під час гідролізу АМФ. Активність АМФ-дезамінази визначали за накопиченням аміаку.

Результати. Результати досліджень встановили, що у мітохондріях нирок тварин за умов споживання низькопротеїнового раціону достовірно зменшується вміст АМР на тлі збереження вмісту АТФ і АДФ порівняно з контролем. Водночас у нирках тварин за умов споживання низькопротеїнового раціону зберігаються показники активностей 5′-нуклеотидази, АМФ-дезамінази та F_oF_1-АТФ-ази на рівні показників контролю. Проте у щурів, яких утримували на низькопротеїновій/високосахарозній дієті, відбувається виснаження пулу всіх аденілових нуклеотидів на тлі підвищення гідролазної активності F_oF_1-АТФ-ази, АМФ-деаміназної та 5′-нуклеотидазної активностей. 5′-Нуклеотидазна активність у тварин цієї дослідної групи досягає максимальних значень, тобто за умов споживання низькопротеїнового/високосахарозного раціону посилюється деградація АМФ за участі 5′-нуклеотидази.

Висновки. Надмірне споживання сахарози на тлі аліментарного дефіциту протеїну супроводжується виснаженням пулу аденілових нуклеотидів у мітохондріях і максимально вираженим підвищенням активностей ензимів катаболізму пуринів у цитозольній фракції нирок щурів, наслідком чого може бути дисбаланс енергозабезпечення клітин нирок. Отримані результати відкривають перспективи для розробки стратегії корекції порушень енергетичного обміну в нирках за умов нутрієнтного дисбалансу.

Ключові слова: нутрієнти, нирки, F_oF_1-АТФ-аза, 5′-нуклеотидаза, АМФ-дезаміназа

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