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O. L. Appelhans[1] https://orcid.org/0000-0002-2344-6502
O. O. Pakhomova[1] https://orcid.org/0009-0008-9796-9331
O. O. Protunkevych[2] https://orcid.org/0000-0002-9909-8778
M. F. Konovalov[1] https://orcid.org/0009-0003-6298-6098
O. B. Solomatin[1] https://orcid.org/0000-0003-4837-7520

DISORDERS OF LIPID METABOLISM AND ACTIVITY OF ENZYMES OF KREBS CYCLE AND PENTOSE PHOSPHATE PATHWAY IN THE MODELLING OF METABOLIC ACIDOSIS AND ALKALOSIS IN RATS

[1] Odesa National Medical University, Odesa, Ukraine
[2]Odessa Polytechnic National University, Odesa, Ukraine

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O. L. Appelhans[1], E. O. Pakhomova[1], O. O. Protunkevych[2], N. F. Konovalov[1], A. B. Solomatin[1]

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[1]Odesa National Medical University, Odesa, Ukraine
[2]Odessa Polytechnic National University, Odesa, Ukraine

The aim was to investigate changes in the orientation of cholesterol metabolism and the spectrum of lipids, as well as the activity of key enzymes of the Krebs cycle and the pentose phosphate pathway, regulators of the NADPH/$NADP^+$ ratio, in tissues of rats with disturbances of acid-base state – metabolic acidosis and alkalosis modelled with the aim of unbalanced nutrition: high-protein and carbohydrate (sucrose) diets.

Materials and methods. Metabolic acidosis and alkalosis were modelled in rats sticking to diets with excessive ammonium chloride and sucrose. Acid-base balance and lipoprotein cholesterol content were studied in rat blood using conventional methods, then the animals were decapitated, and lipid content and enzyme activity (NADP-dependent IDH and MDH, G6PD and lipase) were determined in liver tissue, cardiac and thigh muscles using TLC.

Results and discussion. The development of metabolic acidosis and alkalosis under the influence of diets with excessive ammonium chloride and sucrose was noted. The diet with ammonium chloride promoted lipolysis, activation of lipase, accumulation of under oxidised FFA and cholesterol, decreased the activity of NADP-dependent ICH in all tissues observed and the activity of NADP-dependent MDH in the tissues of the thigh and cardiac muscles of rats, and increased the activity of G6PD in the liver. The sucrose diet increased lipogenesis, cholesterol biosynthesis, and accumulation of FFA in the tissues of the heart and liver, and activated NADP-dependent MDH and IDH in all tissues observed of rats.

Keywords: diet with ammonium chloride and sucrose, acid-base disorders, lipids, G6PD, NADP-dependent IDH and MDH, lipase.

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О. Л. Аппельханс[1], О. О. Пахомова[1], О. О. Протункевич[2], М. Ф. Коновалов[1], О. Б. Соломатін[1]

ПОРУШЕННЯ ОБМІНУ ЛІПІДІВ ТА АКТИВНОСТІ ФЕРМЕНТІВ ЦИКЛУ КРЕБСУ І ПЕНТОЗОФОСФАТНОГО ШЛЯХУ У РАЗІ МОДЕЛЮВАННЯ МЕТАБОЛІЧНОГО АЦИДОЗУ І АЛКАЛОЗУ У ЩУРІВ

[1]Одеський національний медичний університет, м. Одеса, Україна
[2]Національний університет «Одеська політехніка», м. Одеса, Україна

Мета – дослідити ліпідний обмін, а також активність Г6ФД, НАДФ-залежних ІЦДГ та МДГ у тканинах щурів у разі моделювання метаболічного ацидозу та алкалозу за допомогою дієт з хлористим амонієм та сахарозою. обидві дієти підвищують вміст холестерину і порушують обмін ліпідів. Встановлено стан метаболічного ацидозу при впливі дієти з хлористим амонієм, активацію ліполізу та накопичення ВЖК, зниження активності НАДФ-ІЦДГ у всіх досліджених тканинах і НАДФ-МДГ у стегновому та серцевому м'язах та активацію Г6ФД у печінки. У разі розвитку метаболічного алкалозу під впливом сахарозного раціону активується ліпогенез та біосинтез ВЖК у тканинах серця та печінки та НАДФ-МДГ і НАДФ-ІЦДГ у всіх тканинах щурів.

Ключові слова: дієти з хлоридом амонію та сахарозою, зміни КОС, ліпіди, Г6ФД, НАДФ-залежні ІЦДГ та МДГ.

Introduction

Nutritional factors, in particular, excessive consumption of animal proteins, fats and sugar, occupy a leading position among the causes of stress reactions [1]. The basis of integrative regulation and protection of the body, which ensures the formation of adaptive responses, are physiological and biochemical processes, the implementation of which is carried out in close interaction of a number of systems associated with the acid-base homeostasis of the body [1; 2]. Under conditions of stress and more intensive work of physiological, immunological and biochemical mechanisms, compensatory consumption of the main classes of organic compounds in the body occurs.

The analysis of the peculiarities of various aspects of metabolism in humans and animals and the acid-base state

allows us to draw conclusions about interrelated changes in biochemical metabolism and acid-base state [1; 2]. In the experiment, diets containing an excess of animal fats and proteins result in metabolic acidosis [2; 3], accompanied by electrolyte imbalance and deficiency of Ca^{2+} and K^{+} ions, increased secretion of glucocorticoids and catecholamines, and, accordingly, a decrease in insulin, which indicates a diabetogenic situation in the body [3]. In a sucrose diet, an alternative state of the acid-base state and metabolism is observed, with glucose oxidation activated and insulin secretion, fatty acid biosynthesis, and lipid peroxidation increased [3; 4].

Cholesterol is contained in the body in very large quantities – 150 g – and is a necessary structural component of membranes, with a molar ratio of phospholipids/cholesterol in tissues of 1.0. The body receives cholesterol both from food and is synthesised *de novo* from acetyl-CoA. Cholesterol biosynthesis is one of the longest metabolic pathways and occurs in three main stages: formation of mevalonate, squalene and cholesterol itself. The first irreversible reaction that occurs with a significant loss of free energy – NADPH and is an important regulatory step in the cholesterol biosynthesis chain is the reduction of HMG-CoA to mevalonate [5].

There is a correlation between unbalanced diet and hypercholesterolaemia. Adaptive changes in the body under the influence of a high fat load, including beef lard and cholesterol (19 and 2% of the total diet, respectively), are accompanied by increased immune and redox processes, activation of the inflammatory response, increased triglycerides, LDL in the blood, activation of lipid peroxidation, etc. [6]. Thus, an unbalanced diet leads to disorders in lipid and cholesterol metabolism, but the mechanisms of these disorders with simultaneous changes in the acid-base state deserve further study.

The aim of the study was to investigate changes in cholesterol metabolism and lipid spectrum, as well as the activity of glucose-6-phosphate dehydrogenase, NADP-dependent malate dehydrogenase, NADP-dependent isocitrate dehydrogenase, in rat tissues under acid-base disorders – metabolic acidosis and alkalosis, which were modelled by unbalanced diet: high-protein and carbohydrate (sucrose) diets.

Materials and methods of the study

White Wistar rats of at least 30 days of age (body weight – 100–130 g) were used in the experiment for 60 days. Three groups of animals were separated: a control group, which was kept on a standard vivarium diet, and two experimental groups, which received diets with excessive ammonium chloride and sucrose. Metabolic acidosis was modelled by keeping white rats on a diet with an excess of ammonium chloride at the rate of 4 mg/g body weight [2]. Metabolic alkalosis was determined in the blood of rats when the experimental animals were kept on a diet with an excess of sucrose according to S.A. Nikitin and M.T. Bugaeva [4]. At the end of the experiment, blood was taken to study the acid-base balance and determine the content of lipoprotein cholesterol by conventional methods, animals were decapitated and tissues were taken for biochemical studies: heart and thigh muscle, liver. The acid-base status was determined in the blood of animals taken under vaseline oil using a microanalyser from Radelkis (Hungary). Indicators of acid-base balance were determined by nomograms.

In the tissue homogenates taken for the study, the activity of glucose-6-phosphate dehydrogenase [7], NADP-dependent malate dehydrogenase and NADP-dependent isocitrate dehydrogenase [8], and lipase activity [9] were determined spectrophotometrically by the rate of decrease or increase in the corresponding coefficients.

The lipid spectrum of tissues was analysed by thin-layer chromatography (TLC) on 10×5 plates (Silufol, Czech Republic) [10]. Lipids were extracted from 1.0 cm^3 of tissue homogenate, with the addition of 2.5 cm^3 of ethanol and 5.0 cm^3 of hexane, after shaking, the hexane layer was removed to another tube and the hexane was evaporated. The dry residue was dissolved in 0.1 cm^3 chloroform and applied to 10.0 µl chromatography plates according to the scheme. Lipid fractionation was carried out in the system "hexane: diethyl ether: ice-cold acetic acid" (35:15:2) by double elution. The plates were sprayed with a 5% alcohol solution of phosphorus-molybdenum acid, then placed at 100–200 °C for 5–7 min with the appearance of dark blue lipid spots on a green-yellow background [10]. The obtained chromatograms were scanned on a densitometer and the content was calculated using a computer program in comparison with the corresponding witnesses. The experimental material was processed using Microsoft Excel statistical software packages.

All studies were carried out within the framework of research work, state registration number 0116U008927, 2017–2019; the work was performed at the Department of Human Anatomy of ONMedU in compliance with the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" of 18.03.1986, as amended on 02.12.2005; "General Ethical Principles for Experiments on Animals", adopted by the 5th National Congress on Bioethics (Kyiv, 2013).

Research results and their discussion

Table 1 shows that an excess of ammonium in the diet of rats leads to a decrease in blood pH and a significant sharp decrease in the level of carbon dioxide and bicarbonate in the blood of rats, which is diagnosed as a partially compensated metabolic acidosis.

Consumption of excessive sucrose by rats leads to opposite changes in the acid-base balance of blood. Thus, the content of carbon dioxide in the blood of animals increases significantly, the pH value of the blood shifts to the upper limit of the norm. An increase in pH, carbon dioxide and bicarbonate content in rats is observed only during the first week of the experiment, and then these indicators decrease, which characterises the development of secondary metabolic acidosis, which masks the primary triggering mechanisms of acid-base homeostasis disorders. According to the literature, ammonium ions reduce the functioning of the Krebs cycle, resulting in the accumulation of underoxidation products, which causes the development of acidosis in tissues [2].

The shift in acid-base balance in an unbalanced diet reflects the oxygen content in the blood. An excess of

Table 1

Indicators of acid-base balance in the blood of rats in the modelling of metabolic acidosis and alkalosis (n=30, M±m)

The studied groups	pH	pCO_2	HCO_3^-	pO_2	Diagnosis
Control group	7.31±0.02	37.4±1.38	18.2±0.61	37.7±1.59	Normal, limit of normal
Diet with ammonium chloride	7.25±0.040	24.0±1.89*	10.9±0.69*	20.5±0.80*	Partially compensated metabolic acidosis
Diet with sucrose	7.44±0.03*	76.1±7.99*	48.6±4.88*	52.0±9.60	Partially compensated metabolic alkalosis

Note: * – indicates significant differences compared to the control group (p≤0.05–0.001).

ammonium in the diet causes a decrease, and sucrose increases the oxygen content in the blood (Table 1). This fact is explained by an increase in the ability of haemoglobin to bind oxygen with a sufficient content of carbon dioxide in the blood [11].

Thus, the intake of excessive ammonium or sucrose in the body of rats leads to shifts in the acid-base balance of an alternative nature.

A significant increase in the level of total cholesterol and LDL cholesterol was found in the blood plasma of experimental animals both under the influence of a diet with an excess of ammonium and sucrose diet (Table 2).

An increase in triglyceride content was observed under the influence of the sucrose diet, while under the influence of ammonium excess diet the triglyceride content decreased. The decrease in HDL cholesterol content in the blood of rats under the influence of both diets may indicate that unsaturated phospholipid chains were oxidised in the processes of lipid peroxidation.

Consumption of diets with a high carbohydrate content leads to a shift in the concentration of hydrogen ions in the body of experimental animals, with an increase in respiration and CO_2 release, which can lead to the development of respiratory alkalosis [12]. Under the influence of a diet with an excess of sucrose, an increase in total lipids, cholesterol and triglycerides, and insulin secretion in the blood was observed in the early period [13].

In all the tissues of rats studied, both those on the diet with excess ammonium and those on the sucrose diet, an increase in free cholesterol and a decrease in phospholipids were observed (Table 3), with greater changes in these parameters observed under the influence of the sucrose diet. This direction of metabolic processes was reflected by a decrease in the phospholipids / free cholesterol ratio.

The content of triglycerides decreased in all studied tissues of rats with a diet with excess ammonium, while under the influence of sucrose diet it increased in the tissues of the heart muscle and liver (Table 3). At the same time, free fatty acids (FFAs) accumulated in all studied tissues of rats with an ammonium-excess diet, while the sucrose diet contributed to the accumulation of FFAs in the heart muscle and liver tissues (Table 3). It is known that lipolysis is also activated during muscle work and fasting, which is accompanied by an increase in the concentration of FFAs in the blood, which in this situation act as a source of energy [5].

The multilevel control of cellular metabolism, which ensures the maintenance of homeostasis in changing environmental conditions, includes as one of the main factors of regulation the changes in the so-called redox state of nicotinamide coenzymes, which can be judged by the ratio of oxidised and reduced metabolites. An increase in this ratio activates IDH, MDH, and other enzymes of the Krebs cycle, and shuttle mechanisms for the transport of reducing equivalents from mitochondria to the cytoplasm. A high rate of glucose oxidation and fatty acid synthesis is achieved at high values of the $NADPH/NADP^+$ ratio [2; 5].

In heart muscle tissue (Figure 1), under the influence of an ammonium diet and the development of metabolic acidosis, the activity of H6PD, one of the regulators of which is the hormone insulin, decreases. An increase in the activity of H6PD in the heart muscle during sucrose dieting indicates an increase in the oxidation of the environment and an increase in insulin activity.

The activity of G6PD did not change in the thigh muscle tissue. In the liver tissues of rats with a diet with an excess of ammonium chloride (Figures 2, 3), a slight activation of G6PD was observed with the development of metabolic acidosis in the body. The sucrose diet, unlike the ammonium diet, did not change the activity of G6PD (Figures 2, 3) in the liver.

Changes in the activity of NADP-dependent isocitrate dehydrogenase (IDH) under the influence of unbalanced diets are complex (Figures 1–3). Under the influence of a diet with ammonium chloride, the development of

Table 2

Serum lipoprotein cholesterol and total cholesterol content in rats with modelling of metabolic acidosis and alkalosis (n=30, mmol/l, M±m)

The studied groups	HDL cholesterol	LDL cholesterol	Total cholesterol	Triglycerides
Control group	0.79±0.081	0.902±0.279	1.654±0.108	1.06±0.076
Diet with ammonium chloride	0.528±0.0498*	2.667±0.142*	3.87±0.51*	0.65±0.098*
Diet with sucrose	0.584±0.012*	3.68±0.397*	4.885±0.275*	3.79±0.24*

Note: * – indicates significant differences compared to the control group (p≤0.05–0.001).

Table 3

Lipid content in rat tissues in the modelling of metabolic acidosis and alkalosis (n=30, μmol/g tissue)

The studied groups	Phospholipids	Free cholesterol	Phospholipids / free cholesterol	Free fatty acids	Triglycerides
Thigh muscle					
Control group	1.90±0.09	1.62±0.06	1.17±0.07	0.91±0.02	0.96±0.02
Diet with ammonium chloride	0.65±0.03*	3.64±0.22*	0.2±0.02*	3.34±0.13*	0.03±0.015*
Diet with sucrose	0.54±0.03*	5.33±0.15*	0.10±0.013*	0.67±0.02	1.04±0.03
Cardiac muscle					
Control group	28.19±0.89	3.25±0.13	8.90±0.44	9.25±0.24	4.62±0.21
Diet with ammonium chloride	16.25±0.69*	7.07±0.11*	2.31±0.10*	19.03±0.36*	0.51±0.02*
Diet with sucrose	9.36±0.19*	10.17±0.22*	0.93±0.03*	16.52±0.27*	20.28±0.27*
Liver					
Control group	33.54±1.18	8.42±0.14	3.99±0.16	9.20±0.15	6.32±0.2
Diet with ammonium chloride	18.25±0.16*	16.21±0.33*	1.13±0.02*	26.29±0.29*	0.65±0.02*
Diet with sucrose	7.28±0.15*	25.62±0.49*	0.29±0.01*	14.24±0.32*	24.16±0.26*

Note: * – indicates significant differences compared to the control group (p≤0.05–0.001)

Fig. 1. Activity of TCA and PPP enzymes in rat heart muscle under unbalanced nutrition

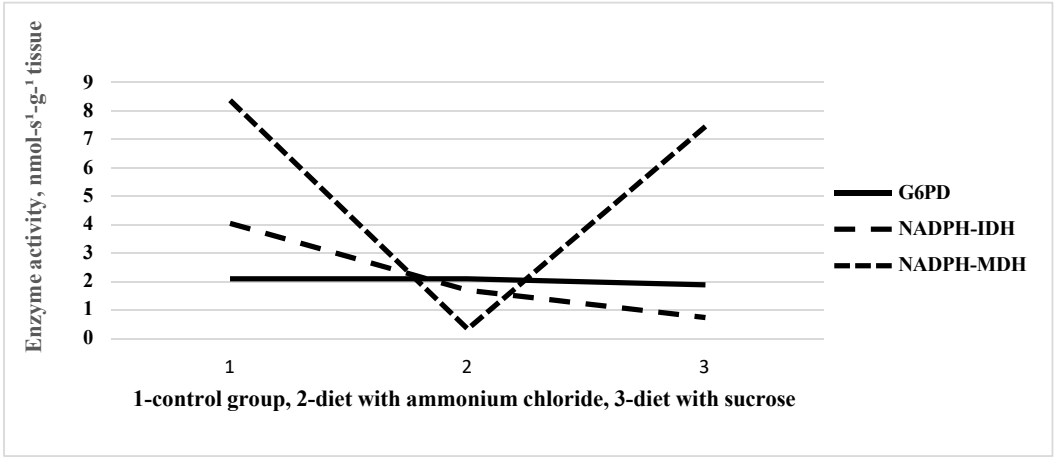

Fig. 2. Activity of TCA and PPP enzymes in the thigh muscle of rats with an unbalanced diet

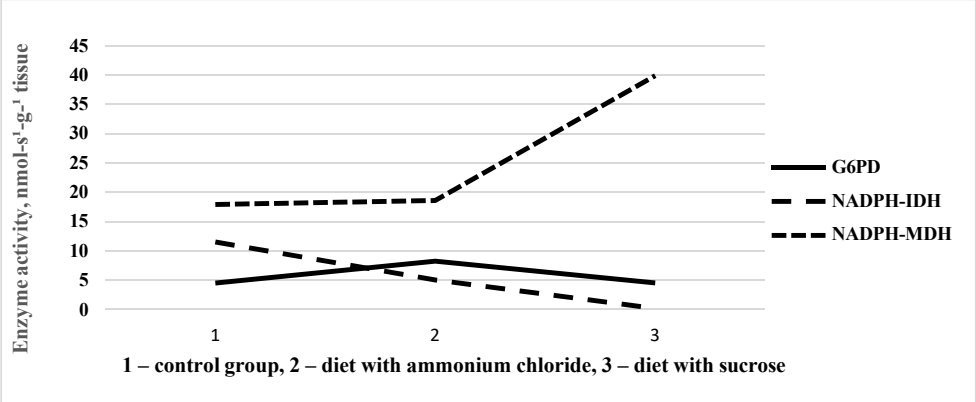

Fig. 3. Activity of TCA and PPP enzymes in the liver of in the liver of rats with an unbalanced diet

metabolic acidosis and the accumulation of reduced equivalents (Table 1), the activity of NADP-dependent IDH in heart and thigh muscle tissues decreased, but in liver tissues its activity did not differ from control values. Under the influence of sucrose diet, there was also a decrease in the activity of NADP-dependent IDH in the tissues of the thigh muscle and liver, but it did not differ from normal values in the tissues of the heart muscle (Figures 1–3).

There is evidence that during calorie restriction in a mouse experiment, deacetylase activates mitochondrial forms of NADP-dependent IDH, thereby regulating the glutathione antioxidant defense system, and high concentrations of NADPH inhibit the catalytically active forms of this enzyme [14].

The activity of NADP-dependent malate dehydrogenase (MDH) under the influence of a sucrose diet significantly increases in liver and heart muscle tissues (Figures 1, 2). Changes in MDH activity may be associated with the development of a state of metabolic alkalosis or respiratory acidosis, which are accompanied by a higher level of carbon dioxide in the tissues, which once again confirms the primary alkalosis in a high-carbohydrate diet. Under the influence of a diet with ammonium chloride, the activity of NADP-dependent MDH decreased in the tissues of the thigh and heart muscle (Figures 1, 2).

Figure 4 shows the lipase activity in the tissues of rats fed diets with ammonium chloride and sucrose. Activation of glycolysis and accumulation of oxidized metabolites under the influence of the sucrose diet reduces lipase activity, i.e. inhibits lipolysis and activates NADP-dependent MDH in heart and thigh muscle tissues (Figures 1, 3), indicating an active course of lipogenesis.

It is known that the maximum rate of lipogenesis is observed during the sucrose diet. The main source of NADPH in the cytoplasm, which provides lipogenesis reactions, is G6PD and NADP-dependent MDH. Their activity is strictly correlated with the rate of lipogenesis [5]. The development of metabolic acidosis on an ammonium chloride diet was accompanied by an increase in lipase activity in all studied rat tissues and a decrease in NADP-dependent MDH activity in the tissues of the heart and thigh muscle, indicating activation of lipolysis and accumulation of hydrogen ions.

Despite the fact that NADPH is also formed during the oxidation of malate to pyruvate and carbon dioxide (with the participation of NADP-dependent MDH) and

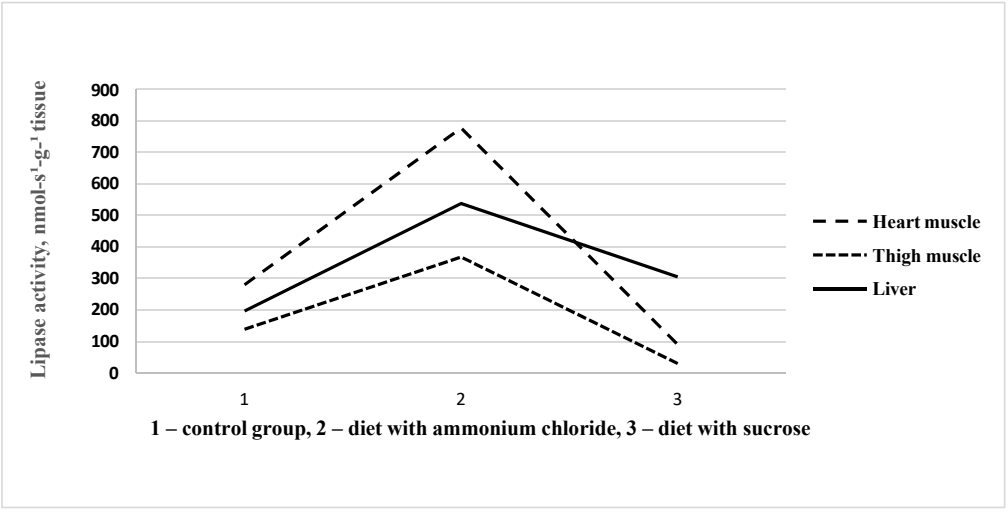

Fig. 4. Lipase activity in rat tissues under unbalanced nutrition

dehydration of isocitrate (with the participation of NADP-dependent IDH), in most cases, the needs of cells are met by the pentose phosphate pathway (PPP).

Glucose-6-phosphate dehydrogenase, a key enzyme of the PPP, is under complex regulatory control and is central to many cellular processes, as it is the main source of NADPH synthesis, including glutathione reduction, antioxidant pathways, nitric oxide synthase, NADPH oxidase, cytochrome p450 system, etc. The activity of G6PD is regulated by many signals that affect transcription, intracellular location and interaction with other proteins; its deficiency reduces the cell's energy reserves and leads to the development of haemolytic anaemia, and dysregulation is associated with cancer, autoimmune diseases and oxidative stress [15].

It is known that the activity of G6PD increases with the development of oxidative stress in rats [15]. G6PD activity is increased in the liver and adipose tissue due to a carbohydrate-rich diet, hyperinsulinaemia, glutathione redox, and NADPH. Deficiency of G6PD in mice on a diet that promotes obesity reduces weight gain and hyperinsulinaemia [16]. Studies of the effect of diets or pharmacotherapeutic interventions to reduce G6PD activity and, consequently, weight gain and obesity have yielded mixed results that require further research [17].

The studies have shown that an increase in total cholesterol, LDL cholesterol in rat serum and free cholesterol in tissues indicate changes in the homeostasis of the body of experimental animals and a tendency to develop atherosclerotic processes in the presence of metabolic acidosis and alkalosis. The increase in the content of these compounds in the development of metabolic alkalosis and increased lipogenesis, decreased lipase activity and activation of TCA and PPP enzymes is undoubtedly confirmed by the activation of cholesterol biosynthesis. However, the increase in LDL cholesterol and free cholesterol in rat liver tissues is probably due to the further development of metabolic acidosis. Lipolysis produces an excess of FFAs, the content of which was increased in all studied rat tissues with metabolic acidosis (Table 3), and then they are transferred to the liver from the tissues and transformed into acetyl-CoA, part of which could be used for cholesterol biosynthesis.

It is known that the aging process is closely linked to lipid metabolism disorders and impaired fatty acid utilization [1, 18], which is greatly contributed to by inactivity and unhealthy diet. Fatty acids entering the cell disrupt the oxidation processes in the mitochondria, which triggers several negative chain reactions associated with age- related diseases and aging in general.

Conclusions

1. Our studies convincingly show that excessive consumption of refined sugar and ammonium chloride with food, as models of high-carbohydrate nutrition and a diet with excessive protein, cause disturbances in body homeostasis with alternative changes in the acid base state and lead to the development of metabolic acidosis in the body due to the maintenance of animals on a diet with ammonium chloride and metabolic alkalosis when animals are kept on a sucrose diet.

2. The development of partially compensated states of metabolic acidosis and alkalosis in experimental animals leads to unidirectional changes in the content of both total and LDL cholesterol and free tissue cholesterol, but with varying degrees of severity and, consequently, the risk of atherosclerosis.

3. The decrease in phospholipid content and the phospholipid / free cholesterol ratio in all studied rat tissues probably indicates a greater intensification of lipid peroxidation under the influence of sucrose diet than ammonium chloride diet. The latter diet activated lipolysis, triglyceride oxidation and accumulation of FFAs, while the sucrose diet, on the contrary, promoted lipogenesis, biosynthesis of triglycerides and free fatty acids and their increase in heart and liver tissues. Imbalances in the content of cholesterol, phospholipids and triglycerides, which are components of cell membranes, affect the structure of vascular endothelial membranes.

4. In metabolic alkalosis, there was an increase in the activity of NADP-dependent MDH in the heart muscle and liver and G6PD in the heart muscle and, accordingly, a more pronounced regeneration of $NADP^+$, and in partially compensated metabolic acidosis, in contrast to the state of metabolic alkalosis, an increase in the activity of the key enzyme of the pentose phosphate pathway – G6PD in the liver. The activity of NADP-dependent IDH decreased in all tissues under metabolic acidosis, while NADP-dependent MDH decreased in thigh and cardiac muscle tissues and did not change in the liver. It can be assumed that different organs respond differently to metabolic acidosis or alkalosis.

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Електронна адреса для листування olena.paxomova@onmedu.edu.ua