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Original Article

Effect of vanadium and chromium citrates on lipid composition in the blood of rats with experimental diabetes

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Abstract

In clinical endocrinology, diabetes is one of the most important pathologies due to the high, ever-increasing prevalence and frequent development of complications, including dyslipidemia. The aim of the research was to investigate the effect of organic compounds of vanadium and chromium citrates at the dose of 0.5 μg/ml and 0.1 μg/ml on the metabolism of lipids in the blood of rats with alloxan-induced diabetes. The total amount of lipids was determined by weighing dry using a residue gravimetric method. The separation of lipids into classes was carried out by thin-layer chromatography. Lipid profile showed that non-esterified cholesterol, phosphatidylserine, and lysophosphatidylcholine increased, and esterified cholesterol and phosphatidylethanolamine decreased in diabetic rats. The content of phospholipid, non-esterified cholesterol and triacylglycerol decreased. However, the content of monoacylglycerols and diacylglycerols increased in the blood of diabetic rats with a daily diet of vanadium and chromium citrates at the dose of 0.5 μg/ml and 0.1 μg/ml. Changes in phospholipid content were also noted. Our results indicate the normalization of lipid metabolism with the additional introduction of vanadium and chromium citrates to the diet of animals with hyperglycemia. This may indicate that the present compounds may be the progression of diabetes and the risk of deterioration.

Keywords: diabetes, lipids, vanadium citrate, chromium citrate, oxidative stress.

Introduction

Diabetes mellitus is a metabolic disorder usually described as persistent hyperglycemia due to defects in pancreatic β-cell insulin production and decreased insulin sensitivity [1]. The disease is associated with disrupting the body's defense systems, including immune and antioxidant defenses, and developing oxidative stress. Under conditions of intensifying free radical formation and insufficiency of the antioxidant system, membranes become targets of highly reactive oxygen species, and the objects of disorganization are lipids. Changes in lipid metabolism and lipoprotein composition due to insulin resistance lead to the formation of low-density lipoprotein cholesterol, which is atherogenic. This can accelerate the development of atherosclerosis and the development of cardiovascular complications in the body on the background of diabetes.

Trace elements present in the body in small quantities are necessary to perform specific functions. They most often function as important cofactors of enzymes and thus help maintain the basic metabolic reactions (glycolysis, citric acid cycle, lipid, and amino acid metabolism) needed to support energy production and life. It should be noted that even a moderate deficiency of trace elements can lead to serious illnesses, including diabetes [2].

This paper pays attention to the elements of Vanadium and Chromium, which are involved in complex biochemical processes associated with insulin concentration, resistance and hyperglycemia in diabetes. From the 1980s, vanadium and its compounds began

to be studied as new drugs in treating diabetes [3]. This element can change the redox potential in a living organism and can have a positive or negative effect on overall antioxidant protection. The mechanism is very variable and depends on the degree of oxidation, dose, type of ligands, the presence of vitamin C, tocopherol and others. In particular, vanadium may reduce hyperglycemia, inhibit glucose-6-phosphatase and improve insulin action by increasing the activity of glucose transporters [4]. There is also evidence to suggest that vanadium in diabetic rats reduces insulin requirements, cholesterol and triglyceride levels [5].

Chromium is an important element for optimal insulin activity. It is necessary for the normal functioning of carbohydrate and lipid metabolism [6]. Chromium (III) is considered a biologically active form necessary for insulin functioning [7]. Chromium increases the binding of insulin to cells due to the activation of insulin receptor kinase and inhibition of phosphotyrosine phosphatase activity [8]. This leads to increased phosphorylation of the insulin receptor and increased sensitivity to the hormone.

Therefore, the study of micronutrients, namely compounds of vanadium and chromium as potential prophylactic and therapeutic agents for diabetes, namely their normalizing effect on the lipid profile, is relevant today.

Material and methods

Apparatus and chemicals used

Our research used vanadium and chromium citrates, which were obtained based on «Nanomaterials and Nanotechnologies» (Ukraine, Kyiv). A method for producing metal compounds with organic acids (particularly citric acid) using aquanatotechnology as proposed; this method is an alternative to the chemical method [9].

Experimental animals and design of experiment

The research was conducted on 40 white laboratory male rats weighing 100–120 g. During the experiment, the animals were kept under standard vivarium conditions at the Institute of Animal Biology of the National Academy of Sciences, maintaining a nutritional and drinking regime at the level recommended by the standards for keeping laboratory animals. The experiment was conducted according to the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes", European Treaty Series – No. 123 (Strasbourg, 1985) and "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001). The protocol of the Bioethics Committee meeting of the Institute of Animal Biology No. 76 was dated by November 6, 2018. The duration of the experimental period was 40 days.

The rats were divided into four groups of eight rats each for investigation:

- Group I (normal control). Rats drank pure water without additives.
- Group II. Rats were consuming a combined solution of vanadium and chromium citrates at the dose of 0.5 μg/ml and 0.1 μg/ml water as an addition to drinking water (the daily dose of water is 20 ml for each rat).
- Group III (diabetic control). Diabetes-induced rats drank pure water without additives.
- Group IV. Diabetes-induced rats were consuming a combined solution of vanadium and chromium citrates at the dose of 0.5 μg/ml and 0.1 μg/ml water in addition to drinking water (the daily dose of water is 20 ml for each rat).

Experimental diabetes

On the 31st day of the study, animals were induced with experimental diabetes mellitus (EDM) by a single intraperitoneal administration of a 5% solution of alloxan monohydrate in the amount of 150 mg/kg of body weight of the animal (as solvent 0.9% NaCl). The animals were given no food during the last 24 hours. Control rats were injected intraperitoneally with 0.9% NaCl.

Collection of samples

Hyperglycemia was detected by measuring glucose blood collected from the tail vein using a portable glucometer ("Gamma-M", UK) on 1, 15, and 30 days and after induced experimental diabetes on 32, 36, and 40 days of experimentation. Animals with a glucose concentration greater than 11.1 mmol/L were selected for the experiment and this indicator was accepted as a successful induction of diabetes mellitus. On the 40th day of the study, we withdrew the animals from the experiment by decapitation after intravenous administration of 2–2.5% sodium thiopental solution for anesthesia. The material for the research was animal blood (heparin was used as an anticoagulant).

Obtaining common lipids

Blood plasma (1 cm^3) was extracted with the chloroform-methanol mixture in the ratio of 2:1 (v/v) according to the Folch method [10]. A 0.74 M of KCl solution was added to clean the lipid extract. The total amount of lipids was determined by weighing dry using a residue gravimetric method [11].

Separation of lipids into classes

The separation of lipids into classes was carried out by thin-layer chromatography on silica gel (silica gel L 5/40 μ, LSL 5/40 μ, Chemapol, Slovakia) as a mobile phase hexane-diethyl ether–acetic acid in a ratio of 70:30:1 (v/v/v) was used [11]. Plates were obtained using the vapors of crystalline iodine. Identification of individual lipids was carried out by Rf values [12]. The developed plates were scanned (HP Scanjet G2710, China). Quantitative analysis and counting of the contents of the lipid classes were performed by computer processing of programs using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool.

Separation of phospholipids

To separate the phospholipids by a thin-layer chromatography method on a silica gel, a solvent system of chloroform-methanol-water in a ratio of 65:25:4 (v/v/v) was used [12]. Crystalline iodine vapors were used as a developer. The identification of individual phospholipids was carried out by Rf values [12]. The obtained plates were scanned. Quantitative analysis and counting of the individual lipids content were performed by computer processing the foregrams using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool. The blood plasma of rats was examined to identify the content of the phosphatidic acid, cardiolipin, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, phosphatidylserine, sphingomyelin, and lysophosphatidylcholine in rats' blood plasma.

Statistical analysis

Digital material obtained during the research was processed with the method of variational statistics using ANOVA. Average arithmetic values (x) and standard error (±SE) were calculated. Changes were considered plausible at $p \leq 0.05$.

Results

Alloxan is used to induce experimental diabetes due to selective damage of the β-cells of the Langerhans of the pancreas producing insulin. Changes in insulin concentration in blood plasma lead to changes in blood glucose levels. In animals from control groups, glucose levels fluctuated during the experiment in the 6.1–7.2 mmol/L range.

In diabetic animals of group III, glucose levels increased significantly by 107.4% on the 32nd day, by 143.5% on the 36th day, and by 175.3% on the 40th day relative to control group I. Also, in the blood of rats of group IV, glucose levels increased by 90.9% on the 32nd day, by 106.5% on the 36th day and by 124.9% on the 40th day compared to the control group ($p<0.05$). However, there was a decrease in blood glucose of animals of group IV relative to group III by 15.2% on the 36th day of the research and by 18.3% on the 40th day ($p<0.05$) (Figure 1).

At the beginning of the research, all animals from the research groups showed a slight difference in their body weight: 116.3±1.2 (group I), 117.4±1.1 (group II), 113.8±1.0 (group III) and 117.1±1.5 (group IV) grams. The body weight of intact animals of group II under the combined influence of vanadium and chromium citrates was slightly higher than control. Bodyweight decreased by 19.8% in group III rats due to EDM induction compared to control group I ($p<0.05$). On the 40th day, the weight of the animals in group IV increased by 9.2% compared to group III ($p<0.05$) and decreased by 12.4% compared to the control ($p<0.05$) (Figure 2).

The combined effect of vanadium and chromium citrates led to a slight decrease in total lipids in groups II and IV animals. The content of total lipids in the blood increased during EDM in animals of group III, which can be explained by the mobilization of lipids from the depot (Table 1).

The content of phospholipids in the blood plasma of intact animals of group II decreased by 18.5% compared to that of group I ($p<0.05$). During EDM in animals of group III, there was a tendency to increase the content of phospholipids in the blood. The content of phospholipids decreased by 5.6% under the influence of vanadium and chromium citrates in diabetic rats of group IV compared to animals of group III ($p<0.05$).

We noted a significant increase in the content of non-esterified cholesterol by 45.2% in animals of group III with EDM relative to the control. Under the influence of vanadium and chromium citrates in group IV diabetic rats, the cholesterol content probably decreased by 24.1% compared to group III.

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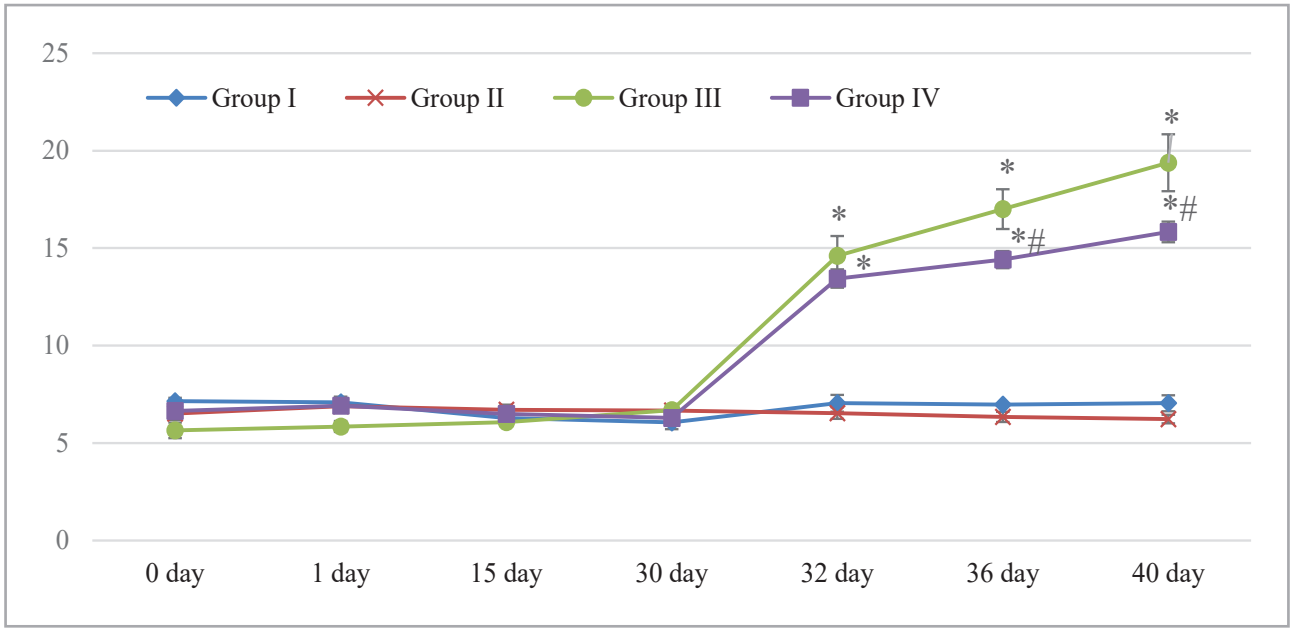

Figure 1: Blood glucose dynamics in rats of the control group, diabetic group, and under the effect of vanadium and chromium citrates (mmol/L). Note: Each value represents the Mean±SEM of the n=8 reading. Probability of indexes of II, III, and IV groups relative to the group I * – $p<0.05$, probability of indexes of IV group relative to III group # – $p<0.05$.

Mono- and diacylglycerols are mediators of the synthesis of triacylglycerols and phospholipids. Compared to the control group, their content significantly increased by 31.6% in the combined effect of vanadium and chromium citrates in group II animals ($p<0.05$). Under the influence of diabetes, this indicator tended to decrease. During the previous feeding of vanadium and chromium citrates, the indicator significantly increased by 103.1% in animals of group IV compared to the indicator in group III.

The relative content of non-esterified fatty acids probably did not change in the blood of experimental animals. The content of triacylglycerols decreased by 12.7 and 26.5% in animals of groups II and IV relative to animals of control group I ($p<0.05$). Also, the content of triacylglycerols decreased by 31.5% in the blood of ani-

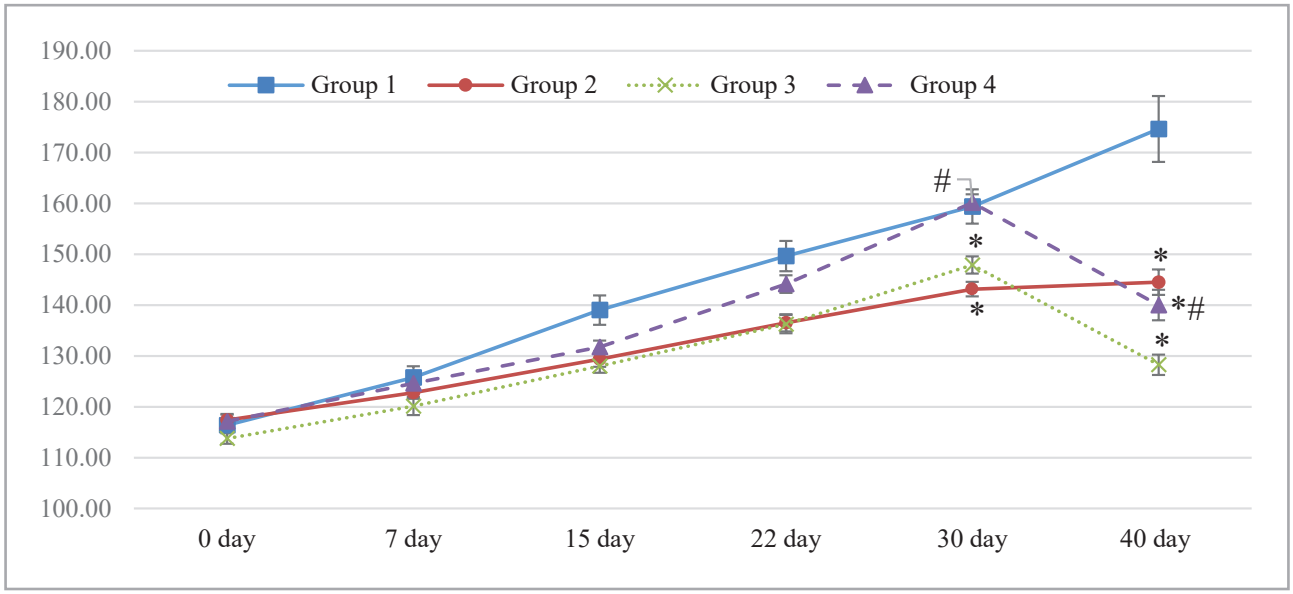

Figure 2: Bodyweight dynamics in rats of the control group, diabetic group, and under the effect of vanadium and chromium citrates (grams). Note: Each value represents the Mean±SEM of the n=8 reading. Probability of indexes of II, III, and IV groups relative to the group I * – $p<0.05$, probability of indexes of IV group relative to III group # – $p<0.05$

Table 1: Lipid profile in the blood plasma of rats with EDM under the effect of vanadium and chromium citrates.

Indicators	Animals groups			
	I	II	III	IV
Total lipids, g/L	7.8±0.3	7.4±0.4	8.2±0.5	7.4±0.4
Phospholipids, %	33.3±1.0	27.1±1.8*	35.1±0.6	33.1±0.4#
Non-esterified cholesterol, %	8.4±0.4	8.6±0.1	12.2±0.8*	9.3±0.7#
Mono- and diacylglycerols, %	6.8±0.9	9.0±0.7*	5.5±0.3	11.1±0.9*#
Non-esterified fatty acids, %	9.9±0.9	7.4±1.1	11.1±0.5	9.9±0.9
Triacylglycerol, %	19.1±0.7	16.7±0.8*	20.5±1.1	14.1±0.7*#
Esterified cholesterol, %	23.6±0.4	25.9±0.4*	20.8±0.6*	22.4±0.8

Note: Each value represents the Mean±SEM of the n=8 reading. Probability of indexes of II, III, and IV groups relative to the group I * – $p<0.05$, probability of indexes of IV group relative to III group # – $p<0.05$.

mals of group IV relative to animals of group III with EDM ($p<0.05$).

The esterified cholesterol content increased by 9.7% in the blood of intact animals of group II ($p<0.05$). This indicator decreased by 12.1% in diabetic rats of group III compared to the control group ($p<0.05$). In animals of group IV, this indicator increased slightly compared to group III.

Studying the relative content of phospholipids, we noted that the content of phosphatidic acid probably did not change in the experimental groups (Table 2).

In group IV animals, cardiolipin content significantly increased by 32.9% compared to group I and 68.9% compared to group III. The phosphatidylethanolamine content significantly decreased by 29.7% in the blood of animals of group III with EDM compared with animals of group I control. An increase in this indicator was observed in animals of group IV by 36.9%, compared with animals of group III ($p<0.05$).

The phosphatidylcholine content significantly decreased in animals of groups II and IV by 15.3 and 12.7%, respectively, compared with animals of group I. In addition, this indicator decreased by 15.8% in animals of group IV compared to animals of group III with EDM ($p<0.05$). The phosphatidylcholine content decreased in groups II and IV due to indirect inhibition of chromium and vanadium citrates of choline kinase activity.

An increase in phosphatidylserine content was observed in the blood of animals of groups II and III, by 74.3 and 96.7%, relative to animals of control group I ($p<0.05$). The content of lysophosphatidylcholine significantly increased by 24.8% in animals of group III with EDM compared to animals of control group I ($p<0.05$).

Table 2: Phospholipids profile in the blood plasma of rats with EDM under the effect of vanadium and chromium citrates.

Indicators	Animals groups			
	I	II	III	IV
Phosphatidic acids, %	9.7±0.3	11.2±0.8	10.4±0.8	11.1±0.6
Cardiolipin, %	4.7±0.4	4.1±0.4	3.7±0.5	6.2±0.3*#
Phosphatidylethanolamine, %	20.9±1.1	22.0±1.0	14.7±0.8*	20.1±0.9#
Phosphatidylinositol, %	8.3±0.4	8.1±0.6	6.6±0.8	6.5±0.8
Phosphatidylcholine, %	39.1±1.0	33.2±0.8*	40.6±0.8	34.2±1.5*#
Phosphatidylserine, %	3.9±1.0	6.9±0.8*	7.7±0.8*	5.8±0.5
Sphingomyelin, %	6.0±0.6	5.4±0.4	7.2±0.3	7.3±0.3
Lysophosphatidylcholine, %	7.3±0.6	9.3±0.5	9.2±0.4*	8.9±0.5

Note: Each value represents the Mean±SEM of the n=8 reading. Probability of indexes of II, III, and IV groups relative to the group I * – $p<0.05$, probability of indexes of IV group relative to III group # – $p<0.05$.

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Discussion

Diabetes is reaching alarming proportions around the world, and despite many years of studying the development of the disease and the use of new modern treatments, the disease continues to progress. Reproduction of the model of diabetes allows us to obtain valuable information not only for understanding the pathophysiology and biochemistry of the disease but also the mechanism of antidiabetic action of various drugs to target their use.

In this research, the alloxan model of diabetes was used. Administration of alloxan in diabetogenic doses to animals causes a maximum increase in blood glucose after 2–4 hours, with subsequent hypoglycemia within a day or two. In the future, if the experimental animal does not die, it develops persistent hyperglycemia, which indicates the development of diabetes [13]. Apparently, alloxan, which can accumulate in pancreatic β-cells and cause their death, led to a decrease in insulin secretion and to the stage of chronic persistent hyperglycemia.

We noted a lower glucose concentration level under the influence of vanadium and chromium citrates in the blood of animals of group IV with EDM compared with animals of group III. This indicates a positive combined effect of Vanadium and Chromium compounds on the blood glucose concentration of diabetic rats. There are literature data that the model of type 1 diabetes mellitus under the action of vanadium improves the functioning of signaling pathways of insulin [14]. The combination of Chromium and Vanadium together gives a significant additive effect on insulin-stimulated glucose uptake into the cell [15].

There was a change in body weight due to impaired energy metabolism in diabetes. Studies have shown a decrease in the body weight of rats by EDM. This is consistent with studies by other authors [16]. This reduction of body weight can be due to the breakdown or degradation of structural proteins and fats due to insufficient use of carbohydrates as a source of energy for diabetes. Intact animals in group II experienced partial weight loss due to vanadium and chromium citrates relative to control. Other researchers have found a decrease in body weight under the action of Vanadium compounds [17], which suggests their possible use as a therapeutic agent in obesity. The action of vanadium may be associated with changes in the level of hypothalamic neuropeptide Y, which is inhibited by insulin and is known to be associated with the regulation of appetite and increased leptin secretion in adipose tissue.

Lipids play an important role in the pathogenesis of diabetes in cardiovascular disease. Lipids are major cell membrane components involved in energy storage, signal transduction across cell membranes, cell growth, and apoptosis. Fat mobilization from the depot increases with diabetes; fatty acids are transported to the liver, which is oxidized and partially provides cells with energy. Excess fatty acids are used to synthesize ketone bodies, fats, phospholipids, and cholesterol. Synthesized fats, phospholipids, and cholesterol are excreted in the blood as low-density lipoproteins or very low-density lipoproteins, which causes hyperlipoproteinemia and can lead to atherosclerosis [18]. Therefore, the level of total lipids in the blood of rats with EDM compared to the control group.

Phospholipids are the main lipid components of the biological membrane. The content of phospholipids is constantly updated in the membrane of a mature erythrocyte during blood circulation. Erythrocytes cannot perform their biosynthesis *de novo* but regulate their composition by passively exchanging intact phospholipids with plasma lipoproteins [19]. An alternative way to reduce phospholipids involves the acylation of fatty acids with either endogenous or exogenous lysophospholipids with their subsequent inclusion in the erythrocyte. It is also known that the intensity of synthesis of this class of lipids, and consequently their content in body tissues, can also be a kind of indicator of protection of body cells from penetration through their membrane of toxicants by sealing the membrane. It should be noted that the combined effect of vanadium and chromium citrates on intact animals led to a probable decrease in phospholipid content compared to the control.

An increase in plasma cholesterol and triacylglycerols caused the increased content of total lipids in rats with EDM. However, their content decreased under the influence of vanadium and chromium citrates in rats' EDM blood. Animal research suggests that Chromium compounds are associated with decreased triacylglycerol levels and lipid accumulation in the liver [20]. Chromium functions in lipid metabolism are associated with increased concentrations of high-density lipoproteins and decreased total cholesterol and low-density lipoproteins due to increased lipase activity. Chromium can increase the activity of lecithin-cholesterol acyltransferase and accelerate the esterification and excretion of cholesterol [21]. In addition, studies show that chromium activates the glucose transporter GLUT4 through a cholesterol-dependent mechanism that lowers cholesterol [22]. Vanadium compounds

modulate several key regulators of lipid metabolism by improving the expression of PPARγ (peroxisome proliferator-activated receptor) and the activation of AMPK (AMP-activated protein kinase) [23]. Vanadium also affects adiponectin expression, a protein that plays an important role in modulating glucose and lipid metabolism in insulin-sensitive tissues [23]. The decrease in the content of non-esterified cholesterol in the blood plasma of rats of group IV indicates an increase in the processes of esterification and hydrolysis of cholesterol in the body under the influence of biologically active substances, in this case, vanadium and chromium citrates.

Decreases in triacylglycerols observed in groups II and IV animals were confirmed by other authors, who found that vanadium compounds reduce high levels of triacylglycerols in serum and liver [24]. The same decrease was observed when using Chromium compounds as a supplement in people with diabetes, making its compounds relevant lipid-lowering agents [25].

Cardiolipin is a phospholipid localized exclusively in the inner mitochondrial membrane and plays an important role in maintaining the optimal mitochondrial function required for oxidative phosphorylation and ATP synthesis. Changes in cardiolipin are associated with various pathophysiological conditions, including diabetes, obesity, heart failure, hyperthyroidism, and aging. The content of cardiolipin decreased in the blood of diabetic rats as a result of oxidative stress and increased under the influence of vanadium and chromium citrates. This phospholipid is highly sensitive to oxidative damage by reactive oxygen species due to its high content of polyunsaturated fatty acids. Cardiolipin is involved in maintaining the respiratory chain's functioning in mitochondria and regulating mitochondrial functions, which are closely related to resistance to resistance.

Despite the small amount of phosphatidylinositol and its derivatives (10–20%), they are important intracellular secondary messengers and are involved in universal signaling systems in cells, including lipid distribution and metabolic processes [26]. The content of this phospholipid tended to decrease in the blood of rats by EDM.

Also, the phosphatidylethanolamine content decreased in the blood of rats with EDM. Phosphatidylcholine is synthesized from this phospholipid, which plays a role in developing insulin resistance. Its content increased in the blood of rats by EDM and normalized under the influence of vanadium and chromium citrates. Moreover, phosphatidylcholine is the most important of the phospholipids. It has a lipotropic regulatory effect and is a structural element of important components of the body (cell membranes, myelin sheaths). It can transfer excess cholesterol from tissues and blood to the liver and promotes its excretion from the body, accelerating redox processes.

Phosphatidylserine is a phospholipid most concentrated in organs with high metabolic activity, such as the brain, lungs, heart, liver, and skeletal muscle. It is located mainly in the inner layer of the cell membrane and has several unique regulatory and structural functions. First of all, phosphatidylserine modulates the activity of receptors, ion channels, enzymes, and signaling molecules and participates in regulating membrane fluidity [27]. Phosphatidylserine content increased in the blood of animals with EDM but decreased due to the flow of vanadium and chromium citrates. The reduction of phosphatidylinositol and phosphatidylserine levels, which are directly involved in activating atypical forms of protein kinase C and PI3K, contributes to restoring insulin sensitivity in the plasma of rats with EDM under the action of vanadium and chromium citrates.

Plasma sphingolipids are potential biomarkers of diabetes and metabolic syndrome. Normalization of sphingomyelin by vanadium and chromium citrates may result in decreased ceramide formation, which blocks the activation of the Akt signaling pathway and, as a result, helps to restore insulin signaling. For example, the plasma levels of ceramides in people with type 2 diabetes are elevated and negatively correlated with the rate of glucose release, indicating a relationship between ceramide levels and insulin resistance [28].

Conclusions

The results of our research showed that adding 0.5 μg/ml of vanadium citrate and 0.1 μg/ml of chromium citrate to the diet of rats with EDM was effective in adjusting blood glucose, body weight and individual lipid metabolism. Therefore, the research results may form the basis for developing new therapeutic approaches for the prevention and treatment of diabetes and dyslipidemia, which arise through the use of appropriate combinations and doses of compounds vanadium and chromium.

Conflict of interest

The authors declare no conflict of interest.

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