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Original Research Article

Effect of Vanadium Citrate on the Lipid Composition in the Blood Plasma of Rats with Experimental Diabetes

Olga Sushko[1*], Ruslana Iskra[2], Andriy Pylypets[3]
[1]PhD Student, Laboratory of Biochemistry Adaptation and Ontogenesis of Animals, Institute of Animal Biology NAAS, Vasylya Stusa Street, 38, L'viv, L'vivs'ka oblast, Ukraine, 79000
[2]Dr., Deputy Director of Research Dr. Sc., The Head of Laboratory of Biochemistry Adaptation and Ontogenesis of Animals, Institute of Animal Biology NAAS, Vasylya Stusa Street, 38, L'viv, L'vivs'ka oblast, Ukraine, 79000
[3]Senior Research Scientist, Candidate of Agricultural Science, Laboratory of Biochemistry Adaptation and Ontogenesis of Animals, Institute of Animal Biology NAAS, Vasylya Stusa Street, 38, L'viv, L'vivs'ka oblast, Ukraine, 79000

***Corresponding author:** Olga Sushko
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Abstract

We investigated the effect of vanadium citrate in the amounts of 0.125, 0.5 and 2.0 μg/ml of water on the indicators of lipid metabolism in the blood plasma of rats with alloxan induced diabetes. Blood plasma was extracted using chloroform-methanol mixture according to the Folch method. The total amount of lipids was determined by weighing the dry residue (gravimetric method). The division of lipids into classes was performed by thin-layer chromatography on silica gel. During the research, we found that the total amount of lipids, phospholipids, non-esterified cholesterol, triacylglycerols, non-esterified fatty acids, as well as the cholesterol-to phospholipid ratio increased in the blood plasma of rats with alloxan induced diabetes. However, the level of diacylglycerols and esterified cholesterol decreased. Given the consumption of vanadium citrate, the total levels of lipids, phospholipids, non-esterified cholesterol, triacylglycerols, and non-esterified fatty acids in the blood decreased, but the level of diacylglycerols and esterified cholesterol increased compared to the rats with experimentally induced diabetes. It was found that lipid metabolism is normalized in the blood of rats with experimentally induced diabetes that were watered with the solution of vanadium citrate in the amount of 0.125 μg/ml of water.

Keywords: Rats, Citrate, Vanadium, Diabetes, Blood, Lipids.

INTRODUCTION

The metabolism of lipids, carbohydrates, proteins is disturbed by diabetes, thus affecting the quality of life and health [1]. This disease is characterized by hyperglycemia, which occurs due to the insufficiency of insulin secretion combined or not combined with the deterioration of the hormone's action [2]. A characteristic sign of diabetes is the increased concentration of lipoprotein in blood, mainly low density lipoprotein, free fatty acids and, most importantly, ketone bodies. This is explained by the fact that edible fats are not deposited in the adipose tissue because cAMP-dependent lipase adipocytes is in the phosphorylated (active) form. Hence, the level of free fatty acids in the blood is higher. Fatty acids are absorbed by the liver and some of them are converted into triacylglycerols, which are part of low density lipoproteins secreted into the bloodstream. Other part of the fatty acids chooses the path of β-oxidation in the liver mitochondria, and the formed acetyl-CoA is used to synthesize ketone bodies.

Deviations of lipid metabolism caused by diabetes mellitus evolve in different ways. This is the effect of hyperglycemia, peroxide oxidation of lipids and fatty acids in vasoconstrictor reactions, the participation of lipoproteins in the formation of immune vascular damaging complexes and the interaction of lipids with platelets and vascular walls. According to the results of recent studies, it has been proved that dyslipoproteinemia during diabetes mellitus, in addition to the development of atherosclerosis, is also involved in the pathogenesis of microangiopathy, as evidenced by the intensification of LPO, the presence of immune complexes, and the participation of lipoproteins in vascular wall defeat.

The mechanism of vanadium's action in the treatment of diabetes is unknown, but this may be explained by the inhibition of synthesis of protein tyrosine phosphatase (PTPase), which is responsible for dephosphorylation of tyrosine residues in proteins. This

leads to the activation of tyrosine kinase and phosphorylation of the insulin receptor substrate (IRS). IRS molecules are key molecules of signaling transduction of insulin and play a central role in maintaining cellular functions. In this case, glucose transportation into the cells and the synthesis of glycogen in tissues are initiated.

Vanadium reduces the amount of cholesterol in the blood, normalizes lipid metabolism, and is effective in complex treatment of cardiovascular diseases and atherosclerosis [3-5].

Therefore, the purpose of the study was to investigate the effects of various doses of vanadium citrate on lipid metabolism indices in the blood plasma of rats with experimental diabetes.

MATERIAL AND METHOD

Animals and Feeding

The research was conducted on 40 white laboratory rats weighing 100-120 g, which were divided into 5 groups: I — control group, II, III, IV and V — experimental groups (with experimentally induced diabetes). 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 rats in the I and II groups drank clean water without additives. Animals in the III, IV and V groups consumed vanadium citrate for one month with drinking water in the quantities of 0.125, 0.5 and 2.0 μg/ml.

In order to induce diabetes, four weeks after the start of the experimental treatment the animals from groups II, II, IV and V have undergone a 24-hour period of hunger, and after that have received intraperitoneal injection of 5% solution alloxan monohydrate ("Synbias", UK) in an amount of 150 mg/kg body weight. Hyperglycemia was detected by measuring glucose blood collected from the tail vein using a portable glucometer ("Gamma-M", UK) on a weekly basis. The duration of the research period was 40 days.

Upon completion of the experiment, the animals were decapitated under mild chloroform anesthesia, without violations of the standards of humane treatment of laboratory animals, and taking into account the generally accepted bioethical norms and in accordance with the international regulations regarding experimental work. 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 [16] and "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics [17].

The research material was blood plasma of rats obtained by centrifugation of heparinized blood at 3000 rpm for 10 minutes.

Obtaining Common Lipids

Blood plasma (1 cm^3) was extracted with chloroform-methanol mixture in the ratio 2:1 (v/v) according to the Folch method [6]. To clear the lipid extract, a 0.74 M KCl solution was added. The total amount of lipids was determined by weighing the dry residue (gravimetric method) [7].

Separation of Lipids into Classes

The separation of lipids into classes was carried out by thin layer chromatography (TLC) on silica gel (silica gel L 5/40 μ, LSL 5/40 μ, Chemapol, Slovakia), using hexane–ethyl ether–acetic acid as a mobile phase in ratio 70:30:1 (v/v/v) [7]. Plates were developed using the vapors of crystalline iodine. Identification of individual lipids was carried out with values of Rf. 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 foregrams using the TotalLab TL120 software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total pool.

The content of non-esterified cholesterol (NEC) and esterified cholesterol (EC), phospholipids (PL), monoglycerols and diacylglycerols (MDAG), triacylglycerols (TAG), and non-esterified fatty acids (NEFA) were identified in the rats' blood plasma.

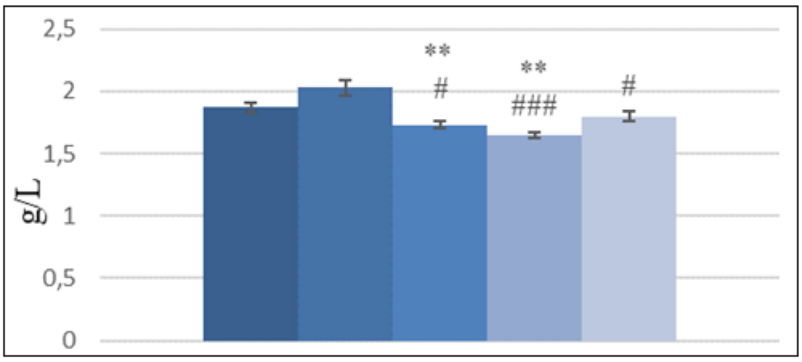
Statistical Analysis

The received digital materials were processed according to the method of variation statistics using the Student's t-test. The average arithmetic values (M) and the mean arithmetic mean errors (± m) were calculated. Changes were considered probable for $P \leq 0.05$. Computer program Microsoft Excel was used for calculating the results.

RESULTS

Analysis of Common Lipids

The experimental studies have revealed that the content of total lipids in the blood plasma of rats has increased by 7.41% for animals from group II, and decreased by 8.46% ($P < 0.01$) for group III, by 12.70% ($P < 0.01$) for group IV, and by 4.76% for group V, as compared with the control group (as shown in Graph-1). Compared to the animals with experimental diabetes from group II, the content of total lipids decreased in the blood of animals from group III by 14.78% ($P < 0.01$), from group IV — by 18.72% ($P < 0.001$), and from group V — by 11.33% ($P < 0.05$).



Graph-1: **Total lipids were extracted with a chloroform-methanol mixture in the ratio 2:1 (v/v) according to the Folch method**

I — control group, II, III, IV and V — experimental groups (with experimentally induced diabetes). Animals from III, IV and V groups consumed vanadium citrate in the amounts of 0.125, 0.5 and 2.0 µg/ml of water, respectively (Note: here and onward *— P < 0.05, ** — P <0.01, *** — P < 0.001 significance of indexes in groups II, III, IV, V compared to group I; #— P < 0.05, ## — P <0.01, ### — P < 0.001 significance of indexes in groups III, IV, V compared to group II).

Analysis of Lipid Classes

The blood plasma of rats was studied for the classes of lipids — phospholipids, non-esterified and esterified cholesterol, diacylglycerols, triacylglycerols, non-esterified fatty acids (as shown in Figure-2).

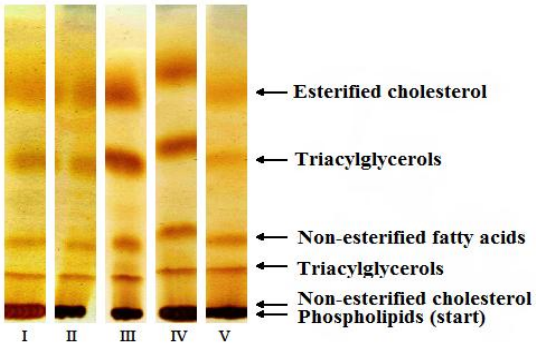


Fig-2: Chromatogram of lipids in the blood plasma of rats. The separation of lipids into classes was performed by TLC on silica gel, hexane-diethyl ether-acetic acid as a mobile phase in the ratio 70:30:1 (v/v/v). I — control group, II, III, IV and V — experimental groups (with experimentally induced diabetes). Animals from III, IV and V groups consumed vanadium citrate in the amounts of 0.125, 0.5 and 2.0 µg/ml of water, respectively

Table: **Relative content of blood lipid classes in the blood plasma of rats with diabetes mellitus (group II) and the influence of vanadium citrate in the amounts of 0.125, 0.5 and 2.0 µg/ml of water (respectively III, IV and V), %**

Indicators	Control group	Research groups			
	I	II	III	IV	V
Phospholipids	28.95±1,08	33,06±1,12	27,85±2,02$^{\#}$	27,13±1,18$^{\#\#}$	26.54±2.97$^{\#}$
Non-esterified cholesterol	8,39±1,57	11,35±1,25	8,22±1,67	9,23±2,83	9.73±2.87
Monoacylgly-cerols and diacylglycerols	10,62±2,42	10,12±0,52	12,57±1,12	15,91±3,97	13.99±1.89
Non-esterified fatty acids	7,09±0,45	8,61±0,55	6,82±0,15**	6,22±1,02*	8.22±1.22
Triacylglycerols	15,51±2,30	20,03±2,73	18,93±2,73	17,72±0,78	18.24±3.88
Esterified cholesterol	24,08±1,12	23,80±1,01	25,61±3,41	24,80±3,11	24.65±0.31$^{*\#}$

The study of lipid classes in the blood plasma of rats with experimental diabetes in group II has revealed an increase in the content of PL by 14.20%, NEC — by 35.28%, NEFA — by 21.44%, and TAG — by 29.14%. However, the study has also revealed a slight decrease in the content of DAG by 4.71% and EC by 1.16% compared to their levels in the blood plasma animals from the control group (as shown in the table).

The research of lipids in the blood plasma of rats from group III has revealed an increase in the content of DAG by 37.83% and EC by 19.39%. However, it has also revealed the decrease of PL

content by 10.33% (P < 0.05), NEC — by 15.52% (P < 0.05), NEFA — by 20.79% (P < 0.05), and TAG — by 5.49%, compared to their levels in the blood plasma of animals with experimental diabetes from group II (as shown in the table).

The research of lipids in the blood plasma of rats from group IV has revealed an increase in the content of DAG by 74.45% and EC by 1.63%, but also a decrease in the content of PL by 12.65%, NEC — by 5.14%, NEFA — by 27.76% (P < 0.05), and TAG — by 1.55%, compared to their levels in the blood plasma of animals with experimental diabetes from group II (as shown in the table).

The research of lipids in the blood plasma of rats from group V has revealed an increase in the content of DAG by 53.40% and the EC by 0.93%, but also a decrease in the content of PL by 14.55%, NEFA — by 4.53%, NEC — by 14.27%, and TAG — by 8.94%, compared to their levels in the blood plasma of animals with experimental diabetes from group II (as shown in the table).

The study of cholesterol/phospholipid ratio has revealed that this index increased in group II by 18.46%, in group III — by 1.83%, in group IV — by 17.39%, and in group V — by 26.50%, compared to their levels in the blood plasma of animals from the control group.

Compared to the animals with experimental diabetes from group II, the ratio of cholesterol/phospholipids decreased in group III by 14.04% and in group IV by 0.90%, but increased by 6.78% in group V. Analysis of the cholesterol/phospholipids ratio in all experimental groups has allowed to identify the normalization of the ratio in animals from group III, which were watered with vanadium citrate in the amount of 0,125 μg/ml of water.

DISCUSSION

Hyperlipidemia often occurs during diseases associated with a violation of energy metabolism, diabetes mellitus in particular. The lack or ineffectiveness of insulin's action during diabetes leads to uncontrolled stimulation of lipase, accompanied by an enhanced process of mobilization of fats from the depot of the body and their oxidation. They enter the bloodstream, causing hyperlipidemia, and enter the organs with insufficient energy levels.

In the animals with experimental diabetes (experimental group II), the concentration of total lipids in plasma has increased compared with the control group. Insufficient intake of carbohydrates, energy expenses that are not compensated by carbohydrates, and also a disruption of glucose metabolism during diabetes mellitus is accompanied by the mobilization of lipids and the possible appearance of ketosis.

Researchers found that patients with type 2 diabetes experience disruption of lipid metabolism in the form of a characteristic "diabetic dyslipidemia": *hypertriacylglycerolemia, increase in low-density lipoprotein content (LDL), lower concentration of high-density lipoprotein cholesterol (HDL cholesterol) [8]. During diabetes, insulin resistance enhances the mobilization of free fatty acids from adipose tissue, thus increasing the production of very-low-density lipoprotein (VLDL) in the liver. As a result, the level of triacylglycerols increases and HDL cholesterol levels decrease.*

It was established that the blood of animals of the experimental group II contained increased levels of phospholipids, which are the main components of blood lipoproteins. They are also part of the bile and some enzymes. Phospholipids are found in the cells of the protein-lipid complexes that form the lipid layer of membranes. They regulate the permeability of membranes involved in the synthesis of protein and the transport of fats from the liver into the adipose tissue. It is also known that the intensity of phospholipid synthesis and, accordingly, their content may be a way using which body cells prevent penetration by thickening their membrane. It is noted that phosphatidic acid, which is a precursor in the synthesis of both phospholipids and triacylglycerols, is synthesized under the influence of glycerol-3-phosphate acyltransferases, which uses predominantly saturated fatty acids, and especially palmitic (C16:0) and unsaturated fatty acids, in particular olein (C18:1), for the synthesis of acyl-CoA.

Rats from experimental group II displayed an increase in the levels of NEC by 15.97%, triacylglycerols — by 14.39% and free fatty acids — by 21.44%. Accumulation of NEC in the cell causes inhibition of the activity of 3-hydroxy-3-methylglutaryl-CoA reductase. This enzyme is the key to regulating the cholesterol biosynthesis rate of the cell and increases the activity of acyl-CoA-cholesterol-acyltransferase. Acyl-CoA-cholesterol-acyltransferase is an enzyme that regulates the esterification of cholesterol, which increases the rate of re-sterilization of cholesterol and suppresses the synthesis of some receptors on the cell surface. In the absence of insulin, oxidation of fatty acids to the end products is disturbed. Acetyl-CoA, which is the starting material in the synthesis of cholesterol, accumulates. In addition to the above, the depletion of liver induced by glycogen leads to intensification of lipolysis in the adipose tissue and increase in the amount of free fatty acids in the blood. Fatty acids are important for many biological functions. Typically, fatty acids are decomposed by beta-oxidation or esterification, and then stored as triacylglycerols. Increasing the level of non-esterified fatty acids, natural

dissociation of oxidative phosphorylation, can lead to regulation of energy metabolism during experimental diabetes mellitus.

Cholesterol metabolism is disturbed by diabetes: synthesis, metabolism, transport and excretion, formation of lipoproteins (especially low-density lipoproteins), their catabolism, and the reception of lipoproteins by arterial cells can be the causes of arteriosclerosis [8].

As it is known, the level of esterification is one of the leading tests in evaluating the functional state of the organism. Therefore, a decrease in the level of esterified cholesterol in the blood plasma of animals with the diabetes mellitus from group II may indicate negative functional changes in the body that occur during this disease.

The low content of diacylglycerols in blood plasma of rats from group II indicates the direction of lipid metabolism towards the synthesis of structural lipids because diacylglycerols are mediators of the synthesis of triacylglycerols and phospholipids.

Adding vanadium citrate into the blood plasma of rats from III, IV and V experimental groups led to the decrease in the levels of total lipids by 14.78%, 18.72%, and 11.33% respectively, compared to the experimental group II. This may indicate their use in adaptive rearrangements of the lipid layer of cell membranes.

The effects of different doses of vanadium have changed the ratio of lipid classes in the blood of animals from experimental groups. In particular, a decrease in the content of phospholipids in the III, IV and V experimental groups indicates an increased activation of their hydrolysis.

The decreased content of NEC in the blood plasma of rats from III and IV experimental groups indicates an increase in the functional activity of tissues. This may indicate changes in the processes of esterification and hydrolysis of cholesterol in the body under the influence of biologically active substances (in this case, vanadium). Also, NEC can be reduced when used for the synthesis of sex hormones and hormones of the adrenal cortical layer.

The decreased content of triacylglycerols in the experimental groups III, IV and V indicates an increase in the β-oxidation of fatty acids and is associated with their formation in the process of glucose metabolism through L-α-glycerophosphate. It is also known that the rate of synthesis of triacylglycerols changes under the influence of hormones. Thus, insulin stimulates the transformation of carbohydrates into triacylglycerol and vice versa.

Studies by other authors have found that vanadium compounds reduce the high levels of triacylglycerols in serum and liver [9]. Additionally, the expression of genes involved in lipid biosynthesis pathways in patients with diabetes mellitus is normalized after the introduction of vanadium compounds [1]. The increase of diacylglycerols in the blood plasma of rats from III, IV, and V experimental groups acts as an indirect confirmation of enzymatic hydrolysis of phospholipids — the main products of this process.

An increase in the content of the esterified cholesterol was observed in blood plasma of rats from III, IV, and V experimental groups, indicating an increase in its synthesis. As a rule, esterified cholesterol is found in cell cytosol and is a spare form of non-esterified cholesterol. Cell lysosomes contain active hydrolase of esterified cholesterol, and the endoplasmic reticulum contains acyl-CoA-cholesterol-acyltransferase. These two enzymes, depending on the conditions and needs of the cell, carry out hydrolysis or synthesis of esterified cholesterol.

Insulin-mimetic properties and antidiabetic effects of vanadium compounds have been widely documented both in vivo and in vitro [10-12]. Vanadium compounds stimulate the synthesis of glycogen and lipogenesis and suppress lipolysis. Ions of vanadium cause insulin-dependent effects in relation to adipocytes in rats — they stimulate glucose uptake and inhibit the release of free fatty acids [13]. Additionally, the researchers found that, in a setting of six weeks, patients with diabetes consuming 100 mg of vanadium on a daily basis have experienced reduced levels of glycosylated hemoglobin, fatty acids, total cholesterol, triacylglycerols and low density lipoprotein.

Phospholipids circulating in the blood, among other functions, perform the role of cholesterol stabilizers in blood plasma. They prevent crystallization of cholesterol, with its falling out of plasma, and deposition on the walls of the blood vessels. The ratio of cholesterol/phospholipids in blood plasma determines the degree of solubility of cholesterol in the blood vessels [14]. An increase in the cholesterol/phospholipid ratio in the animals with experimental diabetes mellitus from group II was established. This may indicate an increase in the microviscosity of blood cell membranes, which is explained by the densification of the lipid bilayer and, accordingly, the decrease in its permeability. Additionally, the content of unsaturated fatty acids is significantly reduced in the membranes of patients with diabetes mellitus. Also, the concentration of saturated increases proportionally, leading to an increase in the rigidity of the membranes and the disruption of a number of their functions. At the same time, a decrease of the cholesterol/phospholipid ratio in III, IV, and V groups indicates a change in the viscosity of the bilayer

membranes with the transition to a more fluid phase and an increase of their permeability [15]. In general, our studies have found that the normalization of lipid metabolism in blood of rats under conditions of experimentally induced diabetes is achieved by adding vanadium citrate in the amount of 0.125 μg/ml of water.

CONCLUSIONS

Experimentally induced diabetes in the blood plasma of rats increases the content of common lipids, in particular, phospholipids, non-esterified cholesterol, triacylglycerols, non-esterified fatty acids, and the cholesterol/phospholipids ratio, but reduces the level of diacylglycerols and esterified cholesterol.

Given the consumption of vanadium citrate in the amounts of 0.125, 0.5 and 2.0 μg/ml of water, the blood plasma of rats displays a decrease in the content of total lipids, phospholipids, non-esterified cholesterol, triacylglycerols, and non-esterified fatty acids, and an increase in the level of diacylglycerols and esterified cholesterol, compared with the animals that have been experimentally induced diabetes.

Normalization of the level of lipid metabolism, including the cholesterol/phospholipid ratio, in the blood of rats with experimentally induced diabetes occurs when adding vanadium citrate in the amount of 0.125 μg/ml of water.

Conflict of Interest: None.

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