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Effects of a high-fat, high-carbohydrate diet on blood cells of rats

Birulina J.G.[1], Ivanov V.V.[1], Buyko E.E.[1, 2], Trubacheva O.A.[1, 3], Petrova I.V.[1], Grechishnikova A.Yu.[1], Nosarev A.V.[1], Gusakova S.V.[1]

[1] *Siberian State Medical University (SSMU)*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *National Research Tomsk Polytechnic University (NR TPU)*
30, Lenina Av., Tomsk, 634050, Russian Federation

[3] *Cardiology Research Institute, Tomsk National Research Medical Center (TNRMC), Russian Academy of Sciences (RAS)*
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To study the effects of a high-fat, high-carbohydrate diet on erythrocytes and platelets of rats.

Materials and methods. Male Wistar rats *(n* = 23) were used for the study. The rats were divided into a control group and an experimental group. The rats from the control group were fed with standard rat chow. The rats from the experimental group had received a high-fat and high-carbohydrate diet for 12 weeks. In the rats, body weight and blood pressure (BP) were measured, an oral glucose tolerance test was carried out, and hematological and lipid metabolism parameters were analyzed. The conductance of erythrocyte K_{Ca}-channels was measured by the potentiometric method, and platelet aggregation was determined by the turbidimetric method.

Results. Feeding the rats with a high-fat, high-carbohydrate diet for 12 weeks resulted in obesity, BP elevation, hyperglycemia, impaired glucose tolerance, and dyslipidemia with pronounced triglyceridemia. In the experimental group, a rise in the number of leukocytes, mainly due to granulocytes, and an increase in the number of platelets and their collagen-induced aggregation were observed. The red blood cell count in the rats of the experimental group did not significantly differ from that of the control group. In the experimental group, multidirectional changes in the membrane potential were observed in response to the stimulation of the K_{Ca}-channels in the erythrocyte membrane with the Ca^{2+} ionophore A23187 or artificial redox systems.

Conclusion. The obtained data indicate that a high-fat, high-carbohydrate diet leads to metabolic and hemorheological disorders that are typical of metabolic syndrome.

Key words: high-fat, high-carbohydrate diet, metabolic syndrome, obesity, dyslipidemia, blood cells, K_{Ca}-channels, aggregation.

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✉ *Birulina Julia G.,* e-mail: birulina20@yandex.ru

Влияние высокожировой и высокоуглеводной диеты на клетки крови крыс

Бирулина Ю.Г.[1], Иванов В.В.[1], Буйко Е.Е.[1, 2], Трубачева О.А.[1, 3], Петрова И.В.[1], Гречишникова А.Ю.[1], Носарев А.В.[1], Гусакова С.В.[1]

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

[2] Национальный исследовательский Томский политехнический университет (НИ ТПУ)
634050, г. Томск, пр. Ленина, 30

[3] Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель – изучить воздействие высокожировой и высокоуглеводной диеты на эритроциты и тромбоциты крови крыс.

Материалы и методы. Исследование выполнено на 23 самцах крыс линии Вистар, которые были разделены на контрольную и опытную группу. Крысы контрольной группы находились на стандартной диете. Крысы опытной группы в течение 12 нед получали высокожировую и высокоуглеводную диету. Животным измеряли массу тела, артериальное давление (АД), выполняли глюкозотолерантный тест, определяли гематологические показатели и параметры липидного обмена. Потенциометрическим методом изучали проводимость K_{Ca}-каналов мембраны эритроцитов, турбидиметрическим – агрегационную способность тромбоцитов.

Результаты. Содержание животных на высокожировой и высокоуглеводной диете приводило к ожирению, повышению АД, гипергликемии, снижению толерантности к глюкозе, дислипидемии с выраженной триглицеридемией. У животных опытной группы происходило увеличение количества лейкоцитов, главным образом, за счет гранулоцитов, повышение числа тромбоцитов и их коллаген-индуцированной агрегации. Количественные показатели клеток красной крови крыс опытной группы не отличались от контрольной группы. В ответ на стимуляцию K_{Ca}-каналов мембраны эритроцитов животных экспериментальной группы с помощью Ca^{2+}-ионофора A23187 или редокс-системы наблюдались разнонаправленные изменения мембранного потенциала.

Заключение. Полученные результаты свидетельствуют, что высокожировая и высокоуглеводная диета приводит к метаболическим и гемореологическим нарушениям, характерным для метаболического синдрома.

Ключевые слова: высокожировая и высокоуглеводная диета, метаболический синдром, ожирение, дислипидемия, клетки крови, K_{Ca}-каналы, агрегация.

Конфликт интересов. Авторы декларируют отсутствие потенциальных и явных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Metabolic syndrome (MS) is one of the most topical problems in modern medicine. This is a combination of metabolic, hormonal, and hemodynamic disorders that increase the risk of cardiovascular diseases [1, 2]. The most significant factors in the MS development are abdominal obesity and insulin resistance [3, 4]. In recent years, the number of obese people has increased significantly, that has resulted in the increased prevalence of MS [5, 6].

Currently, it has been documented that MS is accompanied by various pathological processes of the blood system, which play an important role in the pathogenesis of many complications of cardiovascular diseases [7, 8]. Structural and functional disorders of erythrocytes and platelets contribute to the development of the latter. Disorganization and modulation of physicochemical properties in the erythrocyte membrane in metabolic disorders lead to dysregulation of its cation-transporting function, in which an important role belongs to Ca^{2+}-activated potassium channels – Gardos channels (K_{Ca}-channels), the activity of which determines red blood cell eryptosis [9] and deformability [10]. Dyslipidemia and systemic oxidative stress in MS [11] cause an increase in platelet aggregation and elevate the risk of thrombosis [12].

The need to clarify the fundamental basis of hemorheological disorders and their role in the progression of cardiovascular pathology during MS requires an adequate experimental model of MS. One of the most common methods for modeling MS in animals is a special diet. In this respect, a diet with a high content of fats and carbohydrates proved to be the most effective, reproducing the typical characteristics of MS in animals [13, 14].

Therefore, the aim of the study was to investigate the effect of experimental MS, induced by a high-fat and high-carbohydrate diet, on erythrocytes and platelets of rats.

MATERIALS AND METHODS

An experimental MS was modeled on male Wistar rats (23 rats aged 6 weeks, average weight 226.5 ± 20.9 g). The studies were carried out in compliance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki. The animals were divided into a control group and an experimental group (11 and 12 animals, respectively). The rats of the control group were fed with a standard diet (Delta Feeds, Biopro, Russia, total calories 3,000 kcal / kg). The rats of the experimental group had received a diet containing standard food (66%) with the addition of animal fat (17%), fructose (17%), and cholesterol (0.25%) for 12 weeks. Drinking water was replaced with a 20% fructose solution (total calories 4,400 kcal/kg).

Before and at the end of the study, body weight and blood pressure (BP) (Systola, Neurobotics, Russia) were measured in the animals. At the 12th week of the experiment, a glucose tolerance test (GTT) was performed [15]. Fasted rats (12 h of food deprivation) were injected intragastrically with a glucose solution at a dose of 2 g / kg (D-glucose, Sigma-Aldrich, USA). The concentration of glucose was determined in the blood drawn from the rat tail vein spectrophotometrically at 0, 15, 30, 60, 90, and 120 min of the experiment using an enzymatic kit (Chronolab, Spain).

The animals were euthanized with CO_2 after 12 weeks from the start of the experiment. Blood from the heart was collected in BD Vacutainer® vacuum tubes with anticoagulants: K_2 EDTA (to assess hematological and biochemical parameters) or sodium citrate (to study platelet aggregation). The liver and visceral adipose tissue were isolated and weighed using the analytical balance, and their specific gravity was calculated. Hematological parameters were assessed using an automatic analyzer (BC-2800 Vet, Mindray, China). Blood plasma was obtained by centrifugation of whole blood (4 °C, 8,000 g, 6 min). The blood plasma lipid spectrum of the animals was determined using an automatic biochemical analyzer (Architect c4000, Abbott, USA).

To study the activity of K_{Ca}-channels, the erythrocyte sediment obtained after centrifugation was resuspended in an incubation medium containing 150 mM NaCl, 10 mM glucose, 1 mM KCl, and 1 mM $MgCl_2$. The amplitude of an erythrocyte hyperpolarization response (HR) was determined after addition of 0.5 μM Ca^{2+} ionophore A23187 or the artificial ascorbate (10 mM) – phenazine methosulfate (PMS, 0.1 mM) redox system. Rat platelet aggregation was studied by the turbidimetric method using a laser analyzer (Biola, Russia). Collagen (2 μg / ml) was used as a platelet aggregation inducer. The degree and rate of platelet aggregation were determined according to the average aggregate size curve.

The data were analyzed using the SPSS Statistics 23 software. The data are presented as the mean and standard deviation ($M \pm SD$) for normally distributed variables and as the median (Me) and the interquartile range (Q_1; Q_3) for non-normally distributed variables. To analyze the differences between the samples, Student's t-test or the Mann – Whitney U-test were used. The result was considered statistically significant at $p < 0.05$. Correlation was assessed using the Pearson's correlation coefficient.

RESULTS

Feeding the rats with a high-fat, high-carbohydrate diet for 12 weeks resulted in an increase in blood pressure and specific gravity of the adipose tissue and liver

of the studied animals (Table 1). It was shown that the specific gravity of abdominal fat, including mesenteric, epididymal, and retroperitoneal fatty tissue, increased by 2.5 times in the experimental rats.

The rats of the experimental group showed an increase in the level of triacylglycerols (TAG) in blood plasma by 2.5 times compared with the control group. The total cholesterol (Ch) level in the blood of the animals fed with a high-fat, high-carbohydrates diet increased by 1.5 times. Such an increase in the level of total Ch is determined by a rise in the level of low-density lipoprotein cholesterol (LDL-Ch), while the level of high-density lipoprotein cholesterol (HDL-Ch) did not change (Table 1). A direct relationship was found between the concentration of TAG and an increase in the specific gravity of the adipose tissue ($r = 0.689$, $p = 0.001$), weight of the liver ($r = 0.434$, $p = 0.03$), the content of total Ch, and the specific gravity of the liver ($r = 0.418$, $p = 0.04$). Furthermore, in the animals receiving a special diet, an elevated level of glucose in the blood plasma after fasting was observed (Table 1). A positive correlation was found between the concentration of glucose in the blood and the specific gravity of the adipose tissue ($r = 0.823$, $p = 0.001$).

Table 1

Effects of a high-fat, high-carbohydrate diet on the physiological and biochemical parameters of rats, *M* ± *SD*

Parameter	Group	
	Controls ($n = 11$)	Metabolic syndrome ($n = 12$)
Body weight, g	376.6 ± 28.9	388.2 ± 30.7 ($p = 0.241$)
SBP, mm Hg.	123.2 ± 8.8	139.2 ± 9.1 ($p = 0.01$)
DBP, mm Hg.	86.5 ± 9.3	101.4 ± 12.2 ($p = 0.028$)
Fasting blood glucose, mmol/l	4.0 ± 0.3	5.4 ± 0.5 ($p = 0.001$)
Total Ch, mmol/l	1.7 ± 0.2	2.5 ± 0.3 ($p = 0.009$)
HDL-Ch, mmol/l	± 0.2	8 ± 0.1 ($p = 0.09$)
LDL-Ch, mmol/l	0.7 ± 0.3	1.6 ± 0.4 ($p = 0.03$)
TAG, mmol/l	0.9 ± 0.4	2.3 ± 0.8 ($p = 0.001$)
Specific gravity of the adipose tissue, g	1.1 ± 0.3	2.8 ± 0.4 ($p = 0.001$)
Specific gravity of the liver, g	3.2 ± 0.5	4.3 ± 0.6 ($p = 0.001$)

Note: SBP – systolic blood pressure, DBP – diastolic blood pressure. Here and in Table 2, 3: p – the level of statistical significance in comparison with the controls.

Following GTT, it was found that 30 min after glucose load in the rats of the experimental group, the blood glucose level exceeded the level in the control group by 23.3% ($p = 0.005$); after 60 and 120 min – by 30.9% ($p = 0.001$) and 28.6% ($p = 0.003$), respectively (Figure, *a*). The area under the glucose concentration – time curve ($AUC_{0\text{-}120}$) in the experimental group of animals was 818.2 ± 61.4 mmol / l×120 min and exceeded the control group value (622.1 ± 36.8 mmol / l×120 min, $p = 0.001$) (Figure, *b*).

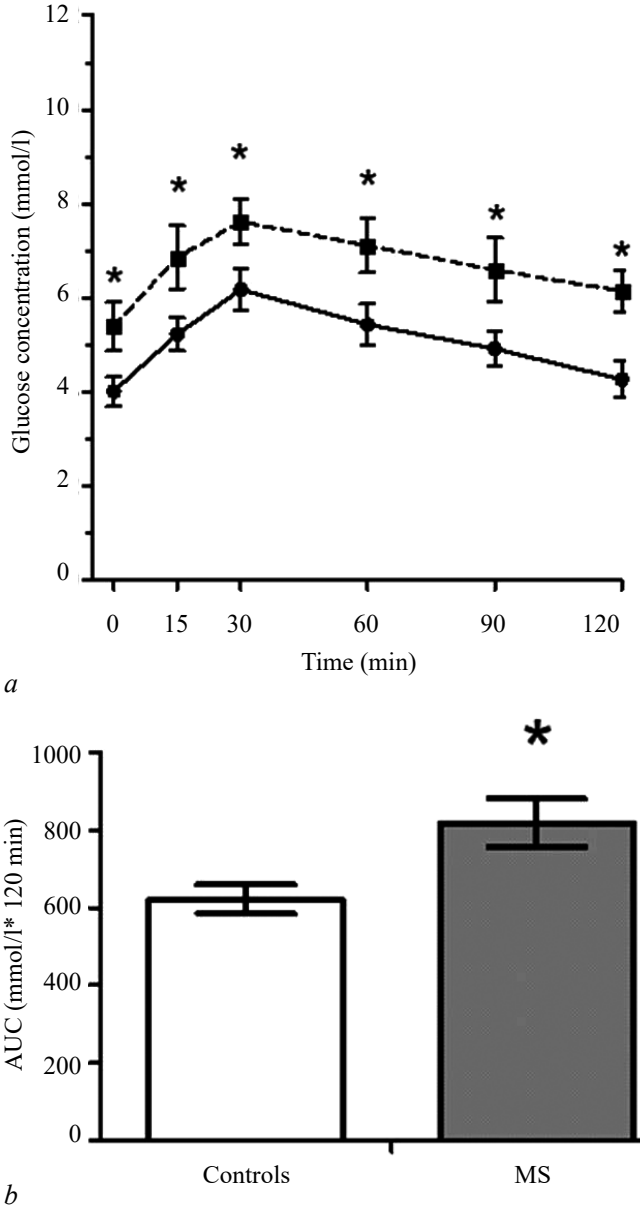

Figure. Changes in the blood glucose concentration in rats (*a*) and the area under the glucose concentration – time curve (*b*) in the oral glucose tolerance test: the control group (solid line) and the experimental group (dotted line). *$p < 0.05$ in comparison with the control group

Evaluation of hematological parameters revealed that in the animals fed with a high-fat and high-carbohydrate diet the leukocyte count increased by 1.5 times in comparison with the control group, mainly due to granulocytes (Table 2). A relationship between the leukocyte count ($r = 0.589$, $p = 0.015$) and the specific gravity of

the adipose tissue was established in the experimental rats. There were no statistically significant changes in the count of red blood cells in the animals of the experimental group. However, the platelet count in the blood was 1.4 times higher than in the rats receiving a standard diet (Table 2).

In the rats fed with a high-fat, high-carbohydrate diet, the amplitude of the erythrocyte HR stimulated by the Ca^{2+} ionophore A23187 decreased compared with the control group (Table 3). In contrast, the amplitude of HR generated by adding the artificial electron-donor system (ascorbate – PMS) to the erythrocyte suspension was elevated in the rats of the experimental group. In the animals of the experimental group, an increase in the degree and rate of collagen-induced platelet aggregation was observed (Table 3).

Table 2

Effects of a high-fat, high-carbohydrate diet on the hematological parameters of rats, *M* ± *SD*

Parameter	Group	
	Controls (n = 11)	Metabolic syndrome (n = 12)
White blood cells, 10^9/l	14.1 ± 3.0	20.7 ± 5.5 (p = 0.022)
Lymphocytes, %	69.7 ± 6.4	63.4 ± 8.9 (p = 0.048)
Monocytes, %	3.7 ± 0.3	3.9 ± 0.7 (p = 0.078)
Granulocytes, %	26.6 ± 5.8	35.9 ± 7.6 (p = 0.001)
Red blood cells, 10^{12}/l	9.4 ± 0.5	9.2 ± 0.8 (p = 0.28)
Hemoglobin, g/l	208 ± 10.9	198 ± 11.5 (p = 0.088)
Hematocrit, %	47.1 ± 2.4	45.6 ± 6.8 (p = 0.072)
Mean cell volume, fl	50.3 ± 1.8	49.3 ± 1.1 (p = 0.118)
Platelets, 10^9/l	1,010.8 ± 70.5	1,450.5 ± 84.6 (p = 0.02)

Table 3

Effects of a high-fat, high-carbohydrate diet on the hyperpolarization response of erythrocytes and collagen-induced platelet aggregation of rats, *Me* (Q_1; Q_3)

Group	Parameter			
	A23187-induced HR, mV	Redox-induced HR, mV	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min
Controls (n = 11)	–38.4 (–40.2; –35.3)	–40.2 (–45.6; –35.5)	1.6 (1.4; 1.8)	1.9 (1.7; 2.6)
Metabolic syndrome (n = 12)	–30.1 (–34.6; –27.6) p = 0.022	–49.1 (–51.3; –44.3) p = 0.015	2.9 (2.4; 4.2) p = 0.008	2.9 (2.7; 5.2) p = 0.011

DISCUSSION

Experimental animal models are used to study the mechanisms of development and progression of metabolic and hemorheological disorders in MS [16–18]. They are aimed at reproducing the characteristic signs of MS, such as overweightness, obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and arterial hypertension [1, 3]. One of the possible ways to induce MS in animals is a high-fat, high-carbohydrate diet.

In our study, it was found that feeding rats with a diet containing animal fat, fructose, and Ch for 12 weeks resulted in obesity, increased BP, hyperglycemia, decreased glucose tolerance, and dyslipidemia with hypertriglyceridemia. In the meantime, a positive correlation between a significant increase in the specific gravity of the liver, total Ch, and TAG level may indicate emergence of metabolic disorders in the liver of the experimental animals. The results are consistent with the literature data on diet-induced MS models [14, 18] and indicate that the chosen diet effectively reproduces the clinical signs of MS.

Experimental and clinical studies carried out in recent years suggest that the pathogenetic mechanisms of MS are inextricably linked with the functioning of the blood system. Thus, it was shown that MS is accompanied by an increase in blood viscosity, aggregation of erythrocytes, a change in the number of red [7, 17] and white blood [19] cells, and an increase in the content and functional activity of platelets [20]. In this work, it was demonstrated that the leukocyte count increased in the animals of the experimental group mainly due to granulocytes. A correlation was also established between the white blood cell count and the specific gravity of the adipose tissue. It is known that obesity, as one of the important components of MS, is accompanied by a chronic inflammatory process in the adipose tissue, infiltrated by leukocytes, with high production of proinflammatory cytokines [15, 19].

Feeding animals with a high-fat, high-carbohydrate diet led to a rise in the content of platelets in the blood and an increase in their collagen-induced aggregation. According to existing data, an increase in the sensitivity of platelets to collagen in experimental rats can be caused by an increase in glycoprotein VI (GPVI) on the cell surface [20, 21], as well as by a change in the content of lipids in platelet membranes due to dyslipidemia [12]. This is inevitably accompanied by activation of phospholipases C and A_2 and protein kinase C followed by phosphorylation of platelet contractile proteins. It was shown that prothrombotic conditions associated with metabolic disorders increase blood clotting, reduce fi-

brinolysis and endothelial thromboresistance, and cause platelet hyperactivity [22].

The quantitative indicators of red blood cells of the experimental animals did not differ from those of the control group. However, after the stimulation of K_{Ca}-channels in the erythrocyte membrane with the Ca^{2+} ionophore A23187 or the artificial electron-donor system (ascorbate – PMS), multidirectional changes in the HR amplitude were observed. This effect can be explained by the increased level of calcium ions in erythrocytes of the rats with MS [9]. Thus, equal addition of Ca^{2+} to a suspension of normal erythrocytes and a suspension with an increased level of calcium ions will cause a decrease in the HR amplitude in the latter. The change in the HR amplitude caused by the Ca^{2+} ionophore and the artificial ascorbate – PMS redox system may be determined by different mechanisms of K_{Ca-}channel opening [23].

In turn, a rise in the amplitude of the ascorbate – PMS-induced HR in the erythrocyte membrane in the animals of the experimental group indicates increased conductance of K_{Ca}-channels in the erythrocyte membrane. At the same time, structural rearrangement of the membrane lipid composition caused by increased cholesterol incorporation and phosphatidylserine externalization significantly affects the activity of Gardos channels, which leads to an increase in microviscosity of the lipid bilayer [10] and a decrease in cell deformability in the vascular bed.

CONCLUSION

It was found that the proposed high-fat, high-carbohydrate diet effectively reproduces the typical symptoms of MS. Analysis of hematological parameters revealed significant changes in white blood cells and platelets of the animals with MS. The erythrocyte membrane of the rats fed with a special diet was characterized by increased permeability to potassium ions, and platelets were characterized by high sensitivity to collagen. The obtained results are valuable for studying the causes ofspe development and progression of cardiovascular diseases associated with MS.

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Authors contribution

Birulina J.G., Ivanov V.V. – conception and design, drafting of the manuscript. Petrova I.V., Grechishnikova A. Yu. – analysis and interpretation of data. Nosarev A.V., Gusakova S.V. – substantiation of the manuscript, final approval of the manuscript for publication. Buyko E.E., Trubacheva O.A. – carrying out of the experiment.

Authors information

Birulina Julia G., Cand.Sci. (Biology), Associate Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0003-1237-9786.

Ivanov Vladimir V., Cand. Sci. (Biology), Head of the Center for Preclinical Research, Central Research Laboratory, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9348-4945.

Buyko Evgeniy E., Laboratory Assistant, Center for Preclinical Research, Central Research Laboratory, SSMU; Post-Graduate Student, Research School of Chemistry & Applied Biomedical Sciences, TPU, Tomsk, Russian Federation. ORCID 0000-0002-6714-1938.

Trubacheva Oksana A., Cand. Sci. (Med.), Physical Education and Health Division, SSMU; Researcher, Department of Functional and Laboratory Diagnostics, Cardiology Research Institute, TNRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-1253-3352.

Petrova Irina V., Dr. Sci. (Biology), Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9034-4226.

Grechishnikova Aleksandra Yu., Assistant, Medical and Biological Cybernetics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9891-2910.

Nosarev Alexey V., Dr. Sci. (Med.), Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0002-0119-9707.

Gusakova Svetlana V., Dr. Sci. (Med.), Head of the Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-5047-8668.

(✉) **Birulina Julia G.,** e-mail: birulina20@yandex.ru

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