ASSESSMENT OF THE EFFECT OF BIOACTIVE NUTRIENTS AND PROBIOTIC MICROORGANISMS ON THE PARAMETERS OF LIPID METABOLISM IN THE BODY

Natalia Rodionova, Evgeny Popov, Natalia Zakharova, Ekaterina Pozhidaeva, Anna Derkanosova, Nikolay Tychinin, Anatoly Khitrov, Mikhail Syromyatnikov

ABSTRACT
The aim of the work is a comparative study of the effect of vegetable nutrients - wheat germ oil (WGO), flour from wheat germ cake (WGC), and animal - concentrated tissue fish oil, origin on lipid metabolism indicators of students and university teachers, as well as an assessment of the probiotic factor in increasing the effectiveness of the target biologically active substances. The data obtained confirm the relation between the nutritional sufficiency of the body and the increase in lipid metabolism effectiveness and atherosclerosis risk reduction in students and teachers of an engineering university with the daily use of bioactive nutrients: WGO, WGC, CTFO and their combinations with the biomass of lactobacilli and bifidobacteria consortium. The lipid metabolism was evaluated based on the analysis of indicators of the total cholesterol (TC) concentrations, high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), atherogenic index (AI). The values of these indicators were recorded before and after daily consuming the study foods for 30 days. The data obtained were analyzed for 3 age groups: 16 – 24, 25 – 44, and 45 – 65. It was found that WGO has the most effective antiatherosclerotic effect; the decrease in the TC level was 6.4%, the increase in the HDL concentration was 13.7%, the decrease in the LDL concentration was 11.3%, the decrease in the TG concentration was 17.1%, and the AI decreased by 22.6%, respectively. With WGC, the changes in the studied parameters were 6.0, 12.3, 13.1, 13.1, and 22.1%, respectively. Together with probiotics WGO effectiveness increased in terms of the reduced TC level (1.9%), increased HDL concentration (8.2%), decreased LDL concentration (2.5%), reduced TG concentration (5.7%), and decreased AI (6.3%) for CTFO by 9.1, 26.1, 14.7, 24.1 and 31.2%, respectively. Thus, the dynamics of lipid metabolism indices objectively testifies to the positive effect of bioactive nutrients on human health indicators.

Keywords: bioactive nutrient; lipid metabolism; LDL cholesterol; HDL cholesterol; triglyceride;

INTRODUCTION
Currently, the need for timely diagnosis of cardiovascular diseases, interpretation of changes in the blood lipid profiles, and effective correction of disorders is beyond dispute. In this regard, it is relevant to search for and assess alimentary factors that provide energy needs of the body utmost normalize its functional state, and implement non-drug methods for correcting lipid metabolism disorders (Arabidze and Tebloev, 2008; Popov et al, 2016; Bouillon and Antonio, 2020; Longo, Ferrari and Zocchi, 2017; Mohammed Farugue, 2018).
Natural vegetal and animal-based resources obtained in the process of their deep processing and probiotic microorganisms, including their consortia, which enhance the effect of target biologically active substances, are the most relevant, effective, and promising means for correcting nutritional status and preventing pathological conditions (Chugh and Kamal-Eldin, 2020; Rodionova et al, 2019a).
A good therapeutic effect in normalizing blood parameters that characterize lipid metabolism for different groups of patients was established when taking biologically active food supplements containing policosanol, which is a mixture of several long-chain alcohols (Rodionova, Popov, and Sokolova, 2016; Rodionova and Alekseeva, 2015; Arruzazabala and Carabajal, 2012; Rodionova et al, 2019b) and omega 3 polyunsaturated fatty acids, in particular eicosapentaenoic and docosahexaenoic acids (Meerson et al, 1993; Rodionova et al, 2016; Isaev and Simonenko, 2016; Heshmati, 2019; Spector and Kim, 2019). Its mechanism of action policosanol is based on the modulation of 3-hydroxy-3-methyl-glutaryl-CoA reductase, ab-sorption of bile acids, and partial utilization
of lactic acid. Policosanol increases receptor-dependent processing of low-density lipoproteins (LDL) by enhancing the LDL-receptor binding affinity, improving the LDL transport to liver cells, thereby significantly increasing the cleavage of LDL cholesterol. In addition to lowering the LDL levels, policosanol has been found to increase high-density lipoprotein (HDL) levels, protect HDL from the damaging effects of free radicals, and inhibit excessive platelet aggregation (Arruzazabala and Carbajal, 2012; Arruzazabala, Valdes and Mas, 2013; Canetti, Morera, and Illnait, 2012; Carbajal, Arruzazabala and Mas, 2012).

Scientific hypothesis
It was clinically proven that probiotic products increased the body’s resistance to various external and internal factors (Angelín and Kavitha, 2020; Centí, 2014; Roobab et al, 2020; Teneva-Angelova et al, 2018, Zuo, Chen and Marcotte, 2020). Concerning such a wide range of positive clinical effects, a hypothesis was put forward about the feasibility of studying the biomass influence on the homeostasis in the human body when introduced into the diet.

MATERIAL AND METHODOLOGY
Samples
Wheat germ is a valuable source of policosanol (Vishnyakov et al, 2020; Boukid et al, 2018). This by-product of flour milling includes up to 15% oil with a policosanol content of 1.5-8.0 mg/100 g (Rodionova et al, 2016; Popov et al, 2016). When separating wheat germ oil by cold pressing (at 150 MPa), the protein-carbohydrate component (cake) remains with a residual oil content of 6-8%; this cake is processed into flour, resulting in Vitazar dietary supplement. This flour contains up to 35% of protein, including all essential amino acids, up to 47% of carbohydrates, represented by glucose, maltose, sucrose, oligosaccharides, pentosans, polysaccharides. It is a source of biologically active substances: vitamins (B1, B2, B3, B6, B9, PP), macro- and microelements (Zn, Mn, Mg, Ca, K, Fe, Na, Se, P) and policosanol content of 0.1-0.7 mg/100 g. The amount of cake in oil production is up to 95% of the feedstock; it is an inexpensive food bioresource with valuable functional properties (Rodionova et al, 2016; Rodionova, Popov and Sokolova, 2016).

Concentrated tissue fish oil (CTFO), the Eikolon dietary supplement, is a source of polysaturated fatty acids of the omega-3 class, including eicosapentaenoic and docosahexaenoic acids. It is an active bioregulator of metabolic processes, obtained with the use of gentle modes of the physical impact that preserve the native properties of the lipid fraction to the uttermost (Isaev and Simonenko, 2016). Installed impact of CTFO on the normalization of lipid metabolism indicators, immunological parameters in cardiac ischemia, cardiac muscle contractile function and arrhythmias, rheological parameters of blood, and disseminated sclerosis correction (Jamshidi et al, 2020; Yang et al, 2020; Zamroziewicz et al, 2017).

The biomass of lactobacilli and bifidobacteria, introduced additionally into the diet, having a viable cell concentration of 109 CFU/g enables to solve a wide range of tasks on selective stimulation of the immune system, influencing the main links of disease pathogenesis, enhancing the essential drug action. Additional effects were also revealed, such as hypolipidemic, antiarrhythmic properties, gastrointestinal motility regulation, and reinfection prevention.

Male and female students and university members of the University voluntarily participated in these four experimental groups aged 16 to 65. The participants spent at least 6 hours daily in the same conditions – the premises of the university. All the subjects had not previously used the bioactive nutrients under study. They had no concomitant diseases impairing lipid metabolism (liver and kidney diseases, hypothyroidism, obesity) and were not subject to regular medical check-ups. The study was carried out in the autumn to exclude changes associated with the seasonality of nutrition and physical activity. Smoking and pregnancy were exclusionary criteria for study entry (Grigoriev, 2015). Each member of the experimental group signed informed consent to participate in the trial.

Chemicals
The following supplements were investigated as correctors of lipid metabolism: WGO and WGCF (manufactured by Pultat LLC, RF); CTFO (manufactured by NPP Trinita LLC, RF).

Animals and Biological Material:
The biomass of the lactobacilli and bifidobacteria consortium – Streptococcus thermophilus, Casesubsp. Rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactococcus fermentum, Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium adolescentis, with a viable cell content of n 109 CFU/g.

Instruments
Cobas 6000 diagnostic immunoassay analyzer (produced by F. Hoffmann-La Roche Ltd, Switzerland; distributed by LLC MedTechnic, Russia).

Laboratory Methods
The reliability of the obtained parameters was assessed according to the Mann-Whitney nonparametric U-test (Grigoriev, 2015). Volunteer patients were divided into four groups. The experimental groups amounted to 180 persons, of which 92 are men and 82 are women; the same number of subjects (180 persons) was in the control groups that did not consume the test products (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Bioactive nutrients</th>
<th>Patients' age, years</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>16-24</td>
<td>25-44</td>
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<tr>
<td>WGO</td>
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<td>WGCF</td>
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<td>WGO-containing probiotic emulsion</td>
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<td>CTFO-containing probiotic emulsion</td>
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The first group was given 3.5 g of WGO, the second group consumed WGCF in an amount providing 3.5 g of oil, which corresponded to 50 g in the composition of a confectionery (Rodionova, Popov and Rodionova, 2019). The third group consumed a probiotic emulsion containing 3.5 g of WGO, and the fourth group took a probiotic emulsion containing 6.5 g of CTFO combined with 10 g of biomass of probiotic microorganisms (Zuo, Chen and Marcotte, 2020).

**Description of the Experiment**

**Sample preparation:** To study the fermentation processes, consortia of probiotic microorganisms were activated, in 100 ml sterilized (temperature 121 ±2 °C, exposure 13 ±2 min) and cooled to a temperature of (39 ±1 °C) skim milk, 0.7 g of dry sour-dough was added. To activate microbial cells, the concentrate was thoroughly mixed and kept for 4.0 h at a temperature of (37 ±1 °C). After 1 and 2 h after the start of activation, the bacterial suspension was stirred again (by shaking) to evenly distribute the bacterial cells throughout the mass. The resulting activated concentrate (without the formation of a clot) immediately after activation was introduced with stirring into 900 g of pasteurized (92 ±2 °C, exposure 2 – 8 min) and chilled (37 – 42 °C) milk. Fermentation was carried out at temperatures in the range (37 – 42 °C), with an interval of 1 hour, the titratable and active acidity of the medium was monitored until the formation of a clot, the standard fermentation time, taking into account the acid-forming properties of the strains of Lacto- and Bifidobacteria included in the consortium was 12 – 14 hours. The titratable acidity of the test samples was 80 – 100 °T, pH = 4.61 – 4.65.

- **Number of samples analyzed:** 736.
- **Number of repeated analyses:** 36*3=2208.
- **Number of experiment replication:** 1.

The amount of added wheat germ oil (3.5 g / day) corresponded to the satisfaction of 50% of the body's daily need for vitamin E. Contraindications to the use of wheat germ oil are the period of exacerbation of cholecystitis, gastritis, hepatitis, or cholelithiasis, as well as individual intolerance.

The mass of the injected concentrated tissue fish oil (6.5 g / day) was determined by 50% of satisfying the daily requirement of the body in omega-3 polyunsaturated fatty acid. Contraindication to admission is a pronounced exacerbation of chronic cholecystitis and pancreatitis, individual intolerance.

The stability of emulsions was at least 70 – 75% because of the high exopolysaccharide activity of the biomass (Rodionova, Popov and Rodionova, 2019; Zuo, Chen and Marcotte, 2020). These food forms were consumed by volunteers regardless of food intake, without correction of the basic diet. The duration of the experimental studies was 30 days; at the beginning and the end of the intake of bioactive nutrients, the studied parameters were controlled. On the day of sampling, the investigated bioactive nutrients were not given to the subjects; the time interval between using the investigated products and sampling was no less than 24 hours.

Blood was analyzed by a hardware automatic method using a Cobas 6000 diagnostic immunoassay analyzer (F. Hoffmann-La Roche Ltd, Switzerland), which is based on the electrochemiluminescence method. The atherogenic index (AI) was determined by calculation. Blood was collected from the cubital vein at least 12 hours after the last meal. The lipid metabolism parameters were monitored before and after 30 days of taking the test product (Grigoriev, 2015).

**Statistical Analysis**

The reliability of the data presented is confirmed by the values of the Mann-Whitney test (Table 3). Statistica 13 software (developed by StatSoft Russia) was used for statistical analysis.

The results obtained by the authors agree with experimental data on the study of the impact of policosanol, isolated from the sugar cane wax mass, on the stabilization and subsequent approximation to the norm of the lipid metabolism parameters in the human body. A comparative assessment of the biocorrective influence of WGO and WGCF on the lipid metabolism parameters, in the case of an identical concentration of the target biologically active substance, showed that WGCF has a more intense effect on the studied parameters.

**RESULTS AND DISCUSSION**

All performed calculations show p<0.05.

Analysis of lipid metabolism indicators for three age groups before the start of the experiment showed that the TC level in the first two groups of the surveyed participants was within the borderline level – 5.03 mmol/l; in the third group, it exceeded the norm: 5.82 mmol/l (norm <5.2 mmol/l). The HDL content in the subjects’ blood serum was below the norm: 1.21 mmol/l (norm >1.42 mmol/l) in all groups. The LDL content was within the norm: 3.12 mmol/l in the first two groups, and the third group, it exceeded the norm: 3.61 mmol/l (norm <3.37 mmol/l). The TG concentration was within the normal range: 1.66 mmol/l in the first group; while in the second and third group it exceeded the norm: 1.98 mmol/l (norm ranging within 0.14-1.82 mmol/l). In the first age group, the atherogenic index (AI) was within the normal range (2.44 units), while in the second and third age groups it exceeded the norm amounting to 3.71 units (norm ranging within 2.0-3.0 units). Thus, certain lipid metabolism disorders of varying degrees were recorded practically in all age groups. It is known that the significance of the HDL concentration in the body is primarily determined by their participation in the cholesterol homeostasis in blood serum and tissues due to their involvement in the so-called reverse cholesterol transport (Meerson et al, 1993; Jamshidi et al, 2020; Longo, Ferrari and Zocchi, 2017).

Evaluation of changes in lipid metabolism after 30 days of taking WGO and WGCF showed that the TC level decreased by 6.4 and 6.0%, the HDL concentration increased by 13.7 and 12.3%, the LDL concentration decreased by 11.3 and 13.1 %, the TG level decreased by 17.1 and 13.1% with a corresponding decrease in AI by 22.6 and 22.1%, respectively (Figure 1).

Previous experimental studies on the correction of lipid metabolism disorders in patients with arterial hypertension showed that after 30 days of taking CTFO, there was a fixed decrease in TC concentration by 7.5%, TG by 23%, AI by 23.6%, LDL by 12.9% and an increase in HDL by 15.6% (Meerson et al, 1993; Jamshidi et al, 2020).
When comparing the results obtained with the patients’ age as a result of the reception WGO and WGCF, a 6.8% decrease in the TC level was recorded in the first age group with the LDL concentration decreasing by 6.9%, TG by 18.2%, AI by 22.9%, and the HDL concentration increasing by 13.3%. In the second group, the HDL concentration increased by 12.2%, TC, LDL, TG decreased by 5.9, 13.9, and 16.5%, respectively, the AI value decreased by 21.5%. In the third group, there was a decrease in the TC, LDL, and TG concentration by 6.1, 15.1, 16.6%, respectively, an increase in the HDL concentration by 13.8%, and a positive correction of the AI values by 22.4% (Figure 2 and Figure 3).

The calculated values of the Mann-Whitney test do not exceed its critical parameters for each age group, which indicates the reliability of the revealed differences in the values of the studied indicators and suggests the adequate effectiveness of the WGO and WGCF influence on the lipid metabolism parameters in the human body (Table 2).

The possibility of increasing the performance of target biologically active substances through the use of probiotic components that perform the functions of transfer and targeted delivery in various body systems made it possible to proceed to the next stage of the study – a comparative assessment of the corrective action of WGO- and CTFO-containing probiotic emulsions (Roobab et al, 2020; Teneva-Angelova et al, 2018; Zuo, Chen and Marcotte, 2020).

It was found that the combined use of the investigated bioactive nutrients with probiotic microorganisms is specific for each bioactive nutrients under study (Figure 4). The CTFO-containing probiotic emulsion showed a more pronounced impact on lipid metabolism compared to the WGO-containing emulsion. (Isaev and Simonenko, 2016; Rodionova et al, 2016; Vishnyakov et al, 2020; Boukid et al, 2018; Arabidze and Tebloev, 2008; Popov et al, 2016). In the case of WGO- and CTFO-containing probiotic emulsion intake, TC level decreased by 8.3 and 9.1%, HDL concentration increased by 21.9 and 26.1%, LDL concentration decreased by 13.8 and 14.7%, TG level decreased by 22.8 and 24.1%, positive AI correction was 28.9 and 31.2%, respectively, which indicates an increase in the nutrient correction effectiveness in the presence of probiotic microorganisms (Meerson et al, 1993; Jamshidi et al, 2020; Longo, Ferrari and Zocchi, 2017). The WGO and CTFO effectiveness increased in terms of the decreased TC level by 1.9 and 1.6 %, increased HDL concentration by 8.2 and 10.5 %, decreased LDL concentration by 2.5 and 1.8 %, decreased TG concentration by 5.7 and 1.1 %, and reduced AI values by 6.3 and 7.6 %.

The results of experimental studies allow for the statement of the vital need to expand the range of products containing combinations of bioactive nutrients and probiotic microorganisms in a viable form. (Meerson et al, 1993; Jamshidi et al, 2020; Grigoriev, 2015; Rodionova et al, 2019c; Angelin and Kavitha, 2020; Yang et al, 2020; Zamroziewicz et al, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017). The development of formulations and technologies for these products is an urgent task at the present stage of developing food technologies that have the properties of pharmaceuticals intended for preventing pathological conditions, providing health preservation, and improving the quality of life. (Rodionova, Popov and Sokolova, 2016; Arruzazabala and Carbajal, 2012) (Arruzazabala, Valdes and Mas, 2013; Canetti, 1997; Yang et al, 2020; Zamo, 2017).
Figure 1 Concentrations in blood: a – blood TC level, mmol/l; b - HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d - AI, units. 1 (4) – prior to WGO (WGCF) intake; 2 (5) – 30 days after WGO (WGCF) intake; 3 (6) – control group (during the entire period); 7 – reference interval.

Figure 2 Concentrations in blood: a – blood TC level, mmol/l; b - HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d – A I, units. 1, 4, 7 – prior to WGO intake in the 1st, 2nd, and 3rd age groups; 2, 5, 8 – 30 days after WGO intake in the 1st, 2nd, and 3rd age groups; 3, 6, 9 – control group (during the entire period); 10 – reference interval.
Figure 3 Concentrations in blood: a – blood TC level, mmol/l; b – HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d – AI, units. 1, 4, 7 – prior to WGCF intake in the 1st, 2nd and 3rd age groups; 2, 5, 8 – 30 days after WGCF intake in the 1st, 2nd, and 3rd age groups; 3, 6, 9 – control group (during the entire period); 10 – reference interval.

Figure 4 Concentrations in blood: a – blood TC level, mmol/l; b – HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d – AI, units. 1 (4) – prior to WGO (CTFO) intake; 2 (5) – 30 days after WGO (CTFO) intake; 3 (6) – control group (during the entire period); 7 – reference interval.
**Figure 5** Concentrations in blood: a - blood TC level, mmol/l; b - HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d – AI, units. 1, 4, 7 – prior to the WGO-containing probiotic emulsion intake course in the 1st, 2nd and 3rd age groups; 2, 5, 8 – 30 days after the WGO-containing probiotic emulsion intake course in the 1st, 2nd, and 3rd age groups; 3, 6, 9 – control group (during the entire period); 10 – reference interval.

**Figure 6** Concentrations in blood: a – blood TC level, mmol/l; b - HDL/LDL cholesterol level, mmol/l; c – TG level, mmol/l; d – AI, units. 1, 4, 7 – prior to the CTFO-containing probiotic emulsion intake in the 1st, 2nd and 3rd age groups; 2, 5, 8 – 30 days after the CTFO-containing probiotic emulsion intake in the 1st, 2nd and 3rd age groups; 3, 6, 9 – control group (during the entire period); 10 – reference interval.
CONCLUSION
The results of experimental studies illustrate the dependence of the patients’ age and the achieved antiatherosclerotic effect on the intake of the probiotic forms under study. As a result of the reception WGO and CTFO in the first age group, the outcomes demonstrated a decrease in the TC level, LDL concentration, TG level, and AI values by 9.2, 9.6, 23.1, 27.8%, respectively, and an increase in the HDL concentration by 18.2%. The second group showed a decrease in the TC level by 8.4%, an increase in the HDL concentration by 19.8%, a decrease in the LDL concentration by 15.5%, a decrease in the TG concentration by 20.8%, and a positive correction of AI values by 30.2%. In the third group, the TC level decreased by 8.9%, with a decrease in the LDL concentration by 17.7%, the TG level by 24.6%, AI values by 22.6%, and an increase in HDL concentration by 23.4%.

The results of experimental studies illustrate a decreased level of TC, an increased concentration of HDL, a decreased concentration of LDL, a decreased concentration of TG, and AI reduction in all age groups when WGO, WGOM, and CTFO are added to the main diet. Evaluation of the combined influence of the viable forms of probiotic microorganisms together with the studied bioactive nutrients on the lipid metabolism indicators showed a synergistic effect in all age groups. It is fair to state that the bioactive nutrients under study, WGO, WGCF, and CTFO, can be attributed to alimentary factors of non-entropic action, ensuring the restoration of the equilibrium state of the body systems responsible for lipid metabolism.

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**Funds:** This research was supported by the Russian Science Foundation (agreement No. 19-76-10023).

**Acknowledgments:** -

**Conflict of Interest:** No conflict of interest.

**Ethical Statement:** The design project of the study was subjected to an ethical review conducted by the ethical committee at the Voronezh State Medical University named after N.N. Burdenko of the Ministry of Health of the Russian Federation (approved by order of the rector No. 303 of 04.10.2006). The draft of this study was approved at a meeting of the committee of the ethics committee.

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