UDC 616.341-008.6-06:[616.98:578.823.91]-053.4-074
DOI: 10.14739/2310-1210.2021.5.231265

Pathogenetic role of intestinal microflora in carbohydrate malabsorption syndrome in early-aged children with rotavirus infection

N. V. Vorobiova *A,B,C,D, **O. V. Usachova** A,E,F, **A. H. Kaplaushenko** C,E,F

Zaporizhzhia State Medical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim is to evaluate the effect of metabolic activity of intestinal microflora on laboratory manifestations of carbohydrate malabsorption syndrome in early-aged children with rotavirus infection by studying the main intestinal metabolites – short-chain fatty acids in faeces.

Materials and methods. 60 breastfed children with rotavirus infection aged 1–24 months were examined. The total amount of carbohydrates in faecal samples was determined using Benedict's test, and the detection of short-chain fatty acids in faeces was carried out using liquid chromatography in the dynamics of the disease – on the 3rd, 5th and 10th day.

Results. It was established, that the amount of reducing sugars in faeces decreased with the increasing concentration of intestinal microflora metabolites during the entire period of rotavirus infection. In patients with ≤0.5 % level of carbohydrates in faeces, the total content of short-chain fatty acids was the highest and exceeded by 2.0, 1.8 and 1.7 times the indicators of children with Benedict's test values >0.5 % on the 3rd, 5th and 10th days of the disease, respectively ($P < 0.05$). A decrease in the metabolic activity of the intestinal microbiota in the above category of children occurred mainly due to a deficiency in the production of acetic and propionic acids ($P < 0.05$). In the acute period of the disease, an imbalance in the intestinal microbiota infrastructure was noted towards a deficiency of obligate anaerobes that was expressed in a decrease in the anaerobic index ($P < 0.05$). The severity of these disorders influenced the severity of carbohydrate malabsorption: with an increase in the level of undigested sugars in faeces >0.5 %, the anaerobic index values were 3 and 5 times lower than at a carbohydrate level ≤0.5 % on the 3rd and 5th days of the disease, respectively ($P < 0.05$).

Conclusions. It has been found that early-aged children with rotavirus infection have structural and functional disorders of the intestinal microflora, which influence the severity of carbohydrate malabsorption syndrome. The lower the saccharolytic activity of intestinal bacteria, the more pronounced the manifestations of this syndrome during the entire period of the disease.

Key words: rotavirus infection, early age children, carbohydrate malabsorption syndrome, gut microbiota, short-chain fatty acids.

Zaporozhye medical journal 2021; 23 (5), 683-690

***E-mail:** Natahich19881988@gmail.com

Патогенетична роль кишкової мікрофлори в синдромі мальабсорбції вуглеводів у дітей раннього віку з ротавірусною інфекцією

Н. В. Воробйова, О. В. Усачова, А. Г. Каплаушенко

Мета роботи – оцінити вплив ферментативної активності кишкової мікрофлори на лабораторні прояви синдрому мальабсорбції вуглеводів при ротавірусній інфекції в дітей раннього віку шляхом вивчення основних кишкових метаболітів – коротколанцюгових жирних кислот (КЖК) у фекаліях.

Матеріали та методи. Обстежили 60 дітей із ротавірусною інфекцією віком 1–24 міс. на грудному вигодовуванні. Проведено визначення загальної кількості вуглеводів у фекаліях за допомогою проби Бенедикта та детекцію коротколанцюгових жирних кислот у випорожненнях методом рідинної хроматографії в динаміці хвороби – на 3, 5, 10 добу.

Результати. Встановили, що зі збільшенням кількості метаболітів кишкової мікрофлори знижувалася кількість неперетравлених цукрів у випорожненнях протягом усього періоду ротавірусної інфекції. У пацієнтів із рівнем вуглеводів у калі ≤0,5 % сумарний пул КЖК був найбільшим, перевищуючи у 2,0, 1,8 та 1,7 раза показники дітей зі значеннями проби Бенедикта >0,5 % на 3, 5 та 10 хвороби відповідно ($p < 0,05$). Зниження ферментативної активності кишкової мікробіоти в останній групі дітей відбувалося переважно внаслідок дефіциту продукції оцтової та пропіонової кислот ($p < 0,05$). У гострий період хвороби визначили дисбаланс інфраструктури кишкової мікробіоти – дефіцит облігатних анаеробів, що виявилось у зниженні анаеробного індексу ($p < 0,05$). Ступінь цих порушень впливав на виразність мальабсорбції вуглеводів: при підвищенні рівня неперетравлених цукрів у фекаліях >0,5 % спостерігали у 3 та 5 разів нижчі показники анаеробного індексу на 3 та 5 добу відповідно, ніж при рівні екскретованих цукрів ≤0,5 % ($p < 0,05$).

Висновки. У дітей раннього віку з ротавірусною інфекцією відбуваються структурні та функціональні порушення кишкової мікрофлори, що впливають на виразність синдрому мальабсорбції вуглеводів. Чим нижча ферментативна активність сахаролітичних кишкових бактерій, тим виразніші прояви цього синдрому протягом усього періоду хвороби.

Ключові слова: ротавірусна інфекція, діти раннього віку, синдром мальабсорбції вуглеводів, мікрофлора кишечника, коротколанцюгові жирні кислоти.

Запорізький медичний журнал. 2021. Т. 23, № 5(128). С. 683-690

Патогенетическая роль кишечной микрофлоры в синдроме мальабсорбции углеводов у детей раннего возраста с ротавирусной инфекцией

Н. В. Воробьёва, Е. В. Усачёва, А. Г. Каплаушенко

Цель работы – оценить влияние ферментативной активности кишечной микрофлоры на лабораторные проявления синдрома мальабсорбции углеводов при ротавирусной инфекции у детей раннего возраста путём изучения основных кишечных метаболитов – короткоцепочечных жирных кислот (КЖК) в кале.

Ключевые слова: ротавирусная инфекция, дети раннего возраста, синдром мальабсорбции углеводов, микрофлора, короткоцепочечные жирные кислоты.

Запорожский медицинский журнал. 2021. Т. 23, № 5(128). С. 683-690

Материалы и методы. Обследовали 60 детей с ротавирусной инфекцией в возрасте 1–24 мес. на грудном вскармливании. Проведено определение общего количества углеводов в кале с помощью пробы Бенедикта и детекция короткоцепочечных жирных кислот в кале методом жидкостной хроматографии в динамике болезни – на 3, 5 и 10 сутки.

Результаты. Установлено, что с увеличением количества метаболитов кишечной микрофлоры снижалось количество непереваренных сахаров в испражнениях в течение всего периода ротавирусной инфекции. У пациентов с уровнем углеводов в кале ≤0,5 % суммарный пул КЖК был наивысшим, превышая в 2,0, 1,8 и 1,7 раза показатели детей со значениями пробы Бенедикта >0,5 % на 3, 5 и 10 сутки болезни соответственно ($p < 0,05$). Снижение ферментативной активности кишечной микробиоты в последней группе детей происходило преимущественно за счёт дефицита продукции уксусной и пропионовой кислот ($p < 0,05$). В острый период болезни отмечен дисбаланс инфраструктуры кишечной микробиоты в виде дефицита облигатных анаэробов, что выражено в снижении анаэробного индекса ($p < 0,05$). Степень этих нарушений влияла на выраженность мальабсорбции углеводов: при повышении уровня непереваренных сахаров в кале >0,5 % значения анаэробного индекса были в 3 и 5 раз ниже, чем при уровне углеводов ≤0,5 % на 3 и 5 сутки болезни соответственно ($p < 0,05$).

Выводы. У детей раннего возраста с ротавирусной инфекцией отмечены структурные и функциональные нарушения кишечной микрофлоры, которые влияют на выраженность синдрома мальабсорбции углеводов. Чем ниже сахаролитическая активность кишечных бактерий, тем более выражены проявления данного синдрома в течение всего периода болезни.

Rotavirus infection (RVI) is the global leading cause of diarrhea-associated morbidity and mortality among children younger than 5 years [1,2]. It causes about 450.000 deaths per year in children under 5 years and millions of hospitalizations due to the development of severe dehydrating gastroenteritis [3].

Pathogenetic features of RVI are the study subject for a lot of researchers at the present stage [4–7]. One of the main pathogenetic mechanisms of rotavirus gastroenteritis is carbohydrate malabsorption syndrome, which develops due to decreased surface activity of enterocyte disaccharides, dysfunction of the sodium-dependent glucose transporter and leads to osmotic diarrhea [4]. It has been shown that intestinal carbohydrate metabolism disorders, mainly due to lactase deficiency, play a major role in causing and maintaining rotavirus diarrhea from the height of the disease, and especially at the end of the first to the beginning of the second week of gastroenteritis [5]. It is believed, that clinical manifestations of carbohydrate malabsorption syndrome in children can vary depending on the combined effect of several factors: the level of lactase-phlorizin hydrolase (LPH) activity, the amount of lactose that comes with food, individual intestinal sensitivity and condition of the intestinal flora [8,9]. This is also evidenced by the fact that lactose maldigesters with the same fermentation rate in the small intestine and the same time of intestinal transit have different intensity of lactose malabsorption (from mild symptoms to diarrhea) [9] and proves the influence of other factors, such as metabolic activity of the intestinal microbiota, on the development of this syndrome. In addition, it is known that the osmotic type of diarrhea develops only when the amount of carbohydrates entering the colon exceeds the ability of the microflora to ferment them due to microbial digestion deficiency [4]. In our opinion, the study of the intestinal microbiota functional status, as an additional pathogenetic factor in the development of osmotic diarrhea in children with rotavirus gastroenteritis, is important and appropriate because it will allow adequate correction and prescription of pathogenetic therapy in the dynamics of the disease.

Microbiota of the lower sections of the intestinal tract is represented by obligate and facultative anaerobes (aerobes), which harvest energy from incomplete oxidation of organic compounds, resulting in the formation of final products – short-chain fatty acids (SCFA) [7]. SCFA are carboxylic acids with a chain length of up to 6 hydrogen atoms. The major SCFAs formed by the gut bacteria are acetate (C2), propionate (C3) and butyrate (C4) which account for approximately 90 % of all SCFAs. The main source of SCFAs are carbohydrates, but aminoacids valine, leucine and isoleucine are protein breakdown products, which can be converted to isobutyrate, isovalerate and isocapronic acid, which make a small (5 %) contribution to the total production of SCFA [7,10].

C2 is the main SCFA in the colon. The major part of it is synthesized by most intestinal bacteria, both anaerobes, such as *Prevotella spp., Ruminococcus spp., Bifidobacterium spp., Bacteroides spp., Clostridium spp., A. muciniphila, B. hydrogenotrophica* and facultative anaerobes including *Streptococcus, E. coli, Staphylococcus, Proteus* and others from uncleaved oligosaccharides by hydrolysis of acetyl-CoA [10,11]. Although propionate-producers are distributed across several phyla, only a few bacterial genera are able to form propionate, and unlike acetate, the utilized propionate pathways are more conserved and substrate specific. Colon bacteria form C3 in three ways: succinate, acrylate and propanediol in strictly anaerobic conditions. Its producers are *Veilonella, Propionibacterium, Bacteroides, Fusobacterium, Clostridium, Gaffkya,* etc. [7,10]. C4 production, like C3, is more conserved and substrate specific. The main synthetic pathway of C4 formation in the intestine is the butyryl-CoA route, which uses such obligate-anaerobic microorganisms, as *Fusobacterium*, *Eubacterium*, *Coprococcus*, *Bacteroides*, *Megasphaera*, *Clostridium* [7,10,11].

Aim

The purpose is to evaluate the effect of metabolic activity of intestinal microflora on laboratory manifestations of carbohydrate malabsorption syndrome in early-aged children with rotavirus infection by studying the main intestinal metabolites – short-chain fatty acids in faeces.

Materials and methods

The prospective study involved 60 children with rotavirus gastroenteritis aged 1–24 months (63.3 % – boys, 36.7 % – girls), who were treated in the Department number 4 of the Municipal Institution "Zaporizhzhia Regional Infectious Clinical Hospital" of the Zaporizhzhia Regional Council. There were the criteria for inclusion in the study:

hospitalization within the first two days from the onset of the disease, detection of rotavirus antigen in the faeces by immunochromatographic method (CITO TEST ROTA test system, Pharmasco, Ukraine), no excretion of pathogenic bacterial flora in the faeces, no congenital or chronic immunocompromised pathology, being breastfed, availability of the informed parental consent for their child participation in a clinical trial. Children were divided into three subgroups by age: 1–6 months – 23.3 % (14 children), 6–12 months – 30.0 % (18 children), 12–24 months – 46.7 % (28 children).

Daily clinical examination, including analysis of the intensity and dynamic changes of the disease leading symptoms, and the standardized range of the clinical and laboratory studies were performed for all children of the study group. The severity of rotavirus gastroenteritis was determined by the Vesikari scale [12]. 70.0 % of children had a severe course of RVI, 23.3 % – moderate-severe, 6.7 % – mild course.

The severity of carbohydrate malabsorption in the RVI dynamics was assessed by the total level of excreted carbohydrates in the faeces, which was determined semi-quantitatively at the beginning of the disease (the 3rd day), during fever (the 5th day) and convalescence (the 10th day) using Benedict's test based on the detection of reducing oligosaccharides (glucose, galactose, lactose, fructose, maltose) [13].

Metabolic activity of intestinal microflora was studied by determining its main metabolites – SCFA: acetate, propionate and butyrate in coprofiltrates. The SCFA spectrums were determined using the method of liquid chromatographic analysis in parallel with the determination of the total level of carbohydrates in the faecal samples (on the 3rd, 5th and 10th day of RVI). Testing was conducted at the Training and Laboratory Center of Zaporizhzhia State Medical University at the Department of Physical and Colloidal Chemistry (headed by Doctor of Pharmaceutical Sciences, Professor Kaplaushenko A. H.). The quantification of SCFA in faeces included two stages: coprofiltrate preparation and chromatographic analysis. The analysis was performed using a highly efficient liquid chromatographic system with mass spectrometric detection (HPLC–MS), consisting of a degasser (Agilent Technologies, Japan), a binary pump (Agilent Technologies, Germany), an autosampler (Agilent Technologies, Germany), a column thermostat (Agilent Technologies, Germany), diode-array detector (Agilent Technologies, Germany), Open LAB CDS software (Certificate of technical competence No. 33/18 dated 26.12.2018, valid until 25.12.2023).

The quantitative measurement of the SCFA concentration was carried out by comparing the peak areas of the determined substances with the peak area of the internal standard with a known concentration. Analytical standards of acetic (CH_3CO_2H, CAS Number 64-19-7, GC area ≥99.8 %), propionic (CH3CO2COOH, CAS Number 79-09-4, GC area ≥99.8 %) and butyric ($CH_3CH_2CH_2COOH$, CAS Number 107-92-6, GC area ≥99.5 %) acids (Sigma-Aldrich, USA) were used as the etalons.

Absolute content of acetic (C2), propionic (C3) and butyric (C4) acids were determined in faecal samples, as these metabolites make up 90–95 % of the total quantity of SCFA and are products of anaerobic carbohydrate fermentation [11,14]. Next, the total acids pool (C2 + C3 + C4), the relative concentration of each acid (that is equal to the ratio of the absolute concentration of this acid to the total concentration of all SCFA) and the values of the anaerobic index ((C3 + C4) / C2) were calculated in the study. The total concentration of SCFA (C2 + C3 + C4) in the faeces reflects the integrated metabolic activity of the intestinal microbiota in relation to carbohydrates, and its increase or decrease indicates the corresponding changes in the amount and / or enzymatic activity of the microflora. As an estimate of the redox balance in the lower intestine, an anaerobic index (AI) was used, which reflects the degree of anaerobiosis of the environment, since the producers of the least reduced acetic acid are most facultative anaerobes and some aerobes, while other SCFA (propionic and butyric) are metabolites of obligate anaerobes [4,14].

The indicators of healthy children were considered to be normal physiological concentrations of SCFA and carbohydrates in faeces. For this purpose, a comparison group was formed, which included 30 breastfed children, representative by age and sex, without signs of gastrointestinal dysfunction, who were examined by determining the faecal concentrations of SCFA and the level of reducing sugars in the faeces.

For statistical processing of the obtained data, a database was formed in Microsoft Excel 2010, on the basis of which further analysis of the results was performed using the packages of the program Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). The type of data distribution was determined using the Shapiro–Wilk test (null hypothesis about the normality of the distribution was rejected at a level of $P < 0.05$). Because the quantitative values had a distribution that was different from normal, nonparametric methods were used. Descriptive statistics were expressed as median (Me) and interquartile range (IQR: Q25–Q75). To assess the validity of the differences between the quantitative features in two independent groups, the Mann-Whitney criterion was used, in several independent groups – the Kruskal–Wallis test was performed. The reliability of the difference in the dynamics of the disease was assessed by the Wilcoxon test. The difference at $P < 0.05$ was considered statistically significant. The strength and direction of the relationship between quantitative values were determined using the Spearman correlation coefficient (r).

Results

In the way of evidence that carbohydrate malabsorption syndrome is an important link in the pathogenesis of RVI, the data obtained in the study suggest that it was diagnosed at the onset of the disease (the 3rd day) in 83.3 % (50/60) of children. In the dynamics of the disease, an increase in the total level of reduced sugars in faeces was noted in all children of the study group.

As a result of the study, the relationship between the severity of carbohydrate malabsorption and the metabolic activity of the intestinal microflora was revealed from the first days of RVI. Thus, on the 3rd day of RVI, a moderate inverse correlation between the total level of carbohydrates and the total SCFA concentration in the faeces was established ($r = -0.44$, $P < 0.05$). That is, with the increase in the amount of the intestinal microflora metabolites, which

Table 1. Comparative characteristics of the faecal concentration of SCFA on the 3rd day of RVI depending on the total amount of carbohydrates in the faeces (n = 60), Me [Q25; Q75]

Metabolites of intestinal microflora on the 3rd day of RVI		The level of carbohydrates in the faeces			P Kruskal–Wallis	Healthy children (n = 30)
		≤0.5 % (n = 29)	0.6–1.0 % (n = 12)	>1.0 % (n = 19)		
Total pool of SCFA, μmol/l		497.64 [256.88; 928.40][2]	226.16 [112.13; 356.01][1,2]	278.43 [207.60; 333.24][1,2]	P > 0.05	978.60 [681.83; 1286.05]
Concentration of each SCFA, μmol/l	Acetic	451.86 [350.68; 907.76][2]	218.92 [104.89; 348.32][1,2]	273.38 [203.57; 316.28][1,2]	P > 0.05	861.17 [606.26; 993.61]
	Propionic	14.62 [4.66; 109.88][1,2]	7.23 [0.00; 14.93][1,2]	4.37 [0.00; 17.97][1,2]	**P < 0.05**	97.49 [74.73; 183.85]
	Butyric	0.00 [0.00; 6.67][2]	0.00 [0.00; 0.15][2]	0.00 [0.00; 0.00][2]	P > 0.05	32.99 [14.43; 53.21]
Anaerobic index		0.06 [0.01; 0.17] [2]	0.02 [0.00; 0.05][1,2]	0.02 [0.00; 0.07][1,2]	P > 0.05	0.20 [0.11; 0.34]

[1]: P < 0.05 the difference is significant compared with children with carbohydrate level in the faeces ≤0.5 % according to the Mann–Whitney criterion; [2]: P < 0.01 compared with healthy children.

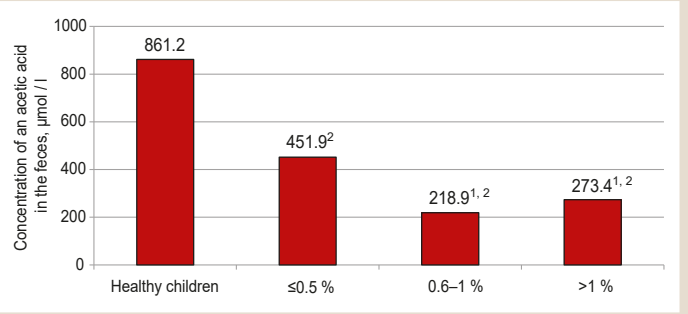

Fig. 1. Comparative characteristics of acetic acid concentrations in children depending on the total level of carbohydrates in the faeces on the 3rd day of RVI (n = 60).

[1]: P < 0.05 the difference is significant compared to children with the carbohydrate level in the faeces ≤0.5 % according to the Mann–Whitney criterion; [2]: P < 0.01 compared to healthy children.

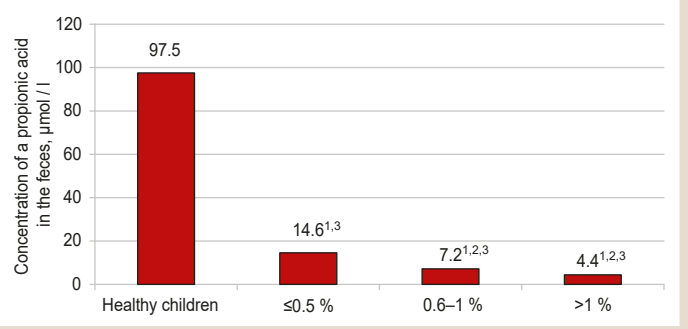

Fig. 2. Comparative characteristics of propionic acid concentrations in children depending on the total level of carbohydrates in the faeces on the 3rd day of RVI (n = 60).

[1]: P < 0.05 – the difference is significant according to the Kruskal-Wallis criterion; [2]: P < 0.05 – compared to children with the carbohydrate level in the faeces ≤0.5 % according to the Mann-Whitney criterion; [3]: P < 0.01 – compared to healthy children.

is an indicator of its overall enzymatic ability, the amount of undigested sugars in the faeces decreased. A similar dependence was observed with the concentrations of acetic (r = -0.41, P < 0.05) and propionic (r = -0.38, P < 0.05) acids.

To demonstrate these relationships clearly, we analyzed the total level of SCFA, as well as the absolute and relative concentrations of each acid and the value of the AI in patients depending on Benedict's test values on the 3rd day of RVI (*Table 1*). It was found that in patients with a minimum level of undigested sugars in the faeces – ≤0.5 %, the total pool of SCFA was the largest – 497.64 [256.88; 928.40] μmol/l, exceeding 2.2 and 1.8 times the values of children with faecal carbohydrate levels of 0.6–1.0 % and >1.0 % (P < 0.05), respectively, however, not reaching healthy children indicators (P < 0.01).

Absolute concentrations of the main metabolites of saccharolytic microflora, namely acetic and propionic acids, in this category of children were also significantly higher than those in patients of the second and third subgroups (P < 0.05), amounting to 451.86 [350.68; 907.76] μmol/l and 14.62 [4.66; 109.88] μmol/l, respectively. That is, compared to patients with minimal malabsorption of oligosaccharides, the decrease in the metabolic activity of the intestinal microbiota in patients with moderate (0.6–1.0 %) and high (>1.0 %) level of undigested sugars in the faeces, was mainly due to deficiency of C2 and C3 production in the first days of RVI. Thus, at this time of the disease, C2 concentrations in children with Benedict's test >0.5 % (second and third subgroups) were 2 and 1.5 times lower, respectively, than those in the first subgroup (P < 0.05) and as much as 4.3 and 3.5 times, respectively, lower than normal for this age (P < 0.01) (*Fig. 1*), reflecting a significant quantitative and functional deficiency of saccharolytic intestinal bacteria – producers of C2, both strict anaerobes (*Prevotella* spp., *Ruminococcus* spp., *Bifidobacterium* spp., *Bacteroides* spp., *Clostridium* spp., *A. muciniphila, B. hydrogenotrophica)* and facultative anaerobes (*Streptococcus* spp., *E. coli*, *Staphylococcus* spp., *Proteus* spp., etc.) in these patients.

Fig. 2 shows that the concentration of C3 also decreased with increasing excretion of oligosaccharides in the faeces (P < 0.05 in groups according to the Kruskal–Wallis test). In children with medium (0.6–1.0 %) and high (>1.0 %) levels of faecal sugars, the amount of C3 was twice and three times lower, respectively, than that in patients with minimal carbohydrate excretion (P < 0.05) and was only 1/13 and 1/22, respectively, of the level in healthy children (P < 0.01). All of this indicated a particularly pronounced insufficiency of obligate C3-producing anaerobes (*Veilonella*, *Propionibacterium*, *Bacteroides*, *Fusobacterium*, *Clostridium*, *Gaffkya*) of the epithelial layer within the intestinal mucosa in patients with high levels of excreted sugars, and emphasized their important role in the intestinal carbohydrate metabolism.

Faecal concentrations of C4 in children in the first days of RVI were very low – 0.00 [0.00; 0.58] μmol/l, against 32.99 [14.43; 53.21] μmol/l in healthy children (P < 0.01). It indicated a significant inhibition of activity of its producers – obligate anaerobes, such as *Fusobacterium*, *Eubacterium*, *Coprococcus*, *Bacteroides*, *Megasphaera*, *Clostridium*, *Peptococcus* in children with RVI in the early stages of the disease. A significant difference between the amount of C4 in children groups with different levels of undigested

sugars in the stool was not found, there was only a tendency to increase with a decrease in Benedict's test (P = 0.064 in groups according to the Kruskal–Wallis test). Thus, C4 in the faeces was not detected at all in 75 % of patients with the highest values of excreted sugars (>1.0 %) (*Table 1*).

Along with a decrease in the metabolic activity of saccharolytic intestinal bacteria, children of the study group showed a violation of the large intestine microbiota composition from the first days of RVI. Structural imbalance of the intestinal microflora was expressed in a statistically significant decrease in the relative concentrations of C3 and C4 (metabolites of anaerobic bacteria populations) and an increase in the proportion of C2 in the total pool of SCFA, and, accordingly, a decrease in AI compared to the healthy children. The degree of intestinal microecological imbalance in the form of its anaerobiosis decrease was comparable to the change in the amount of undigested sugars excreted in the faeces (*Fig. 3*).

During the height of the disease (on the 5th day), there was a deepening of the difference in the intestinal microbiota metabolic activity in children with different values of undigested sugars in the stool. Because none of the studied parameters in children with carbohydrate levels >0.5 % at this time of the disease did not differ statistically, they were combined into one group (n = 32). Exactly in this group of patients, there was a sharp depletion of the total pool of SCFA – 311.56 [160.16; 549.66] μmol/l, against 978.60 [681.83; 1286.05] μmol/l in the healthy children (P < 0.01), which reflected a sharp decrease in the fermentation of oligosaccharides by intestinal bacteria (*Table 2*). There was no statistically significant increase in C2 level relative to the first days of RVI in this group of children (P > 0.05 by the Wilcoxon test). C3 concentrations remained almost at the initial level (P > 0.05) and were very low – 6.65 [0.15; 10.45] μmol/l (P < 0.01 relative to the normal values). C4 in the minimum amount was detected only in 12.5 % (4) of patients.

In children with low carbohydrate excretion (≤0.5 %), almost 2 times higher total amount of SCFA was observed – 569.42 [380.00; 813.86] μmol/l, than that in children of the previous group (P < 0.05) in the height of the disease. It increased in this period mainly due to an increase in the C3 concentration, which was 8 times higher than the level of C3 in children with a more pronounced violation of carbohydrate metabolism (>0.5 %) (P < 0.01).

Analysis of the structural imbalance of the intestinal environment during the peak of RVI showed that all children had a decrease in AI (P < 0.01 compared with the healthy children), due to an increase in the proportion of C2 and a decrease in the relative concentrations of C3 and C4, which reflected the persistence of functional and quantitative deficiency of anaerobic bacteria. However, in the group of children with a low level of excreted sugars in the faeces (≤0.5 %) in the dynamics (on the 5th day) of the disease, the proportion of C2 decreased slightly (relative to the first days of RVI), reaching the upper normal limit, and amounted to 91.8 %, while the relative concentration of C3 increased to 7.7 %, reaching the lower normal limit for this age (*Fig. 4*). AI in them rose in dynamics to 0.085 [0.029; 0.230], being 5 times higher than that in children with severe carbohydrate malabsorption (P < 0.01), however, remaining more than 2 times lower than AI in the healthy children.

Table 2. Comparative characteristics of the faecal concentration of SCFA on the fifth day of RVI depending on the total amount of carbohydrates in the faeces (n = 60), Me [Q25; Q75]

Metabolites of intestinal microflora on the 5th day of RVI		The level of carbohydrates in the faeces		Healthy children (n = 30)
		≤0.5 % (n = 28)	>0.5 % (n = 32)	
Total pool of SCFA, μmol/l		569.42 [380.00; 813.86][1,3]	311.56 [160.16; 549.66][3]	978.60 [681.83; 1286.05]
Concentration of each SCFA, μmol/l	Acetic	442.05 [291.35; 750.82][1,3]	306.89 [153.36; 543.98][3]	861.17 [606.26; 993.61]
	Propionic	53.97 [6.22; 97.95][2,3]	6.65 [0.15; 10.45][3]	97.49 [74.73; 183.85]
	Butyric	1.33 [0.00; 15.87][2, 3]	0.00 [0.00; 0.00][3]	32.99 [14.43; 53.21]
Anaerobic index		0.085 [0.029; 0.230][2,3]	0.017 [0.000; 0.041][3]	0.200 [0.110; 0.340]

[1]: P < 0.05 compared to the children with carbohydrate level in the faeces >0.5 % according to the Mann–Whitney criterion; [2]: P < 0.01 compared to the children with carbohydrate level in the faeces >0.5 %; [3]: P < 0.01 compared to the healthy children.

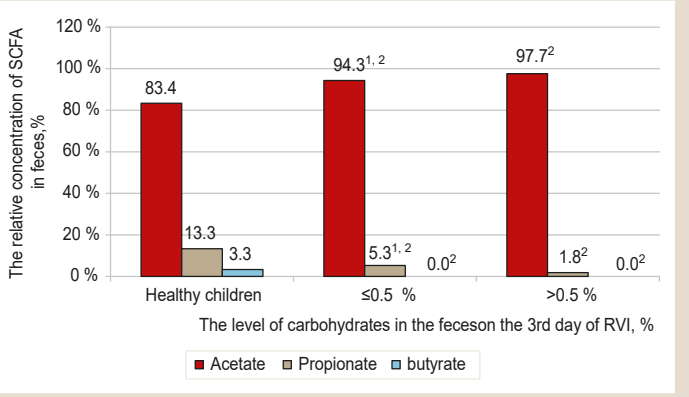

Fig. 3. Comparative characteristics of relative concentrations of SCFA in children with RVI (n = 60) on the third day of illness depending on the concentration of carbohydrates in the faeces.

[1]: P < 0.05 the difference is significant compared to the children with carbohydrate level in the faeces >0.5 % according to the Mann–Whitney criterion; [2]: P < 0.01 compared to the healthy children.

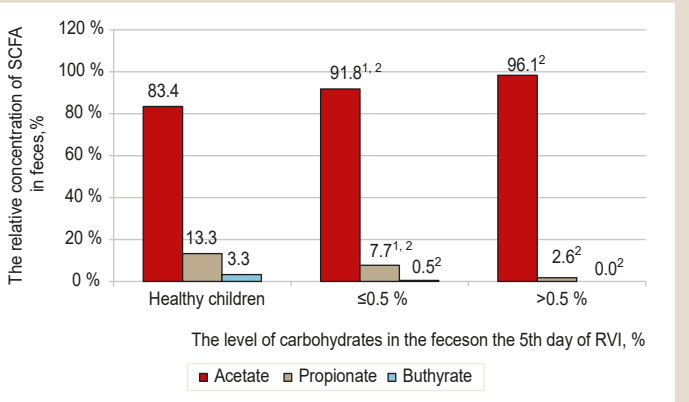

Fig. 4. Comparative characteristics of relative concentrations of SCFA in children with RVI (n = 60) on the 5th day of illness depending on the concentration of carbohydrates in the faeces.

[1]: P < 0.01 the difference is significant compared to the children with carbohydrate level in the faeces >0.5 % according to the Mann-Whitney criterion; [2]: P < 0.01 compared with the healthy children.

Analysis of the quantitative composition of intestinal metabolites in patients in the convalescent period of RVI showed a slight increase in the metabolic activity of saccharolytic bacteria relative to the acute period of RVI in all patients, however, with a persisting statistically significant

difference in the total pool of SCFA between the groups with the level of excreted sugars in the faeces ≤0.5 % and >0.5 % – 660.35 [391.71; 769.93] μmol/l against 397.43 [314.75; 968.51] μmol/l, respectively (P < 0.05). It should be noted that in both groups, these indicators remained significantly lower than those in the healthy children (P < 0.01) reflecting the continuous disorders of colon metabolic status, even in the late stages of the disease. Absolute concentrations of C2 and C3 were still statistically different in the groups, amounting to 586.47 [364.59; 749.00] μmol/l and 46.11 [17.13; 133.24] μmol/l, respectively, in patients with a residual sugar level in the faeces ≤0.5 %, which was 1.5 and 2 times higher, respectively, than that in children with faecal sugar levels >0.5 % (P < 0.05).

It was noted that the concentration of C3 in patients with severe carbohydrate malabsorption increased slightly, amounting to 23.36 [7.91; 41.63] μmol/l, compared to the previous values only on the 10th day of RVI, which indicated a very slow recovery of functional and quantitative deficiency of its producers – obligate anaerobes (*Veilonella*, *Propionibacterium*, *Bacteroides*, *Fusobacterium, Clostridium*, etc.) in this children category. Dynamic changes in the amount of C4 even in the convalescent period of the disease were not observed in both subgroups. They remained minimal and did not differ statistically – 0.00 [0.00; 3.61] μmol/l and 0.31 [0.00; 1.86] μmol/l in the first and second subgroups, respectively (P > 0.05).

It was found that on the 10th day of RVI, the difference in relative concentrations of all SCFA and AI in children with different values of Benedict's test was diminished. Thus, AI was almost the same in patients of the first and second subgroups, amounting to 0.093 [0.023; 0.250] and 0.087 [0.010; 0.130], respectively, remaining more than twice lower than the age norm (P < 0.01). That is, in the late stages of RVI, the severity of oligosaccharide malabsorption depended largely on the decrease in the metabolic status of the intestinal microflora.

Discussion

It is known that undigested lactose in the small intestine is hydrolyzed by β-galactosidase of colon bacteria to galactose and glucose. 80.6 % of faecal bacteria synthesize the enzyme β-galactosidase that indicates its high activity in the colon. Galactose is metabolized to glucose by the Leloir pathway with further fermentation to SCFA, CO_2, H_2 and CH_4 [9]. The osmotic load in the colon is increased about eightfold by fermentation of lactose to SCFA [8]. However, it is believed that SCFA are rapidly absorbed through the colon mucous membrane (their absorption rate is 6.1–12.6 μ mol/cm^2 x g), only 2–4 % of volatile acids is excreted in the feces [9,15,16]. From the literature review, published by B. Misselwitz, M. Butter et al., it is known, that osmotic diarrhea occurs only in case of insufficient fermentation of undigested lactose or its metabolites (glucose, galactose) by intestinal microflora or inefficient removal of terminal carbohydrate metabolites (SCFA) due to low absorption capacity of the colon. A higher enzymatic capacity of the colon microbiota relative to lactose and monosaccharides may reduce the intensity of malabsorption syndrome manifestations with normal absorption of volatile acids [8]. This statement is supported by the study results [4]: in RVI children with normal levels of residual carbohydrates in the faeces (0.25 ± 0.06 %), the total concentration of SCFA observed in the acute period of the disease was twice as high as the normal value, as a result of a sharp rise in sugar fermentation by intestinal bacteria. In all children with complete fermentation of sugars, normalization of stool occurred before the 5th day of illness.

In addition, the presence of intestinal microbiota structural and functional changes [16] and their role in increasing the severity of RVI clinical manifestations, intestinal microbiota are noted in a number of modern literature sources studying the intestinal microbiocinosis state in children with rotavirus gastroenteritis [4,7,17]. However, significant differences in the results of SCFA detection have been identified by some researchers. Thus, L. LI, D. Huang showed a lowering of only the level of lactic acid (*Bifidobacterium* and *Lactobacillus metabolites*) in infants with RVI in the absence of changes in total pool of SCFA and C2–C4 concentrations [16]. Other researchers emphasized a significant decrease in the total amount of volatile acids and absolute concentrations of each of them (C2–C5) in the acute period of RVI [7,17], demonstrating a sharp decrease in anaerobic metabolism of oligosaccharides as a result of impaired intestinal microbiocinosis. Meskina E. R. has proved, that the deficiency of microflora metabolic activity in the acute period of RVI is associated with impaired carbohydrate metabolism in the large intestine in children: with increasing content of excreted sugars, concentrations of acetic and propionic acids decreased, the main products of bacterial fermentation of carbohydrates (r = -0.34/0.35, P < 0.01) [4]. However, we have not found any information in the scientific works about the influence of the intestinal microflora structural and functional state on the manifestations of carbohydrate malabsorption syndrome in the dynamics of RVI in children.

The results of our study have confirmed the previous data and proved that dysbiotic changes in the intestine develop in all patients in the early stages of RVI, which is expressed in a decrease in absolute concentrations of all saccharolytic microflora metabolites: C2–C4 and a significant decrease in AI. Our results are confirmed by modern literature data about low Shannon's and Simpson's indices in young children with RVI, which characterize the species diversity of the intestinal microbiota [16]. The degree of the intestinal microflora metabolic and structural disorders is correlated with an increase in the amount of undigested sugars in the faeces, which, according to the literature [4,5], is the pathogenetic basis for the osmotic diarrhea development in RVI. The lowest values of SCFA and AI concentrations in patients with significant (>0.5 %) faecal levels of residual carbohydrates indicate a pronounced violation of the oligosaccharide fermentation by bacteria and structural changes in the intestinal environment. A significant reduction in the C3 and C4 profiles in this group of children during the entire period of the disease (up to the 10th day) with increased C2 relative concentration may indicate long-term inhibition of C3 and C4 producers – obligate anaerobes and rise in the proportion of aerobic (optional-anaerobic) opportunistic bacteria in the microbiocynosis.

It was shown in the study [4], that only children with a past history (recently suffered from acute intestinal in-

fections and acute respiratory viral infections) of RVI had an increase in residual sugar in the stool (1.1 ± 0.08 %, P < 0.001) in the midst of a sharp decrease in saccharolytic intestinal microbiota function, which was expressed in the deficiency of the SCFA general pool, while in children without premorbid conditions, the microflora responded to disaccharide deficiency by hyperreactivity and completely metabolized undigested carbohydrates. We have observed the development of carbohydrate malabsorption syndrome associated with structural and metabolic imbalance of intestinal microflora of varying severity in all children of the study group (however, as in the previous study, the degree of increase in the faecal residual sugar was inversely correlated with the total number of saccharolytic microflora metabolites in the acute period of RVI). Such differences can be explained by a larger sample of the study group by age (0–14 years) in the study [4], because it is known that children older than 1 year have less lactose load (due to diet), higher metabolic capacity and diversity of intestinal microbiota, than infants [18].

Conclusions

1. There is a decrease in the intestinal microbiota saccharolytic activity and structural imbalance in early-aged children with RVI during the entire period of the disease (from the 3rd to and including the 10th day), which is expressed in a decrease in the total amount of SCFA, absolute concentrations of C2, C3 and C4 – major metabolites of saccharolytic bacteria, and a decrease in the AI (P < 0.01).

2. The severity of carbohydrate malabsorption syndrome is correlated with the degree of violation of oligosaccharide fermentation by intestinal bacteria from the first days of RVI and up to the convalescence period: the level of undigested sugars in the faeces >0.5 % is associated with 2.0, 1.8 and 1.7 times lower total concentrations of SCFA on the 3rd, 5th and 10th days of illness (P < 0.05), respectively, due to primarily decrease in acetic and propionic acids, in comparison to children with low indicators of Benedict's test – ≤0.5 % (P < 0.05).

3. The degree of intestinal anaerobiosis reduction influences the severity of the intestinal carbohydrate metabolism disorders in the early stages of RVI and in the height of the disease: increased level of undigested sugars in the faeces >0.5 % is associated with 3 and 5 times lower values of AI on the 3rd and 5th days, respectively, than in minimal sugar excretion (≤0.5 % in the faeces) (P < 0.05) and more than 10 times lower AI in comparison to the healthy children (P < 0.01).

4. The rise in the total amount of reducing sugars in the stool reflects not only the severity of violation of the oligosaccharide fermentation and absorption by the small intestinal enterocytes, but also the ability of intestinal microbiota to metabolize them.

Prospects for further research are to study the effect of functional and structural disorders of the intestinal microbiota on the clinical manifestations of carbohydrate malabsorption syndrome in early-aged children with RVI, as well as to study the degree of these disorders depending on age and premorbid conditions.

Acknowledgments
The authors would like to thank Associate Professor of the Department of Physical and Colloidal Chemistry, Zaporizhzhia State Medical University, B. O. Varynskyi for participation in the study.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 11.05.2021
Після доопрацювання / Revised: 09.06.2021
Прийнято до друку / Accepted: 16.06.2021

Information about authors:
Vorobiova N. V., MD, PhD student of the Department of Pediatric Infectious Diseases, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-7610-8067
Usachova O. V., MD, PhD, DSc, Professor, Head of the Department of Pediatric Infectious Diseases, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-8413-027
Kaplaushenko A. H., Dr.hab., Professor, Head of the Department of Physical and Colloidal Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0003-3704-5539

Відомості про авторів:
Воробйова Н. В., PhD аспірантка каф. дитячих інфекційних хвороб, Запорізький державний медичний університет, Україна.
Усачова О. В., д-р мед. наук, професор, зав. каф. дитячих інфекційних хвороб, Запорізький державний медичний університет, Україна.
Каплаушенко А. Г., д-р фарм. наук, професор, зав. каф. фізколоїдної хімії, Запорізький державний медичний університет, Україна.

Сведения об авторах:
Воробьёва Н. В., PhD аспирант каф. детских инфекционных болезней, Запорожский государственный медицинский университет, Украина.
Усачёва Е. В., д-р мед. наук, профессор, зав. каф. детских инфекционных болезней, Запорожский государственный медицинский университет, Украина.
Каплаушенко А. Г., д-р фарм. наук, профессор, зав. каф. физколлоидной химии, Запорожский государственный медицинский университет, Украина.

References

[1] Troeger, C., Khalil, I. A., Rao, P. C., Cao, S., Blacker, B. F., Ahmed, T., Armah, G., Bines, J. E., Brewer, T. G., Colombara, D. V., Kang, G., Kirkpatrick, B. D., Kirkwood, C. D., Mwenda, J. M., Parashar, U. D., Petri, W. A., Jr., Riddle, M. S., Steele, A. D., Thompson, R. L., Walson, J. L., … Reiner, R. C., Jr. (2018). Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea Among Children Younger Than 5 Years. *JAMA Pediatrics, 172*(10), 958-965. https://doi.org/10.1001/jamapediatrics.2018.1960

[2] Crawford, S. E., Ramani, S., Tate, J. E., Parashar, U. D., Svensson, L., Hagbom, M., Franco, M. A., Greenberg, H. B., O'Ryan, M., Kang, G., Desselberger, U., & Estes, M. K. (2017). Rotavirus infection. *Nature Reviews. Disease Primers, 3*, Article 17083. https://doi.org/10.1038/nrdp.2017.83

[3] Badur, S., Öztürk, S., Pereira, P., AbdelGhany, M., Khalaf, M., Lagoubi, Y., Ozudogru, O., Hanif, K., & Saha, D. (2019). Systematic review of the rotavirus infection burden in the WHO-EMRO region. *Human Vaccines & Immunotherapeutics, 15*(11), 2754-2768. https://doi.org/10.1080/21645515.2019.1603984

[4] Meskina, E. R. (2015). Sindrom mal'absorbtsii uglevodov u detei s virusnym gastroenteritom [Carbohydrate malabsorption syndrome in children with viral gastroenteritis]. *Al'manakh klinicheskoi meditsiny*, (42), 79-86. https://doi.org/10.18786/2072-0505-2015-42-79-86 [in Russian].

[5] Vorobiova, N., & Usachova, E. (2021). Influence of carbohydrate malabsorption syndrome on the clinical course of rotavirus infection in

children at an early age. *Georgian Medical News*, (2), 120-125. http://dspace.zsmu.edu.ua/handle/123456789/14143

[6] Smiyan, O. I., Smiyan-Horbunova, K. O., Loboda, A. M., Popov, S. V., Bynda, T. P., Vysotsky, I. Yu., Sichnenko, P. I., Petrashenko, V. O., Man'ko, Yu. A., Kosarchuk, V. V., & Gordienko, O. V. (2018). Macro- and microelements determination in children with rotavirus infection. *Zaporozhye medical journal*, *20*(3), 371-374. http://dx.doi.org/10.14739/2310-1210.2018.3.130456

[7] Nezhoda, I. I., Naumenko, O. M., & Nikulchenko, O. V. (2019). Letki zhyrni kysloty yak metabolichni markery porushennia mikrobiotsenozu kyshechnyku pry rotavirusnii infektsii u ditei [Volatile fatty acids as metabolic markers of intestinal microbiocenosis in children with rotavirus infection]. *Infektsiini khvoroby*, (3), 24-32. https://doi.org/10.11603/1681-2727.2019.3.10632 [in Ukrainian].

[8] Misselwitz, B., Butter, M., Verbeke, K., & Fox, M. R. (2019). Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. *Gut, 68*(11), 2080-2091. https://doi.org/10.1136/gutjnl-2019-318404

[9] He, T., Venema, K., Priebe, M. G., Welling, G. W., Brummer, R. J., & Vonk, R. J. (2008). The role of colonic metabolism in lactose intolerance. *European Journal of Clinical Investigation*, *38*(8), 541-547. https://doi.org/10.1111/j.1365-2362.2008.01966.x

[10] Nogal, A., Valdes, A. M., & Menni, C. (2021). The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes*, *13*(1), Article 1897212. https://doi.org/10.1080/19490976.2021.1897212

[11] Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de Los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Frontiers in Microbiology, 7*, Article 185. https://doi.org/10.3389/fmicb.2016.00185

[12] Shim, D. H., Kim, D. Y., & Cho, K. Y. (2016). Diagnostic value of the Vesikari Scoring System for predicting the viral or bacterial pathogens in pediatric gastroenteritis. *Clinical and Experimental Pediatrics*, *59*(3), 126-131. https://doi.org/10.3345/kjp.2016.59.3.126

[13] Kasırga, E. (2019). The importance of stool tests in diagnosis and follow-up of gastrointestinal disorders in children. *Türk Pediatri Arşivi, 54*(3), 141-148. https://doi.org/10.14744/TurkPediatriArs.2018.00483

[14] Boronina, L. G., Fedotova, G. V., Samatova, E. V., & Vakhlova, I. V. (2018). Gazozhidkostnaya khromatografiya i traditsionnye metody izucheniya kishechnoi mikrobioty u detei pervogo goda zhizni [Gas-liquid chromatography and traditional methods of study of intestinal microbiotes in children of first year of life]. *Vestnik ural'skoi meditsinskoi akademicheskoi nauki, 15*(5), 733-743. https://doi.org/10.22138/2500-0918-2018-15-5-733-743 [in Russian].

[15] Bridgman, S. L., Azad, M. B., Field, C. J., Haqq, A. M., Becker, A. B., Mandhane, P. J., Subbarao, P., Turvey, S. E., Sears, M. R., Scott, J. A., Wishart, D. S., Kozyrskyj, A. L., & CHILD Study Investigators. (2017). Fecal Short-Chain Fatty Acid Variations by Breastfeeding Status in Infants at 4 Months: Differences in Relative versus Absolute Concentrations. *Frontiers in Nutrition*, *4,* Article 11. https://doi.org/10.3389/fnut.2017.00011

[16] Li, L., Huang, D., Nevin, A., Fei, P., & Guo, L. (2019). Fecal Microbiota, Lactic Acid and Short Chain Fatty Levels of Infants Following Rotavirus Infection Revealed by Illumina Miseq High-Throughput Sequencing and HPLC Method. *Jundishapur Journal of Microbiology, 12*(6), Article 68389. https://doi.org/10.5812/jjm.68389

[17] Babayeva, B. A., & Valiyeva, K. T. (2019). [Diagnostic value the short chain fatty acids at viral diarrheas at children of the first year of life]. *Journal of Azerbaijan Perinatology and Pediatrics, 5*(1), 61-64.

[18] Zatevalov, A. M., Selkova, E. P., Gudova, N. V., & Oganesyan, A. S. (2018). Vozrastnaya dinamika produktsii korotkotsepochechnykh zhirnykh kislot kishechnoi mikrobiotoi u patsientov, ne imeyushchikh gastroenterologicheskikh zabolevanii [Age-related changes in production of short-chain fatty acids by gut microbiome in patients without gastroenterological diseases]. *Al'manakh klinicheskoi meditsiny*, *46*(2), 109-117. https://doi.org/10.18786/2072-0505-2018-46-2-109-117 [in Russian].