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Metabolic Profiles of the Gut Microbiota in Patients with Different Stages of Metabolic Dysfunction-Associated Fatty Liver Disease

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Aim: to study the metabolic activity of the intestinal microbiota depending on the stage of metabolic dysfunction-associated fatty liver disease (MAFLD).
Materials and methods. The study included 85 patients with MAFLD (27 patients with steatosis without steatohepatitis and fibrosis, 42 patients with steatohepatitis, 16 patients with cirrhosis as an outcome of MAFLD, Child — Pugh class A–B) and 20 healthy people who formed the control group. The level and spectrum of short-chain fatty acids (SCFA) were determined by gas-liquid chromatography.
Results. It was found that patients with MAFLD at the stage of steatosis and cirrhosis of the liver have unidirectional changes in the metabolic activity of the intestinal microbiota. We established a decrease in the absolute concentrations of SCFA — their total content, the level of acetate, propionate, butyrate, a decrease in the level of isoacids. The SCFA profiles showed an increase in the proportion of acetate and a decrease in propionate and butyrate. Moreover, changes in the named parameters of SCFAs are aggravated with progression to liver cirrhosis. At the stage of steatohepatitis, we identified two subgroups of patients with different levels of metabolic activity of the microbiota. Patients whose microbiota metabolism for SCFA production was high had correspondingly elevated SCFA levels. And, on the contrary, patients in whom the metabolic activity of the microbiota was reduced were characterized by a steady decrease in SCFAs and disease progression to liver cirrhosis. In the study, we showed an inverse correlation between the calculated prognostic indices of NFS and FIB-4, elastography values with the total level of SCFA, the level of acetate, propionate, butyrate. Thus, a decrease in the content of SCFA for patients with MAFLD can be considered as a prognostic marker of an unfavorable course of liver disease.
Keywords: MAFLD, liver cirrhosis, short-chain fatty acids, gut microbiota, gut-liver axis
Conflict of interest: the authors declare no conflict of interest.

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Метаболические профили микробиоты кишечника у пациентов с разными стадиями

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Цель: изучение метаболической активности микробиоты кишечника в зависимости от стадии метаболически ассоциированной жировой болезни печени (МАЖБП).
Материалы и методы. В исследование включены 85 пациентов с МАЖБП (27 пациентов со стеатозом без стеатогепатита и фиброза, 42 пациента со стеатогепатитом, 16 пациентов с циррозом в исходе МАЖБП, класс А–В по шкале Чайлда — Пью) и 20 здоровых людей, составивших контрольную группу. Уровень и спектр короткоцепочечных жирных кислот (КЦЖК) определяли методом газожидкостной хроматографии.
Результаты. Было установлено, что у больных МАЖБП на стадии стеатоза и цирроза печени отмечаются однонаправленные изменения метаболической активности микробиоты кишечника. Установлено снижение абсолютных концентраций КЦЖК: их общего содержания, уровня ацетата, пропионата, бутирата, снижение уровня изокислот.

В профилях КЦЖК выявлено повышение доли ацетата и снижение долей пропионата и бутирата. При этом изменения названных параметров КЦЖК усугубляются при прогрессии в цирроз печени. На стадии стеатогепатита мы выделили две подгруппы пациентов с разным уровнем метаболической активности микробиоты. Пациенты, у которых метаболизм микробиоты в отношении производства КЦЖК был высок, имели, соответственно, повышенный уровень КЦЖК. И, напротив, пациенты, у которых метаболическая активность микробиоты была снижена, характеризовались неуклонным снижением КЦЖК и прогрессией заболевания в цирроз печени. В исследовании мы показали обратную корреляционную зависимость расчетных прогностических индексов NFS и FIB-4, значений эластографии с общим уровнем КЦЖК, уровнем ацетата, пропионата, бутирата. Таким образом, снижение содержания КЦЖК для пациентов с МАЖБП можно рассматривать как прогностический маркер неблагоприятного течения заболевания печени.

Ключевые слова: МАЖБП, цирроз печени, короткоцепочечные жирные кислоты, микробиота кишечника, ось «кишка — печень»

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Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) is one of the most prevalent forms of chronic liver disease worldwide. The spectrum of MAFLD manifestations ranges from steatosis and steatohepatitis to MAFLD-related cirrhosis and even hepatocellular carcinoma [1–7]. Today, MAFLD is considered a multifactorial disease, one of the components of which is the disruption of the human microbiota. It is well-known that the microbiota in the gastrointestinal tract interacts with the liver through the "microbiota — gut — liver" axis. This interaction involves not only bacteria and immune cells activated by bacteria but also bacterial metabolites, the most common and studied of which are short-chain fatty acids (SCFAs) — acetate, propionate, butyrate [8–12]. They are formed as a result of bacterial fermentation of indigestible carbohydrates in the intestines. SCFAs, as one of the important sources of energy, play a vital role in the trophological provision of the intestinal epithelium, energy metabolism, and regulation of many physiological functions of the human body [3, 5–10].

Previous studies have reported a relationship between SCFAs and the pathophysiology of MAFLD [6–14]. It is discussed that the mechanism of action of altered microbiota in MAFLD can be realized both by affecting glucose homeostasis and lipid metabolism and by inducing and maintaining the inflammatory process in the liver, which can influence the progression of MAFLD from steatosis to cirrhosis [5–11]. Furthermore, intestinal dysbiosis and alterations in SCFAs production contribute to increased epithelial permeability, endotoxemia, insulin resistance, systemic inflammation, and obesity [2–13]. In our previous study, we demonstrated that in MAFLD at the stage of steatohepatitis, a high level of SCFAs is associated with a low level of liver fibrosis in these patients [14].

Aim of the study: to investigate the changes in SCFAs levels during the transition from steatosis without steatohepatitis and fibrosis to steatohepatitis with varying levels of SCFAs, and ultimately to the stage of cirrhosis.

Materials and methods

This study was conducted in accordance with the WMA Declaration of Helsinki and was approved by the Ethics Committee of Sechenov University (No. 01-22 of 24 January 2022). This study was conducted from 10 December 2022 to 25 March 2023. The diagnosis was established based on a liver examination, biopsy data, or a complex of clinical, laboratory, and instrumental data [11–13].

The study included patients aged 18 to 75 years with MAFLD who came to the clinic with signs of liver disease. The diagnosis of MAFLD was established upon detection of fatty liver degeneration according to elastometry data; in the presence of signs of metabolic disorders (fasting blood glucose level — 5.6–6.9 mmol/L; glucose tolerance test (glucose level in the blood after 2 hours) — 7.8–11.0 mmol/L; glycated hemoglobin (HbA1c) — 5.7–6.4 % or triacylglycerol — ≥ 1.70 mmol/L; high-density lipoproteins (HDL) — < 1.0 mmol/L and < 1.3 mmol/L for men and women, respectively) [12]; and upon exclusion of other causes of liver damage, including the denial of alcohol consumption in hepatotoxic doses (< 30 g of ethanol per day for men and < 20 g per day for women were allowed) for at least 3 months. To exclude other etiologies of liver diseases, all patients were tested for the presence of viral hepatitis markers (HBsAg, HBeAg, Anti-HBc-total, Anti-HBe, Anti-HBs, Anti-HCV-total,

Anti-HAV-IgM, Anti-HAV-IgG), autoimmune hepatitis markers (ANA, SMA and Anti-LKM1), as well as hemochromatosis (iron saturation of transferrin) and Wilson's disease (ceruloplasmin level, Kayser — Fleischer rings, daily amount of copper in urine, free copper level in blood plasma). If the results of these tests were positive, patients were excluded from the study.

The study did not include patients who, over the past 3 months, had taken drugs that affect the composition of the intestinal microbiota (probiotics, prebiotics, antibiotics, prokinetics, and proton pump inhibitors), as well as those with concomitant diseases that cause changes in the composition and metabolic function of the intestinal microbiota (except for diabetes mellitus, hypertension, and hyperuricemia in patients with MAFLD).

Of the total number of 186 patients, 107 did not meet the criteria: other etiology of liver damage (n = 89), the use of drugs that affected the composition of the gut microbiota (n = 18).

We determined normal levels of SCFA in feces based on the results of the control group, which participated in our previously published study [15]. The control group consisted of healthy volunteers with no gastrointestinal tract issues; no concomitant diseases of the respiratory, urinary, endocrine, and cardiovascular system; and no oncological diseases, who went to the clinic for a preventive examination. The subjects in the control group did not use drugs that affect the gut microbiota.

A total of 85 patients with MAFLD were included: 27 patients with steatosis without steatohepatitis and fibrosis, 42 patients with steatohepatitis, 16 patients with cirrhosis as an outcome of MAFLD, Child — Pugh class A–B, and 20 healthy people from the control group. Knowing that in patients at the stage of steatohepatitis the level of SCFAs changes in different directions [14], we considered it necessary to separate them into subgroups of steatohepatitis with a high level of SCFAs (n = 24) and steatohepatitis with a low level of SCFAs (n = 18).

Fecal samples were collected from all study participants and kept at –80 C°until further analysis. Analysis of SCFAs in fecal samples was performed using high-performance liquid chromatography. The analysis procedure was described by us previously [15].

Liver stiffness measurements and liver fatty infiltration were determined using Fibroscan (iLivTouch FT 100, Wuxi Hisky Company, China). Liver steatometry and elastometry were performed sequentially in all patients using M and XL sensors at the same measurement point [14]. The controlled attenuation parameter (CAP) values used to define the presence and degree of steatosis corresponding to stages S0 (steatosis < 10 %), S1 (steatosis from 10 % to < 33 %, mild) and S2 (steatosis from 33 % to < 66 %, moderate) were more than 248, 268 and 280 dB/m, respectively, whereas a CAP value > 280 dB/m indicated stage S3 (steatosis ≥ 66 %, severe). Liver stiffness was expressed as the average of 10 measurements (in kPa) performed at a depth of 25 to 65 mm. The threshold value for defining the presence of fibrosis was a liver stiffness > 7.6 kPa.

Statistical data processing was carried out using the STATISTICA 10 (StatSoft Inc., USA). Data are presented as median with interquartile range. The significance of differences between the two groups was assessed by using the Mann — Whitney method. Differences in categorical variables were determined using Fisher's exact test. Differences in categorical variables were determined using Fisher's exact test. Correlation analysis was performed using Spearman's method. Differences were considered significant when the probability of making a type I error was $p < 0.05$.

Results

We considered the following indicators of the control group (Me [interquartile range]): age, years — 56 [52–59]; Male/Female ratio — 9/11; fecal SCFA, mg/g — 10.2 [9.76–10.7]; fecal acetate (C2), mg/g — 5.87 [5.65–6.04]; fecal propionate (C3), mg/g — 1.77 [1.70–1.83]; fecal butyrate (C4), mg/g — 1.69 [1.66–1.77]; fecal isoacids (Cn), mg/g — 0.62 [0.59–0.64]; fraction of acetate, % — 61.8 [56.0–67.5]; fraction of propionate, % — 19.1 [8.7–19.8]; fraction of butyrate, % — 17.1 [15.3–21.0], fraction of isoacids, % — 5.9 [5.8–6.0], ratio of total isomers to acids (IsoCn/Cn) — 0.07 [0.07–0.07]. There was no significant difference between the general group of patients and controls in terms of age and sex distribution ($p > 0.05$).

The patient group with liver cirrhosis was slightly older than the other groups. Patients with cirrhosis had higher levels of total bilirubin, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and serum glucose than patients with steatosis and steatohepatitis. Levels of total cholesterol and low-density lipoproteins, uric acid, albumin, hemoglobin and platelets were significantly lower, respectively. The value of alanine aminotransferase was higher in patients with cirrhosis and steatohepatitis than in patients with steatosis. There were no significant differences in the triglyceride levels of all patients (Table 1).

Patients with steatosis, steatohepatitis with low levels of SCFAs, and cirrhosis had reduced

Table 1. Main characteristics of patients with metabolic dysfunction-associated liver diseases
Таблица 1. Основные характеристики пациентов с метаболически ассоциированными заболеваниями печени

	Steatosis ***Стеатоз*** **(*n* = 27)**	**NASH with high SCFA** ***НАСГ с высоким уровнем КЦЖК*** **(*n* = 24)**	**NASH with low SCFA** ***НАСГ с низким уровнем КЦЖК*** **(*n* = 18)**	**Liver cirrhosis** ***Цирроз печени*** **(*n* = 16)**
Age, years *Возраст, годы*	52 [47–58]	50 [46–60]#	54 [52–60.3]&	60 [54–65]□
Male/Female *Мужчины/Женщины*	11/16	13/11	4/14	6/10
Body mass index, kg/m^2 *Индекс массы тела, кг/м^2*	29.7 [28.4–32.1]*	33.8 [30.3–36.7]	31.4 [29.1–33.2]	32.1 [30.7–33.1]□
Waist circumference, cm *Окружность талии, см*	106 [103–111]*	115 [107–120]	111 [103–116]	118 [116–124]▪ □□
Total cholesterol, mmol/L *Общий холестерин, ммоль/л*	5.7 [5.2–6.0]**	4.9 [4.3–5.2]	5.5 [5.3–5.7]	4.4 [3.7–5.2]▪ □
HDL, mmol/L *ЛПВП, ммоль/л*	1.2 [1.1–1.3]**	0.92 [0.89–0.99]#	0.97 [0.82–1.04]&&	1.1 [0.9–1.3]▪
LDL, mmol/L *ЛПНП, ммоль/л*	4.0 [3.3–4.2]**	3.2 [2.8–3.5]	3.7 [3.6–3.9]	2.7 [2.0–3.3]▪ □
Triglycerides, mmol/L *Триглицериды, ммоль/л*	1.69 [1.60–2.33]	1.84 [1.61–2.36]	1.9 [1.5–2.3]	2.14 [1.65–2.65]
Uric acid *Мочевая кислота*	323 [293–353]	323 [300–382]##	370 [356–428]&&	247 [217–274]▪▪ □□
Glucose, mmol/L *Глюкоза, ммоль/л*	5.4 [5.1–6.0]	5.4 [4.7–6.0]#	5.47 [5.50–5.60]	6.7 [5.8–8.1]▪ □
FIB-4	0.95 [0.85–1.05]	0.94 [0.69–1.25]##	1.65 [1.36–3.16]&&	3.18 [2.04–4.81]▪ □□
NFS	–2.15 [–2.44–1.93]	–1.88 [–3.16–0.97]##	–0.89 [–1.30–0.14]&&	0.62 [–0.05–1.48]▪▪ □□
Liver stiffness, kPa *Жесткость печени, кПа*	8.70 [8.10–9.50]	7.84 [6.62–9.36]##	11.54 [10.46–13.33]&&	16.80 [16.15–18.25]▪▪ □□
Total protein, g/L *Общий белок, г/л*	70.0 [69.0–72.0]	70.7 [68.8–73.9]	74 [72–74]&&	70.5 [67.5–76.5]
Albumin, g/L *Альбумин, г/л*	43.0 [42.3–43.7]	44.2 [41.8–45.4]##	44 [43–45]	37.1 [34.2–40.8]▪▪ □□
Total bilirubin, umol/L *Общий билирубин, мкмоль/л*	16.6 [12.4–21.9]*	12.5 [10.9–15.7]#	9.96 [10.02–10.15]&&	20.2 [13.6–27.4]▪▪
ALT, U/L *АЛТ, Ед./л*	23 [20–24]**	49 [36–55]	48 [38–82]&&	42 [31–78]□□
AST, U/L *АСТ, Ед./л*	24 [22–25]**	31 [30–34]##	38 [33–50]&&	49 [43–70]▪ □□
GGT, U/L *ГГТ, Ед./л*	31 [24–42]*	48 [33–57]#	30 [26–35]	60 [53–68]▪▪ □□
Alkaline phosphatase, U/L *Щелочная фосфатаза, Ед./л*	85 [73–93]*	74 [69–83]	83 [73–90]	91 [70–105]
C-reactive protein, mg/L *С-реактивный белок, мг/л*	3.3 [2.8–4.0]	2.7 [1.2–5.3]	3.2 [2.5–3.8]	2.5 [1.7–4.4]
Hemoglobin, g/L *Гемоглобин, г/л*	142 [138–145]	144 [136–152]##	140 [137–145]	130 [124–138]▪ □□
Leukocytes, 10^9/L *Лейкоциты, 109/л*	6.0 [5.8–7.0]	6.8 [5.8–7.8]#	6.8 [6.0–7.7]	5.8 [4.6–6.2]▪
Thrombocytes, 10^9/L *Тромбоциты, 10^9/л*	273 [253–310]	294 [256–336]##	192 [170–223]&&	147 [103–180]▪ □□

Note: NASH — non-alcoholic steatohepatitis; SCFA — short-chain fatty acids; HDL — high-density lipoprotein; LDL — low-density lipoprotein; FIB-4 — fibrosis index-4; NFS — NAFLD fibrosis score; ALT — alanine aminotransferase; AST — aspartate aminotransferase; GGT — gamma-glutamyl transpeptidase; * — < 0.001, ** — < 0.05 when comparing "Steatosis" vs. "NASH with high SCFA", # — < 0.001, ## — < 0.05 when comparing "Liver cirrhosis" vs. "NASH with high SCFA"; & — < 0.001, && — < 0.05 when comparing "Steatosis" vs. "NASH with low SCFA"; ■ — < 0.001, ■■ — < 0.05 when comparing "Liver cirrhosis" vs. " NASH with low SCFA"; □ — < 0.001, □□ — < 0.05 when comparing "Steatosis" vs. "Liver cirrhosis".

Примечание: НАСГ — неалкогольный стеатогепатит; КЦЖК — короткоцепочечные жирные кислоты; ЛПВП — липопротеиды высокой плотности; ЛПНП — липопротеиды низкой плотности; FIB-4 — индекс фиброза-4; NFS (NAFLD fibrosis score) — шкала фиброза при НАЖБП; АЛТ — аланинаминотрансфераза; АСТ — аспартатаминотрансфераза; ГГТ — гамма-глутамилтранспептидаза; * — < 0,001, ** — < 0,05 при сравнении «Стеатоз» vs. «НАСГ с высоким уровнем КЦЖК», # — < 0,001, ## — < 0,05 при сравнении «Цирроз печени» vs. «НАСГ с высоким уровнем КЦЖК»; & — < 0,001, && — < 0,05 при сравнении «Стеатоз» vs. «НАСГ с низким уровнем КЦЖК»; ■ — < 0,001, ■■ — < 0,05 при сравнении «Цирроз печени» vs. «НАСГ с низким уровнем КЦЖК»; □ — < 0,001, □□ — < 0,05 при сравнении «Стеатоз» vs. «Цирроз печени».

Table 2. Absolute and relative content of SCFAs in patients with different stages of metabolic dysfunction-associated fatty liver disease

Таблица 2. Абсолютное и относительное содержание КЦЖК у пациентов с разными стадиями метаболически ассоциированной жировой болезни печени

	Steatosis ***Стеатоз*** **(*n* = 27)**	**NASH with high SCFA** ***НАСГ с высоким уровнем КЦЖК*** **(*n* = 24)**	**НАСГ с низким уровнем КЦЖК** ***NASH with low SCFA*** **(*n* = 18)**	**Цирроз печени** ***Liver cirrhosis*** **(*n* = 16)**
Total SCFA content, mg/g *Общее содержание КЦЖК, мг/г*	6.00 [4.59–7.41]** //	15.36 [13.56–18.09]## //	4.67 [3.36–5.81]& //	3.20 [2.13–4.22]■ □□ //
Acetate (C2), mg/g *Ацетат (С2), мг/г*	3.58 [2.75–5.27]** //	9.32 [7.90–11.02]## //	2.72 [2.07–3.30]& //	2.12 [1.03–2.28]■ □□ //
Propionate (C3), mg/g *Пропионат (С3), мг/г*	0.90 [0.61–1.17]** //	2.76 [2.25–3.54]## //	0.87 [0.61–1.32]//	0.58 [0.46–0.81]■ □ //
Butyrate (C4), mg/g *Бутират (С4), мг/г*	0.80 [0.65–0.99]**//	2.49 [1.77–3.14]## //	0.66 [0.43–0.82]//	0.35 [0.28–0.48]■ □□ //
Isoacids (Cn), mg/g *Изокислоты (Cn), мг/г*	0.39 [0.26–0.45]* //	0.57 [0.31–0.75]#	0.23 [0.17–0.31]& //	0.23 [0.21–0.31]■ //
Fraction of acetate, % *Доля ацетата, %*	71.1 [65.5–77.6]* /	65.5 [62.5–67.6]	65.4 [62.8–67.1]&	62.8 [56.3–66.8]□□
Fraction of propionate, % *Доля пропионата, %*	16.6 [15.0–19.6]* /	18.5 [16.7–21.6]	20.5 [17.7–23.1]&	22.7 [19.8–26.3]□□ /
Fraction of butyrate, % *Доля бутирата, %*	14.3 [13.3–22.7]/	16.1 [14.1–18.9]	14.3 [12.8–15.5]//	12.9 [11.3–15.0]/
Fraction of isoacids, % *Доля изокислот, %*	6.8 [5.4–7.4]** /	3.8 [1.8–5.6]## //	5.0 [3.7–6.2]& /	8.2 [7.4–10.0]■■ □□ //
IsoCn/Cn *IsoCn/Cn*	0.46 [0.29–0.52]* //	0.19 [0.10–0.44]# //	0.35 [0.30–0.38]& //	0.08 [0.07–0.11]■■ □□ //

Note: NASH — non-alcoholic steatohepatitis; SCFA — short-chain fatty acids; ** — < 0.001, * — < 0.05 when comparing "Steatosis" vs. "NASH with high SCFA"; ## — < 0.001, # — < 0.05 when comparing "Cirrhosis" vs. "NASH with high SCFA"; && — < 0.001, & — < 0.05 when comparing "Steatosis" vs. "NASH with low SCFA"; ■■ — < 0.001, ■ — < 0.05 when comparing "Cirrhosis" vs. "NASH with low SCFA"; □□ — < 0.001, □ — < 0.05 when comparing "Steatosis" vs. "Liver cirrhosis"; // — < 0.001, / — < 0.05 when comparing with the control group.

Примечание: НАСГ — неалкогольный стеатогепатит; КЦЖК — короткоцепочечные жирные кислоты; ** — < 0,001, * — < 0,05 при сравнении «Стеатоз» vs. «НАСГ с высоким уровнем КЦЖК»; ## — < 0,001, # — < 0,05 при сравнении «Цирроз печени» vs. «НАСГ с высоким уровнем КЦЖК»; && — < 0,001, & — < 0,05 при сравнении «Стеатоз» vs. «НАСГ с низким уровнем КЦЖК»; ■■ — < 0,001, ■ — < 0,05 при сравнении «Цирроз печени» vs. «НАСГ с низким уровнем КЦЖК»; □□ — < 0,001, □ — < 0,05 при сравнении «Стеатоз» vs. «Цирроз печени»; // — < 0,001, / — < 0,05 при сравнении с группой контроля.

fecal levels of SCFAs, acetate, propionate, and butyrate compared with healthy controls, and these reductions were more pronounced in patients with cirrhosis (Table 2). In the case of patients with steatohepatitis and high levels of SCFAs, the levels of the studied parameters were correspondingly higher than those in the other groups.

The proportion of butyrate in all patients was lower than in healthy controls but did not differ with disease progression. The relative proportion of acetate was increased in steatosis, while the proportion of propionate was increased in cirrhosis.

Fecal isoacid levels were also lower in all patients than in healthy subjects, and they were further

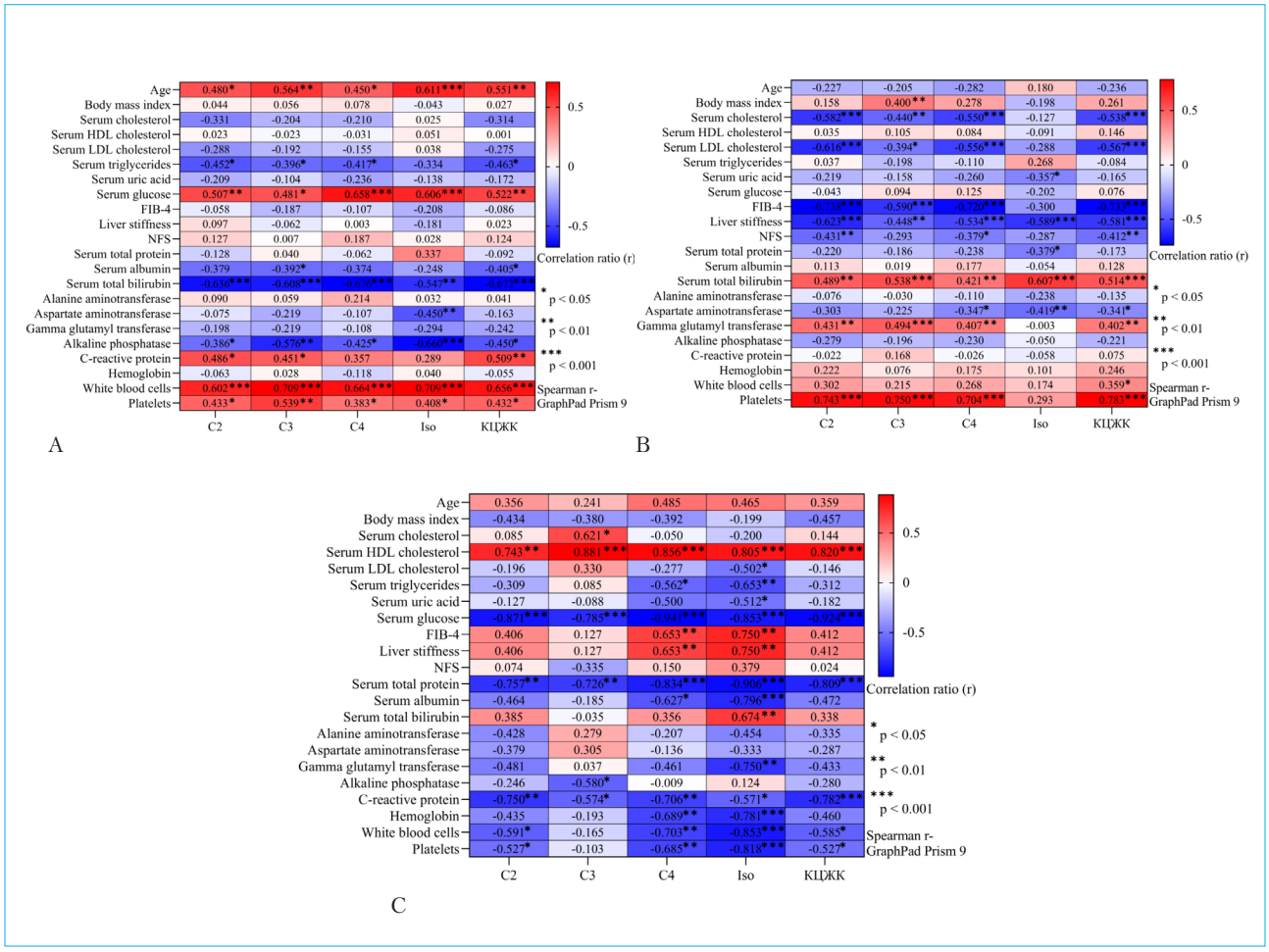

Figure 1. Correlations of absolute levels of acetate (C2), propionate (C3), butyrate (C4), isoacids (Iso) and total SCFA in feces with the main clinical and laboratory parameters of patients with steatosis (A), steatohepatitis (B), and liver cirrhosis (C)

Рисунок 1. Корреляции абсолютных уровней ацетата (C2), пропионата (C3), бутирата (C4), изокислот (Iso) и общего количества КЦЖК в кале с основными клинико-лабораторными показателями пациентов со стеатозом (A), стеатогепатитом (B), циррозом печени (C)

reduced in patients with steatohepatitis with low SCFA levels and patients with liver cirrhosis. The proportion of isoacids was more stable in patients with liver cirrhosis in than in other groups (Table 2).

The level of SCFAs and their fractions had direct and inverse correlations with the main clinical and laboratory characteristics of patients. The results of the correlation analysis are shown in Figure 1.

We analyzed the relationship between the correlation graphs of the SCFA content and the elastometry results, the results are presented in Figure 2.

Discussion

The pathogenesis of MAFLD is complex. The combined effect of many different factors that determine the occurrence of MAFLD are currently being discussed, including genetic, social, environmental and other factors. An important aspect in the modern understanding of the pathogenesis of MAFLD is the recognition of the role of intestinal microbiota disorders [1–5, 10, 13, 15, 16, 17, 18, 19].

In this study, we researched the relationship of SCFAs with the severity of MAFLD in patients with different stages of MAFLD. Our results show that patients with MAFLD at the stage of steatosis and cirrhosis exhibit unidirectional changes in the metabolic activity of the intestinal microbiota. A decrease in absolute concentrations of SCFAs was established — their total content, the level of acetate, propionate, butyrate, and a decrease in isoacids. In the SCFAs profiles of patients with liver steatosis, an increase in the proportion of acetate and a decrease in the proportions of propionate and butyrate were revealed. In patients with cirrhosis, the proportion of propionate increased, and the proportion of butyrate decreased. We hypothesize that this may be associated both with changes in the metabolic activity of the

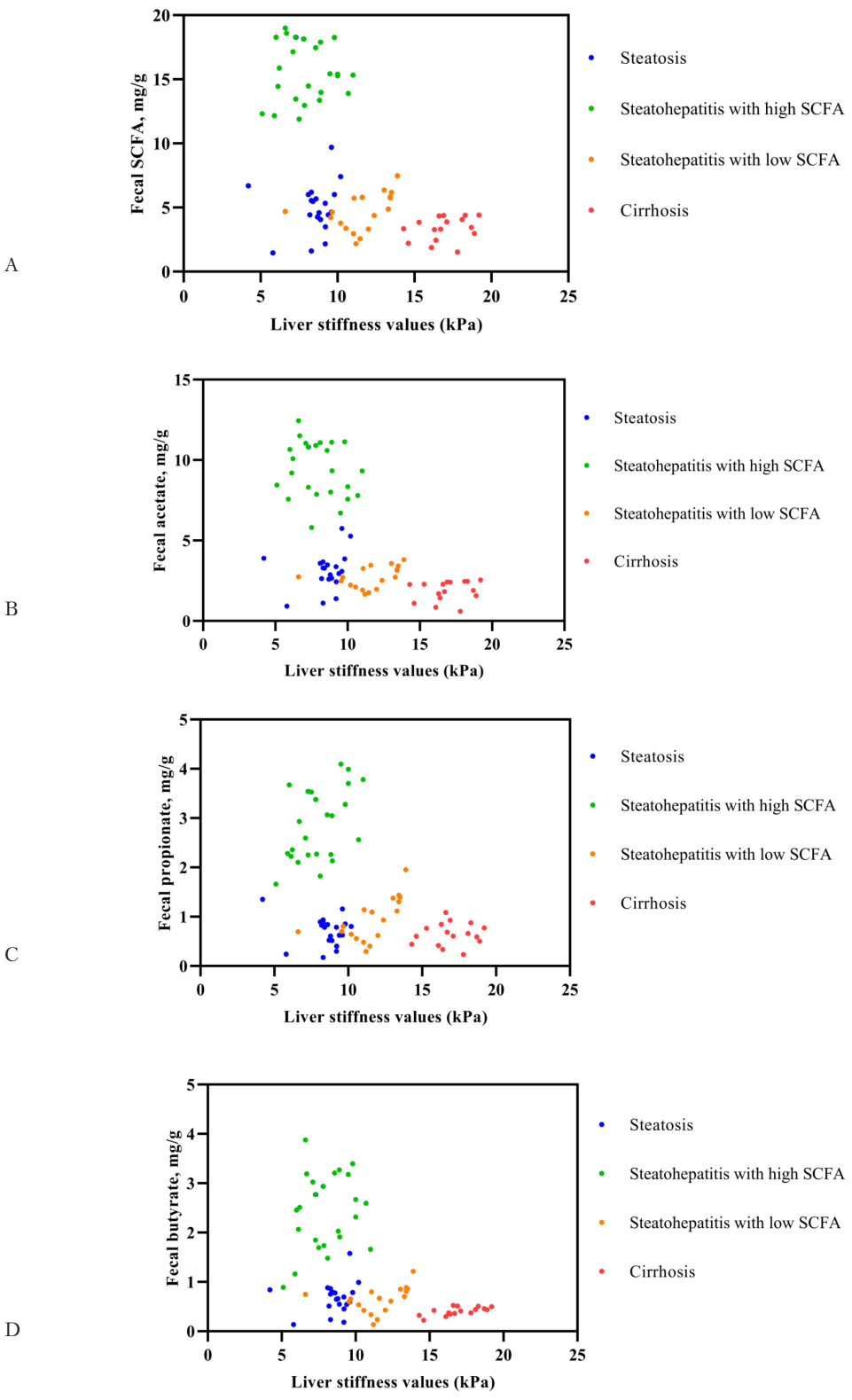

Figure 2. Correlation dependences of the content of SCFA (A), acetate (B), propionate (C), butyrate (D) with the results of elastometry

Рисунок 2. Корреляционные зависимости содержания КЦЖК (A), ацетата (B), пропионата (C), бутирата (D) с результатами эластометрии

microbiota and a manifestation of the impaired utilization of SCFAs by colonocytes.

In our opinion, SCFA levels at the stage of steatohepatitis are extremely important in the progression of MAFLD. Moreover, this fact probably predetermines the further course of the disease and the risk of developing liver cirrhosis. We identified two subgroups of patients with different levels of metabolic activity of the microbiota. Patients whose microbiota metabolism in relation to the production of SCFAs was high had correspondingly elevated levels of SCFAs. In contrast, patients in whom the metabolic activity of the microbiota was reduced were characterized by a steady decrease in SCFAs and the progression of the disease to cirrhosis. In this study, we showed an inverse correlation between the calculated prognostic indices of NFS and FIB-4, the values of elastography with the total level of SCFAs, and acetate, propionate, and butyrate levels. Thus, a decrease in SCFAs for patients with MAFLD can be considered as a prognostic marker of an unfavorable course of liver disease.

Our data confirm the results of studies in which changes in SCFA levels were determined in patients with MAFLD [5, 7, 8, 20, 21]. R. Ganesan et al. observed that SCFA levels are significantly lower in patients with hepatic steatosis, including acetate ($p = 0.03$), butyrate ($p = 0.0008$) and propionate levels [22]. M. Rau et al. assessed the results of a study of patients with MAFLD at the stage of steatohepatitis. Notably, patients with NASH are characterized by a different composition of the intestinal microbiota with higher levels of SCFAs in feces and a higher content of *Fusobacteria* and *Fusobacteriaceae* that produce SCFAs in MAFLD [23]. According to these authors, these changes are associated with the immunological features of the disease, namely, a lower level of resting regulatory T cells (rTreg) ($CD4^+CD45RA^+CD25^{++}$) and a higher Th17/rTreg ratio in the peripheral blood of patients with NASH [23]. An association of a decrease in fecal SCFAs with low microbiota diversity and high intestinal permeability was shown [24–27]. A negative correlation between the TNF-α level and SCFAs as MAFLD progresses was shown [28].

In our study, we determined a change in the ratio of SCFAs, namely, a decrease in the proportion of butyrate, which in our opinion plays a critical role in the progression of MAFLD. The literature also describes that the anti-inflammatory activity of butyrate is associated with the suppression of the LPS-mediated expression of pro-inflammatory genes, the emergence of an anti-inflammatory phenotype, and the induction of apoptosis by pro-inflammatory macrophages. At the molecular level, these cellular phenomena are mediated through AMP-activated protein kinase (AMPK), histone deacetylase (HDACs), and nuclear transcription factor (NF-κB) [29].

Thus, SCFAs may become potential diagnostic, therapeutic, and prognostic markers. To confirm our hypothesis, further studies on a large population of patients and the simultaneous examination of both circulating and fecal SCFAs are necessary.

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