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ORIGINAL ARTICLE

EFFECTS OF DIETARY COMPONENTS ON INTESTINAL SHORT-CHAIN FATTY ACIDS (SCFAs) SYNTHESIS IN HEALTHY ADULT PERSONS FOLLOWING A KETOGENIC DIET

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ABSTRACT

Background. The ketogenic diet (KD) has been used for almost 100 years in the treatment of drug-resistant epilepsy in children - and adults. The intestinal microbiome has a climax character, and the main factor changing its composition and functions is the diet. Both increased biodiversity and the production of short-chain fatty acids (SCFAs) are important indicators of gut barrier function. SCFAs are synthesized by microorganisms through the fermentation of dietary fibre provided with the diet. They are an important element in signal transduction from the digestive system to other tissues. To date, there is little research to determine how the use of KD alters the SCFAs profile of the human stool.

Objective. To assess the SCFAs profile in the stool of healthy and active KD users.

Material and methods. Study group: amateur athletes following KD. Control group: amateur athletes following a regular diet (carbohydrates min. 50%); gender: men and women aged 18-60. Material: stool sample (1x10 g). SCFAs content was determined in stool samples using gas chromtography method. Participants completed a Food Frequency Questionnaire (FFQ) and a 72-hour food diary.

Results. There research has shown differences in the amount of SCFAs, as far as the results obtained from the two groups are concerned. The discrepancies referred to the levels of acetic, butyric, iso-butyric, valeric, and isovaleric acids. *Spearman's* rank correlation analysis showed a strong relationship between the consumption of selected dietary components (vegetables, fruits, red meat, poultry, fish, nuts and seeds, sugar, sugar substitutes, fats) and the SCFAs content in the stool of the study group.

Conclusions. High consumption of cruciferous and leaf vegetables, berries and nuts on a ketogenic diet may have a positive effect on the profile of short-chain fatty acids produced by the gut microbiome. Changing the diet towards a greater supply of plant products may prevent proteolytic fermentation and reduce the negative effects of microbiome changes caused by an oversupply of protein and fat in the ketogenic diet.

Key words: *ketogenic diet, intestinal microbiome, short-chain fatty acids, proteolytic fermentation*

STRESZCZENIE

Wprowadzenie. Dieta ketogeniczna (DK) jest stosowana już niemal 100 lat w leczeniu padaczki lekoopornej u dzieci oraz dorosłych. Dieta jest głównym czynnikiem zmieniającym skład i funkcje mikrobioty. Bioróżnorodność mikrobiomu i produkcja krótkołańcuchowych kwasów tłuszczowych (SCFAs) to ważne wskaźniki funkcji bariery jelitowej. SCFAs są produkowane przez mikrobiom podczas fermentacji włókna pokarmowego z diety. Stanowią ważny element w transdukcji sygnału z jelit do tkanek. Do tej pory istnieje niewiele badań określających zmiany profilu SCFAs w stolcu u ludzi na DK.

Cel. Ocena profilu SCFAs w stolcu zdrowych i aktywnych osób stosujących dietę ketogeniczną.

Materiał i metody. Grupa badana: zdrowe osoby stosujące DK jako swój wybór od minimum miesiąca. Grupa kontrolna: zdrowe osoby stosujące dietę zwyczajową (węglowodany min. 50%); płeć: mężczyźni i kobiety w wieku 18-60 lat.

Materiał: kał (1x 10 g) od osób z grupy badanej i kontrolnej. Zawartości SCFAs w próbkach kału oznaczano metodą chromatografii gazowej. Zastosowano kwestionariusz częstotliwości spożycia (FFQ; ang. Food Frequency Questionaire) oraz 72-godzinny pamiętnik żywieniowy. Analiza statystyczna: Med. Calc 19.2 (Ostend, Belgia).

Wyniki. Wykazano różnice w zawartości SCFAs w stolcu pomiędzy grupami. Różnice dotyczą zawartości kwasów: octowego, masłowego, izomasłowego, walerianowego i izowalerianowego. Analiza korelacji rang *Spearman'a* wykazała silne zależności pomiędzy spożyciem wybranych składników diety (warzywa, owoce, czerwone mięso, drób, ryby, orzechy i nasiona, cukier, substytuty cukru, tłuszcze) a zawartością SCFAs w stolcu grupy badanej.

Wnioski. Duże spożycie warzyw krzyżowych, warzyw liściastych, owoców jagodowych oraz orzechów na diecie ketogenicznej wydaje się korzystnie wpływać na profil SCFAs w stolcu. Zmiana diety w kierunku większej podaży

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produktów roślinnych może zapobiegać fermentacji proteolityczej i niwelować negatywne skutki zmian mikrobiomu wywołane nadmierną podażą białka i tłuszczu na diecie ketogennej.

Słowa kluczowe: *dieta ketogeniczna, mikrobiom jelitowy, krótkołańcuchowe kwasy tłuszczowe, SCFAs, fermentacja proteolityczna*

INTRODUCTION

The ketogenic diet (KD) has been used for almost 100 years in the treatment of drug-resistant epilepsy in children [1, 2, 3] and adults [4, 5, 6]. Currently, KD due to the progressive popularization, most often due to social media, is gaining more and more recognition among people who want to reduce fat mass [15, 16]. The benefits of the ketogenic diet seem to be based not only on weight reduction but according to the latest research, it can be part of the dietary prevention of lifestyle diseases [30].

Low-carbohydrate diets have long been used by athletes to reduce body weight [15]. It turns out, however, that a certain percentage of amateur athletes, as well as a slightly smaller percentage of professional athletes, decide to use KD not only to try to increase their sports achievements but rather for the broadly understood potential health benefits. However, it's worth taking a closer look at the interactions between the ketogenic diet and lifestyle and overall health. The ketogenic diet is based on a significant reduction in carbohydrates in the diet (<50 g per day) and increasing the consumption of high-fat products and total fats [6]. Diet is one of the major regulators of the epigenome [17]. Dietary fibre provided by the diet is a substrate for bacterial synthesis of butyrate and propionate, which exhibit epigenetic effects, and *beta*-hydroxybutyric acid itself is one of the histone deacetylase inhibitors (HDAC) [17, 18]. As a result of bacterial fermentation in the intestinal lumen, various bioactive compounds, both beneficial and unfavourable to the host's health, may be formed [19]. The qualitative composition of the diet seems to have the greatest impact on the composition and functions of the gut microbiota [7, 19]. The intestinal microbiome has a climax character, and the main factor changing its composition and functions is the diet. Both increased biodiversity and the production of short-chain fatty acids (SCFAs) are important indicators of gut barrier function.

SCFAs are synthesized by microorganisms through the fermentation of dietary fibre provided with the diet [7]. They are an important element in signal transduction from the digestive system to other tissues [8, 9]. Short-chain fatty acids are the most common product of the metabolism of microorganisms. SCFAs consist of three most abundant acids: acetic acid (approx. 60%), propionic acid (approx. 25%) and butyric acid (approx. 15%) [19]. The highest concentration of SCFAs is observed in the ascending colon, with slightly lower levels in the transverse and descending colon. One of the more important features of short chain fatty acids appears to be their ability to induce specific metabolic effects along with acting on the host as signalling molecules [8]. SCFAs have the ability to cross the blood-brain barrier, which is an important aspect in the functioning of the gut-brain axis. The metabolic functions of SCFAs include: elimination of lipopolysaccharide induced inflammation, direct modulation of systemic immunity, improvement of insulin sensitivity, regulation of appetite, induction of adipose tissue differentiation (e.g. browning), increase of energy expenditure by enhancing thermogenesis, increasing lipolysis. It should be noted that most studies assessing SCFAs production are carried out on stool samples, which may not be fully reliable due to limitations related to intestinal transit time, intestinal permeability, metabolite transport rate, or the method of collecting and storing the sample itself [27]. To date, there is little research to determine how the use of a ketogenic diet alters the SCFAs profile of the human stool. Therefore, the aim of this study was to assess the SCFAs profile in the stool of healthy and active persons following a ketogenic diet.

MATERIAL AND METHODS

Group characteristics

Healthy and physically active adults participated in the study. The study participants were selected into two groups: the study group (n = 14) and the control group (n = 16).

S*tudy group*: healthy active people voluntarily following a ketogenic diet. *Control group:* healthy active people following a habitual diet, characterized by the consumption of carbohydrates corresponding to a minimum of 50% of energy. The estimated group size was at least 10 people in each group (for the assumed test power of 0.95; p <0.05, G Power test); gender: men and women aged 18-60.

I*nclusion criteria*: individuals who voluntarily wish to submit a stool sample and complete surveys; healthy active people (men and women) who have been on a ketogenic diet for at least a month (study group) and healthy active people (men and women) using habitual nutrition.

E*xclusion criteria:* people> 60 years of age; people with irregular physical activity; people with metabolic disorders (coronary artery disease, hypertension, diabetes); people with liver failure; people diagnosed

with inflammatory bowel disease (Crohn's disease, *Colitis ulcerosa*); people with biliary disorders; obese people; people taking antibiotic therapy for at least 3 months from the start of the study; people who supplemented probiotics, antibiotics and butyric acid in the period preceding the study by less than 3 months.

Surveys

The first step was to conduct a short metric survey and to determine the duration of the ketogenic diet. The subjects of the study gave their formal consent to participate in the study and collect samples. The Food Frequency Questionnaire (FFQ) was conducted and the 72-day food diary was recorded. All data was collected from participants using online survey tools. The consumption of certain groups of food products was recorded with the use of the FFQ questionnaire. The participants completed it considering the period: a) for the test group after switching to the ketogenic diet (up to 12 months back); b) for the control group, the period of the last 12 months. All products and subgroups of food products were divided into the following groups: total fats, coconut oil, animal fat, butter (all types), berries, vegetables (all types), meat and fish products, artificial sweeteners, sugar and honey, alcoholic beverages, sweetened and energy drinks, poultry and rabbit meat, red and game meat, milk, eggs, and natural dairy products, natural unsweetened plant drinks, nuts, seeds and seeds, dried fruit and sweet fruit preserves, polyols and stevia, sweetened dairy products, products cereal (total), snacks (salty, sweet) and fast-food, processed meat products, total fish, fruit and vegetable juices, cream and mayonnaise and dressings, medium-chain fatty acids, vegetable fats (including olive oil and avocado), other vegetables, legumes, green leafy vegetables and cruciferous vegetables, potatoes and sweet potatoes and their derivatives. The ranks from the FFQ questionnaire were converted into the frequency of consumption according to the formula presented in Table 1. Microsoft Excel (Version 16.37 (20051002)) was used to calculate the ranks.

Sampling and analysis

*W*e collected stool samples from participants. The participants were informed about the critical points necessary for the proper storage and transport of the samples. The content of short-chain fatty acids (acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid, valeric acid, caproic acid, isocaproic acid, heptanoic acid) was determined in the stool samples. Any data obtained by analysis in an Agilent technology 1260 A GD gas chromatograph with a flame ionization detector (FID) using a column (DB-FFAP, 30m × 0.53mm × 0.5um). Injection parameters: hydrogen carrier gas with a flow rate of 14.4 ml/min, separation conditions: initial temperature (100°C) / 0.5 minutes, then increase to 180°C and 200°C (5 min). The results were transferred into a Microsoft Excel version 16.37 (20051002) database and presented in both percentages (%) and concentrations (mg/g).

Bioethical Committee

This research received the consent of the Bioethical Committee of the Pomeranian Medical University in Szczecin.

Statistical analysis

The statistical analysis covered the responses from the FFQ questionnaires and the database with the SCFAs analysis results. Statistical analysis was performed using the Med Calc program (19.2.6; Ostend, Belgium). The performed statistical calculations were based on the normal distribution *Shapiro-Wilk* test, the *Spearman's* rank correlation test, and the *Chi*-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Statistical analysis showed significant differences in the content of individual fatty acids between the study group and the control group. Significant diversities were also noticed in the consumption of certain groups of products and certain eating habits. Anthropometric data for both groups are presented

Table 1. The diagram of converting FFQ ranks into frequency of consumption. Based on the FFQ-6 Food Consumption Frequency Questionnaire prepared by *Wądołowska L.* and *Niedźwiedzka E.* [31]

Consumption frequency categories	Ranks assigned to frequency categories	Daily frequency (times / day)
Never or hardly ever	1	0.0
Once a month or less	2	0.025
Several times a month	3	0.100
Several times a week	4	0.571
Everyday	5	1.000
Several times a day	6	2.000

Based on the FFQ-6 Food Consumption Frequency Questionnaire [31].

in the tables below. The individual groups had an approximate gender distribution, and there were more men in the control group (9 vs.7).

The analysis of the auxiliary questions included in the FFQ questionnaire showed that people following the ketogenic diet consumed significantly fewer meals during the day than people eating habitually. The approximate number of meals consumed per day ranged from 1 to 3 in the test group and from 3 to 4 meals in the control group. Additionally, there was a trend (p = 0.068) in which people following the ketogenic diet had a fixed mealtime as opposed to the control group, where half of the participants did not have a fixed eating time. Intermittent fasting consists of eating meals for 8 hours and fasting for 16 hours until the next day. People following the ketogenic diet also used intermittent fasting significantly more often (p = 0.003). Moreover, in the study group, no person reported taking iron supplements, as opposed to the control group, where over 35% of participants took iron supplements. Descriptive statistics for the entire study group broken down by gender showed a strong trend in the percentage (%) of isovaleric acid and the concentration (mg/g) of caproic acid.

The analysis of SCFAs concentrations between the control and intervention groups showed significant differences in the percentage of acetic acid as well as the concentration of isovaleric acid and valeric acid. There was also a strong trend for butyric acid (p = 0.0552) and isobutyric acid (p = 0.0513) between the groups.

Table 2. Anthropometric parameters

	Intervention group			Control group		
	n	Median	25 - 75 P	n	Median	25 - 75 P
Body weight	14	71.0000	61.000 to 82.000	16	74.0000	60.500 to 85.000
Hip circumference	14	93.5000	91.000 to 100.000	16	93.2500	89.500 to 100.000
Waist circumference	14	84.0000	74.000 to 90.000	16	85.0000	76.000 to 90.000
Age	14	28.5000	23.000 to 32.000	16	24.0000	23.000 to 26.000
Height	14	175.5000	168.000 to 184.000	16	176.0000	169.000 to 181.000
BMI	14	23.0500	21.610 to 24.220	16	23.890	21.180 to 25.950
WHR	14	0.9	0.81 to 0.9	16	0.91	0.85 to 0.9

BMI – Body Mass Index; WHR – waist–hip ratio

Table 3. Comparison of SCFAs content between gender

	Women			Men			
	n	Median	25 - 75 P	n	Median	25 - 75 P	P [a]
Acetic acid [%]	14	40.0335	31.838 - 44.748	16	41.0388	33.204 to 46.974	0.5889
Acetic acid [mg/g]	14	0.08778	0.0650 - 0.127	16	0.116	0.0896 - 0.181	0.1637
Propionic acid [%]	14	17.0049	13.694 - 19.943	16	14.9617	13.219 - 23.939	1
Propionic acid [mg/g]	14	0.04311	0.0270 - 0.0656	16	0.04728	0.0331 - 0.0720	0.5746
Butyric acid [%]	14	24.151	18.136 - 27.591	16	24.2946	17.232 - 33.573	1
Butyric acid [mg/g]	14	0.06295	0.0275 - 0.0830	16	0.07562	0.0369 - 0.163	0.2891
Isobutyric acid [%]	14	4.7979	3.675 - 5.870	16	4.8069	3.738 - 5.593	0.8679
Isobutyric acid [mg/g]	14	0.00949	0.00719 - 0.0170	16	0.01253	0.00737 - 0.0184	0.4669
Isovaleric acid [%]	14	7.9259	5.865 - 10.626	16	4.6226	2.536 - 10.052	0.0558
Izovaleric acid [mg/g]	14	0.01621	0.0132 - 0.0267	16	0.01539	0.00860 - 0.0215	0.5466
Valeric acid [%]	14	5.2814	4.463 - 6.142	16	4.4803	3.959 - 6.071	0.2444
Valeric acid [mg/g]	14	0.01092	0.00820 - 0.0209	16	0.01427	0.00882 - 0.0192	0.5746
Caproic acid [%]	14	1.64561	0.238 - 2.802	16	0.2854	0.176 - 0.548	0.0674
Caproic acid [mg/g]	14	0.00225	0.000671 - 0.00501	16	0.0006801	0.000503 - 0.00152	0.0585
Izocaproic acid [%]	14	0.20481	0.106 - 0.284	16	0.1229	0.0695 - 0.230	0.1975
Isocaproic acid [mg/g]	14	0.000469	0.000265 - 0.000910	16	0.0003587	0.000271 - 0.000607	0.5193
Heptanoic acid [%]	14	0.2104	0.0102 - 0.299	16	0.02977	0.0183 - 0.179	0.3184
Heptanoic acid [mg/g]	14	0.000338	0.0000481 - 0.000567	16	0.0001184	0.0000643 - 0.000304	0.5746

Table 4. Comparison of SCFAs content between the intervention group and control group

	Intervention group			Control group			
	n	Median	25 - 75 P	n	Median	25 - 75 P	P [a]
Acetic acid [%]	14	33.6201	29.783 - 41.353	16	43.3003	40.140 - 46.555	0.0034
Acetic acid [mg/g]	14	0.09889	0.0650 - 0.153	16	0.09136	0.0802 - 0.119	0.9369
Propionic acid [%]	14	17.5325	13.648 - 22.118	16	15.1032	13.049 - 20.355	0.3768
Propionic acid [mg/g]	14	0.06016	0.0300 - 0.0776	16	0.03820	0.0267 - 0.0514	0.1493
Butyric acid [%]	14	25.3448	20.150 - 33.131	16	19.0485	15.538 - 25.957	0.1702
Butyric acid [mg/g]	14	0.08301	0.0648 - 0.154	16	0.03719	0.0272 - 0.0775	0.0552
Isobutyric acid [%]	14	5.3615	4.198 - 6.474	16	4.9286	3.758 - 5.808	0.3294
Isobutyric acid [mg/g]	14	0.01732	0.00843 - 0.0241	16	0.01003	0.00714 - 0.0134	0.0513
Isovaleric acid [%]	14	7.8807	4.350 - 9.492	16	6.3459	4.960 - 11.719	0.6920
Isovaleric acid [mg/g]	14	0.02259	0.0142 - 0.0296	16	0.01440	0.0129 - 0.0180	0.0324
Valeric acid [%]	14	5.5977	4.460 - 6.397	16	4.8846	4.108 - 5.667	0.1609
Valeric acid [mg/g]	14	0.01897	0.00898 - 0.0250	16	0.01153	0.00822 - 0.0142	0.0235
Caproic acid [%]	14	0.4627	0.216 - 2.857	16	0.4318	0.238 - 2.060	0.7839
Caproic acid [mg/g]	14	0.001287	0.000618 - 0.00581	16	0.001087	0.000507 - 0.00311	0.3664
Isocaproic acid [%]	14	0.1704	0.0793 - 0.294	16	0.1797	0.107 - 0.249	0.9514
Isocaproic acid [mg/g]	14	0.0005387	0.00025 - 0.000992	16	0.0003417	0.000276 - 0.000585	0.4190
Heptanoic acid [%]	14	0.1072	0.0116 - 0.356	16	0.07301	0.0219 - 0.290	0.9757
Heptanoic acid [mg/g]	14	0.0001552	0.0000525- 0.00059	16	0.0002231	0.0000580 - 0.000526	0.8368

This table shows differences in stool SCFAs content between intervention and control group

There were statistically significant differences ($p < 0.05$) in the consumption of fatty foods (coconut oil, animal fat, butter (all types), medium-chain fatty acids, vegetable fats (including olive and avocado)), berries, sugar and honey, nuts, seeds and seeds, non-berry fruit, dried fruit, and fruit preserves, sweetened dairy products, total cereal products, salty and sweet snacks and fast-food, total fish, vegetable and fruit juices, cream and mayonnaise and dressings, vegetable legumes, green leafy and cruciferous vegetables, and potatoes, sweet potatoes, and their derivatives.

In the study group, a statistically significant negative correlation was observed between the consumption of animal fats and the percentage content of acetic acid, caproic acid, and heptanoic acid. Additionally, a significant positive correlation was found between the consumption of animal fats and the increased percentage of propionic acid and a mean positive correlation for the concentration of isovaleric acid. The consumption of nuts, seeds, and seeds negatively correlated with the percentage of butyric acid. There was a tendency where the consumption of vegetable fats and avocados correlated negatively with the content of iso-butyric acid and positively with the content of isocaproic acid. At the same time, there was a strong positive correlation between the consumption of medium-chain fatty acids and the concentration of butyric acid, and a mean positive correlation for the percentage concentration. There was also an average positive correlation between the consumption of these fats and the concentration and percentage of propionic acid. The declared consumption of medium-chain fatty acids negatively correlated with the percentage of acetic acid and caproic acid, and there was a strong negative correlation with the percentage of heptanoic acid.

In the control group, there was an average negative correlation between the intake of nuts, seeds, and seeds and the percentage of acetic acid. The consumption of coconut oil negatively correlated with the concentration of propionic acid, and the consumption of animal fats negatively correlated with the percentage of caproic acid. There was a weak trend towards increased amounts of valeric acid with increased consumption of animal fats and medium-chain fatty acids.

In the study group, the consumption of red meat, venison, and processed meat products correlated negatively with the percentage of acetic acid. Additionally, the consumption of red meat and venison positively correlated with the content of propionic acid. The consumption of fish positively correlated with the concentration of heptanoic acid.

In the control group, a negative correlation was observed between the consumption of poultry and rabbit meat and the percentage of propionic acid ($RHO = -0.517$; $p = 0.0403$), and a positive correlation ($RHO = 0.562$; $p = 0.0235$) between the consumption of red meat and venison and the concentration of valeric acid. There was also a strong correlation between the consumption of fish and a higher percentage of acetic

Table 5. Consumption of fatty products and SCFAs content in the intervention group

Intervention group								
		Total fats	Coconut oil	Animal fat	Butter	Vegetable fats (incl. avocado and olive oil)	Nuts and seeds	MCT oil
Acetic acid [%]	RHO	-0.281	-0.007	**-0.688**	-0.253	0.467	0.228	**-0.653**
	p	0.331	0.9809	**0.0066**	0.4036	0.0922	0.4328	**0.0113**
	n	14	14	14	13	14	14	14
Acetic acid [mg/g]	RHO	0.108	-0.106	0.333	0.319	-0.213	-0.299	**0.495**
	p	0.713	0.719	0.245	0.2876	0.4655	0.2985	**0.0719**
	n	14	14	14	13	14	14	14
Propionic acid [%]	RHO	0.194	-0.045	**0.823**	0.48	-0.239	-0.091	**0.593**
	p	0.5056	0.8797	**0.0003**	0.0965	0.4098	0.7575	**0.0256**
	n	14	14	14	13	14	14	14
Propionic acid [mg/g]	RHO	0.065	-0.127	0.401	0.211	-0.255	-0.395	**0.545**
	p	0.8257	0.6656	0.1548	0.4881	0.3791	0.1625	**0.044**
	n	14	14	14	13	14	14	14
Butyric acid [%]	RHO	-0.022	-0.028	0.011	0.015	-0.364	**-0.574**	**0.611**
	p	0.9416	0.9239	0.9691	0.9623	0.201	**0.032**	**0.0204**
	n	14	14	14	13	14	14	14
Butyric acid [mg/g]	RHO	0.151	-0.07	0.315	0.334	-0.154	-0.328	**0.788**
	p	0.6056	0.8108	0.2734	0.2649	0.5994	0.252	**0.0008**
	n	14	14	14	13	14	14	14
Isobutyric acid [mg/g]	RHO	0.065	0.052	0.408	0.095	**-0.49**	-0.333	0.421
	p	0.8257	0.8607	0.1472	0.7581	**0.0754**	0.2453	0.1342
	n	14	14	14	13	14	14	14
Isovaleric acid [mg/g]	RHO	**0.497**	0.023	**0.566**	0.118	-0.368	-0.166	0.299
	p	0.0705	0.9365	**0.0348**	0.7008	0.1959	0.5699	0.2993
	n	14	14	14	13	14	14	14
Valeric acid [%]	RHO	0.108	0.04	**0.496**	0.131	-0.249	-0.002	-0.088
	p	0.7134	0.8923	**0.0714**	0.6696	0.3911	0.994	0.7652
	n	14	14	14	13	14	14	14
Caproic acid [%]	RHO	-0.238	0.157	**-0.585**	-0.274	0.04	-0.027	**-0.687**
	p	0.4136	0.5914	**0.028**	0.3655	0.8923	0.9281	**0.0066**
	n	14	14	14	13	14	14	14
Isocaproic acid [%]	RHO	0.022	-0.35	-0.334	0.058	**0.479**	0.419	-0.306
	p	0.9416	0.2203	0.2438	0.8501	**0.0833**	0.1363	0.2867
	n	14	14	14	13	14	14	14
Isocaproic acid [mg/g]	RHO	0.195	**-0.475**	0.234	**0.497**	0.18	0.062	0.45
	p	0.5051	**0.0864**	0.4198	**0.0839**	0.5386	0.833	0.1064
	n	14	14	14	13	14	14	14
Heptanoic acid [%]	RHO	-0.324	-0.162	**-0.628**	-0.376	0.387	0.237	**-0.807**
	p	0.2586	0.5802	**0.0161**	0.2059	0.1713	0.4146	**0.0005**
	n	14	14	14	13	14	14	14

This table shows the correlation between consumption of the fatty products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

Table 6. Consumption of fatty products and SCFAs content in the control group

Control group								
		Total fats	Coconut oil	Animal fat	Butter	Vegetable fats (incl. avocado and olive oil)	Nuts and seeds	MCT oil
Acetic acid [%]	RHO	0.272	0.186	0.313	-0.102	-0.183	**-0.509**	0.108
	p	0.3085	0.4895	0.2371	0.7068	0.4965	**0.0443**	0.6909
	n	16	16	16	16	16	16	16
Propionic acid [mg/g]	RHO	-0.103	**-0.57**	-0.132	-0.312	0.282	0.107	-0.349
	p	0.7034	**0.0212**	0.625	0.2389	0.2901	0.694	0.1846
	n	16	16	16	16	16	16	16
Valeric acid [%]	RHO	-0.008	0.22	-0.125	0.043	-0.003	-0.079	**0.444**
	p	0.9767	0.4124	0.6452	0.8735	0.9911	0.7724	**0.0846**
	n	16	16	16	16	16	16	16
Valeric acid [mg/g]	RHO	-0.436	-0.037	**0.443**	-0.158	-0.141	-0.366	0.267
	p	0.0917	0.8919	**0.0858**	0.5596	0.6025	0.163	0.3165
	n	16	16	16	16	16	16	16
Caproic acid [%]	RHO	-0.084	0.12	**-0.522**	-0.326	0.061	-0.01	-0.371
	p	0.7564	0.6577	**0.0381**	0.2174	0.8235	0.9696	0.1571
	n	16	16	16	16	16	16	16
Caproic acid [mg/g]	RHO	-0.292	0.018	-0.481	-0.418	0.161	-0.083	**-0.449**
	p	0.2716	0.9458	0.0593	0.1076	0.5522	0.7598	**0.0813**
	n	16	16	16	16	16	16	16

This table shows the correlation between consumption of the fatty products and the stool SCFAs content in the control group. RHO – *Spearman's* rank correlation coefficient.

Table 7. Consumption of meat and fish products and SCFAs content in the intervention group

Intervention group					
		Poultry and rabbit meat	Red meat and game	Processed meat products	Fish
Acetic acid [%]	RHO	0	**-0.571**	**-0.55**	-0.009
	p	1	**0.033**	**0.0416**	0.9757
	n	14	14	14	14
Propionic acid [%]	RHO	-0.27	**0.587**	0.372	0.004
	p	0.3513	**0.0273**	0.1905	0.9878
	n	14	14	14	14
Propionic acid [mg/g]	RHO	-0.13	**0.491**	0.17	0.067
	p	0.6569	**0.0744**	0.5621	0.8188
	n	14	14	14	14
Isovaleric acid [%]	RHO	0.096	0.305	0.251	**0.48**
	p	0.7442	0.289	0.3871	0.082
	n	14	14	14	14
Valeric acid [mg/g]	RHO	0.05	**0.48**	0.3	0.027
	p	0.8644	**0.0825**	0.2981	0.9271
	n	14	14	14	14
Heptanoic acid [mg/g]	RHO	**0.508**	0.099	-0.282	**0.582**
	p	**0.0638**	0.7371	0.3288	**0.0289**
	n	14	14	14	14

This table shows the correlation between consumption of the following animal products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

acid (RHO = 0.732; p = 0.0013). In the ketogenic group, a correlation was observed between the consumption of natural unsweetened plant drinks (RHO = 0.657; p = 0.0108) and cream, mayonnaise, and dressings (RHO = 0.558; p = 0.0379) and the percentage of isovaleric acid. Much more correlations were found in the control group, including correlation between consumption of sweetened dairy products and acetic, propionic, butyric, iso-butyric, isovaleric, caproic, and heptanoic acids. The consumption of natural dairy products positively correlated with the percentage of isocaproic acid. The declared consumption of cream, mayonnaise, and dressings correlated positively with the content of butyric acid and negatively with the content of isovaleric acid.

The consumption of berries in the ketogenic group was significantly higher than in the control group. Additionally, significant positive correlations were observed between their consumption and higher amounts of acetic, propionic, iso-butyric, and isocaproic

Table 8. Vegetable and fruit consumption and SCFAs content in the intervention group

Intervention group							
		Non-berry fruit	Berries	Vegetables (all kinds)	Other vegetables	Legumes	Green leafy and cruciferous vegetables
Acetic acid [%]	RHO	-0.281	-0.007	**-0.688**	-0.253	0.467	0.228
	p	0.331	0.9809	**0.0066**	0.4036	0.0922	0.4328
	n	14	14	14	13	14	14
Propionic acid [%]	RHO	0.194	-0.045	**0.823**	0.48	-0.239	-0.091
	p	0.5056	0.8797	**0.0003**	0.0965	0.4098	0.7575
	n	14	14	14	13	14	14
Butyric acid [%]	RHO	-0.022	-0.028	0.011	0.015	-0.364	**-0.574**
	p	0.9416	0.9239	0.9691	0.9623	0.201	**0.032**
	n	14	14	14	13	14	14
Isobutyric acid [mg/g]	RHO	0.065	0.052	0.408	0.095	**-0.49**	-0.333
	p	0.8257	0.8607	0.1472	0.7581	**0.0754**	0.2453
	n	14	14	14	13	14	14
Isovaleric acid [mg/g]	RHO	**0.497**	0.023	**0.566**	0.118	-0.368	-0.166
	p	**0.0705**	0.9365	**0.0348**	0.7008	0.1959	0.5699
	n	14	14	14	13	14	14
Valeric acid [%]	RHO	0.108	0.04	**0.496**	0.131	-0.249	-0.002
	p	0.7134	0.8923	**0.0714**	0.6696	0.3911	0.994
	n	14	14	14	13	14	14
Valeric acid [mg/g]	RHO	0.238	0.021	**0.468**	0.144	-0.403	-0.335
	p	0.413	0.9428	**0.0917**	0.638	0.1531	0.2419
	n	14	14	14	13	14	14
Caproic acid [%]	RHO	-0.238	0.157	**-0.585**	-0.274	0.04	-0.027
	p	0.4136	0.5914	**0.028**	0.3655	0.8923	0.9281
	n	14	14	14	13	14	14
Isocaproic acid [%]	RHO	0.022	-0.35	-0.334	0.058	**0.479**	0.419
	p	0.9416	0.2203	0.2438	0.8501	**0.0833**	0.1363
	n	14	14	14	13	14	14
Isocaproic acid [mg/g]	RHO	0.195	**-0.475**	0.234	**0.497**	0.18	0.062
	p	0.5051	**0.0864**	0.4198	**0.0839**	0.5386	0.833
	n	14	14	14	13	14	14
Heptanoic acid [%]	RHO	-0.324	-0.162	**-0.628**	-0.376	0.387	0.237
	p	0.2586	0.5802	**0.0161**	0.2059	0.1713	0.4146
	n	14	14	14	13	14	14

This table shows the correlation between consumption of the following vegetable and fruit products and the stool SCFAs content in the intervention group. RHO – *Spearman's* rank correlation coefficient.

acid. The consumption of non-berry fruit positively correlated with the amount of butyric acid. A negative correlation was noted between the consumption of legumes and the content of acetic and caproic acid, as well as a positive correlation for propionic acid, poultry and rabbit meat and the percentage of propionic acid (RHO = -0.517; p = 0.0403) and a positive correlation (RHO = 0.562; p = 0.0235) between the consumption of red meat and venison and the concentration of valeric acid. There was also a strong correlation between the consumption of fish and a higher percentage of acetic acid (RHO = 0.732; p = 0.0013). In the ketogenic group, a correlation was observed between the consumption of natural unsweetened plant drinks (RHO = 0.657; p = 0.0108) and cream, mayonnaise, and dressings (RHO = 0.558; p = 0.0379) and the percentage of isovaleric acid. Much more correlations were found in the control group, including correlation between consumption of sweetened dairy products and acetic, propionic, butyric, iso-butyric, isovaleric, caproic, and heptanoic acids. The consumption of natural dairy products positively correlated with the percentage of isocaproic acid. The declared consumption of cream, mayonnaise, and dressings correlated positively with the content of butyric acid and negatively with the content of isovaleric acid.

The consumption of berries in the ketogenic group was significantly higher than in the control group. Additionally, significant positive correlations were observed between their consumption and higher amounts of acetic, propionic, iso-butyric, and isocaproic acid. The consumption of non-berry fruit positively correlated with the amount of butyric acid. Moreover, a negative correlation was noted between the consumption of legumes and the content of acetic and caproic acid, as well as a positive correlation for propionic acid.

The control group was characterized by significant negative correlations between the consumption of fruit, total vegetables, vegetables other than leafy greens and cruciferous vegetables, and legumes, and the content of valeric acid. The consumption of total fruit and berry fruit positively correlated with the content of caproic acid. The consumption of vegetables other than leafy and cruciferous vegetables negatively correlated with the content of isocaproic acid.

In the control group, there were no statistically significant correlations between the consumption of cereal products, potatoes, sweet potatoes, and derivatives and the content of SCFAs. In contrast, in the ketogenic group, statistically significant correlations between the consumption of potatoes, sweet potatoes, and similar products and the content of SCFAs were observed. There were no such correlations for cereal products. In the study group, between these variables there was a negative correlation for acetic acid in percent (RHO = -0.532; p = 0.0501) and positive correlations for acetic acid expressed in mg/g (RHO = 0.577; p = 0.0307), for propionic acid as a percentage (RHO = 0.667); p = 0.0091) and concentration (RHO = 0.765;

Tab. 9. Vegetable and fruit consumption and SCFAs content in the control group

Control group							
		Non-berry fruit	Berries	Vegetables (all kinds)	Other vegetables	Legumes	Green leafy and cruciferous vegetables
Valeric acid [mg/g]	RHO	**-0.578**	0.188	**-0.55**	**-0.553**	**-0.558**	-0.416
	p	**0.019**	0.486	**0.0273**	**0.0262**	**0.0248**	0.1087
	n	16	16	16	16	16	16
Caproic acid [%]	RHO	**0.571**	0.42	0.39	0.085	0.17	0.056
	p	**0.0209**	0.1057	0.1353	0.753	0.5295	0.8362
	n	16	16	16	16	16	16
Caproic acid [mg/g]	RHO	**0.463**	0.504	0.23	-0.111	0.041	-0.112
	p	**0.0708**	0.0465	0.3915	0.6824	0.8814	0.6784
	n	16	16	16	16	16	16
Isocaproic acid [mg/g]	RHO	-0.229	-0.103	0.057	**-0.501**	-0.012	-0.28
	p	0.3944	0.7033	0.8325	**0.0481**	0.9647	0.2943
	n	16	16	16	16	16	16
Heptanoic acid [mg/g]	RHO	0.282	**0.46**	0.092	-0.187	-0.074	-0.296
	p	0.2905	**0.0728**	0.7356	0.487	0.7863	0.2652
	n	16	16	16	16	16	16

This table shows the correlation between consumption of the following vegetable and fruit products and the stool SCFAs content in the control group. RHO – *Spearman's* rank correlation coefficient.

p = 0.0014), for the concentration of iso-butyric acid (RHO = 0.616; p = 0.0191) and for the concentration of valeric acid (RHO = 0.547; p = 0.0431).

DISCUSSION

The stool samples obtained from the test group (ketogenic diet) showed a higher content of isovaleric acid, iso-butyric acid, butyric acid, and a lower amount of acetic acid (compared to the control group - people on a standard diet). The higher fat intake of the ketogenic diet and the drastic elimination of carbohydrates seems to have a big impact on the bacterial metabolome. Isovaleric acid and iso-butyric acid are branched short-chain fatty acids which are the product of proteolytic (putrefactive) fermentation in the large intestine (with the participation of proteolytic microbiota from the *Proteobacteria* group) [20]. Higher content of *Proteobacteria* (in the large intestine) combined with a decrease in the number of microbiota that metabolizes non-digestible carbohydrates (complex glycans, polysaccharides (cellulose, hemicellulose, lignin, resistant starch pectin, and oligosaccharides), monosaccharides, mucins, mucopolysaccharides may be associated with colon cancer progression), inflammation, and the phenomenon of insulin resistance [20, 21]. The large differences in the content of isovaleric acid and iso-butyric acid (BCFAs) obtained in these studies between the groups may indicate differences in proteolytic fermentation, which takes place in a different way depending on the availability of dietary fibre in the diet. The amount of BCFAs increases significantly within 24 hours of consuming more protein. Higher protein intake increases the overall pool of isovaleric acid, which is present in trace amounts in a norm-protein diet [8, 22, 23]. Still little is known about the role of BCFAs in our bodies. It is known from individual reports that some of them can be used as an energy source in the event of an insufficient amount of butyrate (SCFAs are also produced because of proteolytic fermentation (mainly from glutamine and asparagine), but their total pool is much smaller if this fermentation does not come from carbohydrate substrates) [8]. Due to the lack of scientific data regarding the influence of BCFAs, including isovaleric acid on the host's health, the results obtained in this study can only be speculated.
The process of isovaleric acid formation is associated with an increased synthesis of ammonia, which is a toxic product whose content in blood and tissues must be strictly controlled. Ammonia from proteolytic fermentation (along with BCFAs) can be lowered by adding dietary fibree to the diet. Increasing the fermentation of dietary fibree will result in an influx of ammonia to bacterial cells because of the demand for nitrogen, as well as preferential fermentation of carbohydrate substrates. In addition, an acidic environment in the distal colon alone can influence the metabolism of gut bacteria to such an extent that BCFAs synthesis is reduced [8, 22]. However, there are such fractions of dietary fibree that can increase the production of iso-butyric and isocaproic acid. Low pH in the large intestine and the availability of dietary fibree is associated with an increase in bacterial differentiation and a reduction in putrid fermentation. It seems that starch may be of great importance in reducing the intensity of proteolysis, but the process still depends on the pH in the intestinal lumen. BCFAs production is 60% lower at pH=5.5 compared to pH=6.88.20.22. As proved by *Pieper R. et al*., Increasing the amount of dietary fibre from 10 g to 30 g per day resulted in a preferential utilization of fibree over protein, and increased the overall amount of SCFAs, and shortened intestinal transit [23]. It is very important from the point of view of the carcinogenic activity of the metabolites of putrefactive microflora contained in the faeces, as well as from the point of view of the putrid fermentation itself, which takes place in the final sections of the gastrointestinal tract. The shorter the intestinal transit, the less putrid metabolites are likely to be produced. The lower amount of acetic acid in the ketogenic group compared to the test group may be related to the diet (providing a smaller pool of carbohydrates including fibree) and the likely greater absorption of acetate into the circulation. Acetate is trapped in the brain (it crosses the blood-brain barrier) and accumulates in the hypothalamus, changing the expression of the regulatory neuropeptide profile, and thus the regulation of appetite, which could explain the lower number of meals consumed during the day in the study group [8, 9]. Acetic acid produced by bacteria min. *Bacteroidetes* can be used for the synthesis of propionic and butyric acid by *Firmicutes* [24]. It seems that this could to some extent explain the ratio of acetic acid to butyric acid. The greater amount of butyric acid in the stool of the test group may also be due to the much greater consumption of vegetables (mainly leafy and cruciferous) by the ketogenic group compared to the control group, which may mitigate to some extent the effects caused by the greater amount of potentially harmful branched fatty acids. A greater amount of butter acid could also result from the growth of *Akkermansia muciniphila*, which was observed after the implementation of the ketogenic diet in the studies conducted by *Olson et al.* [26] and *Ma et al*. [25]. It should be noted that the very estimation of the degree of short-chain fatty acids production is methodologically problematic because we observe their rapid and continuous uptake by colonocytes, as well as their use for metabolism not only in the host but also in the microbiome. Currently, SCFAs can be performed in faeces and serum, but both measurements have some

limitations. We used stool samples in our study, so it should be remembered that only 5% of the total pool of SCFAs produced is likely to be found in the sample [8, 9].

In the study group, a positive correlation was observed between the consumption of total fat and animal fat and the increased amount of isovaleric acid (compared to the control group). The results are consistent with the high consumption of protein in the diet of athletes. The result confirms previous studies in which the protective role of carbohydrate fermentation (saccharolytic fermentation) was observed. It has been found that the consumption of carbohydrates limits putrefaction, and the fermentation of carbohydrates itself, even though more protein is present in the lumen of the gut, may reduce the production of putrefactive metabolites. The consumption of animal fats in the study group also strongly positively correlated with the content of propionic acid (RHO = 0.823). A similar observation was made for medium-chain fatty acids (MCTs). Propionic acid induces some metabolic functions, such as increasing energy expenditure, leptin expression, reducing energy consumption, increasing PYY (YY peptide) expression, increasing intestinal gluconeogenesis, and hepatic gluconeogenesis. Increased propionate may also be associated with suppression of appetite and its function of glucose production in the process of gluconeogenesis, which is more active during the ketogenic diet [23, 27, 28, 29]. The consumption of medium-chain fatty acids in the ketogenic group was significantly higher than in the control group. MCTs showed correlations with a large amount of short-chain fatty acids, at the same time these correlations were different than those associated with the consumption of long-chain animal fats. The strongest positive correlation was observed between the consumption of MCTs and the increase in the amount of butyric acid. This may coincide with the results of the study by *Rial S. et al* discussing the beneficial effects of MCTs fats in obesity [28]. Microbiome changes induced by MCTs consumption included, among others, the protective effect of MCTs on toxicity and endotoxemia induced by bacterial lipopolysaccharide, improvement of intestinal barrier integrity, increase of sIgA secretion, and decrease of faecal pH. The consumption of medium-chain fatty acids did not correlate with the increase in the amount of isovaleric and iso-butyric acids, which proves their different metabolism compared to animal fats, which showed this correlation. MCTs fats are characterized by fast digestion and absorption and show a different cellular metabolism than long-chain triglycerides [23, 27, 30]. The consumption of vegetables in the ketogenic group was positively correlated with the amount of valeric acid, while in the control group the correlation was negative. This may indicate a significant difference in the composition and function of the microbiome in the face of the drastic elimination of one nutrient (carbohydrates) and a large percentage of another nutrient (fats). Potting fermentation and its products appear to vary with the amount of carbohydrate and fat in the habitual diet. The consumption of berries in the study group negatively correlated with the content of isovaleric acid, which may indicate the potential protective and prebiotic effect of polyphenols contained in colourful and tart berries [8, 27, 30]. The differences in the frequency of consumption of given product groups showed that the ketogenic group consumed vegetables and berries, nuts, seeds and seeds, vegetable fats, and fish significantly more often, which is not insignificant for health. The Mediterranean variant of KD seems to be the most pro-health, but it should still be approached with scepticism if a person wants to use this model of nutrition as usual, without specific health indications [29, 30]. A significant difference in minus was the greater consumption of cream, mayonnaise, and dressings, which may have an adverse effect on the bacterial metabolome due to the high degree of processing of these products, as well as the unfavourable composition (trans fatty acids, disturbed *omega*-3 to *omega*-6 ratio), added sugar, salt, and preservatives). The opposition is significantly lower consumption of salty snacks, sweet snacks, and fast-food products in the ketogenic group, which may indicate greater health and consumer awareness of people implementing this model of nutrition.

CONCLUSIONS

High consumption of cruciferous and leaf vegetables, berries and nuts on a ketogenic diet may have a positive effect on the profile of short-chain fatty acids produced by the gut microbiome. Changing the diet towards a greater supply of plant products may prevent proteolytic fermentation and reduce the negative effects of microbiome changes caused by an oversupply of protein and fat in the ketogenic diet.

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Conflict of interest

The authors declare no conflict of interest.

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