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SCFAs and Their Potential Role in Modulating Cardiometabolic Disease Risk by Interacting with Adiposity Parameters and Diet in Healthy Non-Obese Individuals (a Cross-Sectional Study)

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Abstract: The main objective of this cross-sectional study was to analyze the influence of lifestyle factors (diet, physical activity, sleep), which can affect the concentrations of fecal short-chain fatty acids (SCFAs) and SCFAs potential role in modulating cardiometabolic disease risk by interacting with biochemical and body composition parameters. The study comprised 77 healthy, non-obese individuals, aged 30-45 years who were assessed in the light of SCFAs concentration in stool, diet, physical activity level, and sleep duration. Moreover, body composition measurement and patients' biochemical parameters were included in the analysis. We have indicated a significant negative correlation between several SCFAs [especially acetic acid (AA), isobutyric acid (IBA), butyric acid (BA), isovaleric acid (IVA) and valeric acid (VA)] with BMI, VAT/SAT ratio (visceral to subcutaneous fat ratio) and percentage of fat mass in a group of females enrolled in the study as well as with waist circumference (WC) in case of both sexes included in the study. Moreover, the results of our study acknowledged the importance of a diet in shaping SCFAs profile - we noticed the significant negative associations between energy and fat intake and some SCFAs in males [IBA, IVA, VA, isocaproic acid (ICA)]. In terms of fiber intake, we noticed elevated concentrations of the vast majority of SCFAs and the amount of SCFAs in total in the case of both sexes included in the study. These strong correlations reflect the fact that diet shapes the composition of the gut microbiota and SCFAs (main microbial metabolites) are synthesized from dietary fiber. Furthermore, we noticed that in a group of women, the concentration of AA, propionic acid (PA), and ICA as well as the total concentration of SCFAs showed a significant positive association with their sleep duration. We concluded that SCFAs can have a potential role in modulating cardiometabolic disease risk by interacting with adiposity parameters and diet. In addition, this potential direct link between diet and SCFAs may at least partly contribute to sleep improvement.

Keywords: SCFAs; body composition; VAT/SAT ratio; waist circumference; biochemical results; diet; dietary fiber; physical activity; total sleep time

1. Introduction

Diet determines the gut microbiota structure, which has been identified as one of several potential pivotal factors illustrating dietary impact on health and disease [1,2]. Nonetheless, scrutinizing the link between health and microbiota in humans is demanding due to difficulties in controlling for environmental factors in individuals enrolling in the study. Several comprehensive studies have recently identified interactions between gut microbiota and diet that are associated with various cardiometabolic markers [2–4], however up to this point solely animal studies present some evidence of causality.

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In this study, we scrutinized lifestyle factors [diet, physical activity, sleep] and gut microbiota interaction focusing on the gut-derived microbial metabolites of SCFAs.

SCFAs are the primary end-products of fermentation of non-digestible polysaccharides and are regulatory compounds linked to improved gut health through several effects, including intestinal barrier integrity [5], mucus production [6], and serotonin release [7]. Moreover, SCFAs seem to have a crucial impact on metabolic regulation, and consequently, SCFAs can modulate cardiometabolic risk. Intervention trials in humans with fiber-enriched diets have consistently demonstrated a positive impact on gut microbiota composition, with an increase in SCFA-producing bacteria and SCFAs in stool or blood samples [8–12]. This evidence from human studies seems mainly to support the beneficial role of SCFAs in the regulation of blood glucose, blood lipids, and energy homeostasis. Nevertheless, robust conclusions about causality cannot be made. Moreover, according to several studies, higher SCFAs in stool were associated with negative outcomes such as gut microbiome dysbiosis, obesity, hypertension, and cardiometabolic disease risk factors [13]. Further, SCFAs, as a source of energy, may be associated with the occurrence of overweight or obesity [2]. Consequently, the association of SCFAs with metabolic disorders and obesity is ambiguous.

Therefore, in the present study, we aimed to (1) analyze the stool concentration of SCFAs in healthy non-obese individuals in a sex-based division and (2) analyze the influence of lifestyle factors (diet, physical activity, sleep), which can affect the concentrations of fecal SCFAs and SCFAs potential role in modulating cardiometabolic disease risk, including adiposity parameters such as VAT/SAT ratio, waist circumference (WC) and biochemical parameters.

2. Materials and Methods

2.1. Subjects and data collection

The cross-sectional study was carried out in the Medical University of Warsaw, in 77 healthy, non-obese adults (31 men and 46 women), who took part in the research based on the advertisement. The inclusion criteria were age 30-45 years, no diagnosed chronic diseases, and BMI in the range 18.5 kg/m² - 29.9 kg/m². Exclusion criteria included: pharmacological treatment and any contraindications that apply to body composition analysis (confirmed epilepsy, implanted cardiac pacemaker, defibrillator, and metal endoprosthesis). Body weight and height were measured using a measurement station and column scales Seca 799 (±0.1 kg/cm). Waist circumference was measured using a steel measuring tape, with measurements made halfway between the lower border of the ribs and the iliac crest in a horizontal plane. The Ethics Committee of the Medical University of Warsaw approved the study protocol, and all the participating subjects gave written consent.

2.2. Bioelectrical impedance analysis

We used the bioelectrical impedance method to analyze the body composition of participants. The Bioscan 920-2 device from Maltron Int, UK was used to measure impedance at four different frequencies (5, 50, 100, and 200 kHz) according to the manufacturer's instructions. The participants were required to comply with the guidelines issued by the European Society for Clinical Nutrition and Metabolism (ESPEN) [14]. On the day of the examination, the participants were instructed to fast for at least 2 to 3 hours before the test, avoid physical activity for 12 hours before the test, abstain from alcohol, caffeinated beverages, or fizzy drinks for at least 24 hours before the test, and empty their bladder 30 minutes before the test.

2.3. Biochemical tests

Fasting serum concentrations of insulin, glucose, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and high-sensitivity C-reactive protein were measured in all participants. Homeostatic model assessment of insulin resistance (HOMA-IR) index was used as a measure of insulin resistance and calculated from the fasting glucose and insulin concentrations using the following formula:

HOMA-IR = fasting insulin (μU/L) × fasting glucose (nmol/L)/22.5

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Blood samples were obtained from subjects in the morning after a 12-hour fast without them taking any medications.

2.4. Physical activity (PA) measured by accelerometer

Tri-axial accelerometers (wGT3X-BT ActiGraph LLC, Pensacola, FL, USA) were worn on a waist belt and aligned with the right anterior axillary line and worn always for up to 7 days except when showering, bathing, swimming, or involved in contact sports. A common epoch length was 30-s with a minimum wear time defined as 10 hours/day for 4 of the 7 days, one of which was a weekend day. Wear time was determined by subtracting non-wear period from 24 hours. The non-wear period was defined by an interval of at least 60 consecutive minutes of zero activity intensity counts, with allowance for 1-2 min of counts. Time spent in physical activity of moderate (MPA) or vigorous intensity (VPA), separately or combined (MVPA), was based on the application of count thresholds corresponding to moderate or vigorous intensity activity. Freedson's cut-offs [15] were used to differentiate between the three intensity levels of PA:

- moderate PA (MPA): 1952–5724 counts/min,
- vigorous PA (VPA): ≥ 5725 counts/min,
- moderate + vigorous PA (MVPA) ≥ 1952 counts/min.

Time spent in activity of a defined intensity (MPA, VPA, or MVPA) was determined by summing minutes in a day where the count met the criterion for that intensity. Minutes spent in three intensity levels of PA mentioned above were calculated using the Actilife 5.5 software (ActiGraph, Pensacola, FL, USA).

2.5. Sleep duration measured by accelerometer

Sleep duration and its quality were assessed objectively using an electronic tri-axial monitor (wGT3X-BT ActiGraph LLC, Pensacola, FL, USA) for seven consecutive days. Participants with < 4 nights of data were excluded from the analyses. Data were separated into sleep and wake periods by visual inspection of the actigraphy tracings. The scoring was based on the Cole-Kripke algorithm [16] and calculated using the Actilife 5.5 software (ActiGraph, Pensacola, FL, USA).

2.6. Nutritional value of daily food consumption

The assessment of the participant's diet was based on a 3-day dietary record. Energy, the content of macronutrients, and dietary fiber were calculated using Dieta 6.0 nutritional software (National Food and Nutrition Institute, Warsaw, Poland).

2.7. SCFAs

2.7.1. Chemicals

All short fatty acids standards [(acetic acid-AA (C2), propionic acid- PA (C3), butyric acid-BA (C4), isobutyric acid-IBA (C4), valeric acid- VA (C5), isovaleric acid-IVA (C5), caproic acid - CA (C6), isocaproic acid ICA (C6)], short fatty acids isotope-labeled standards (acetic acid-13C2, propionic acid-D6, butyric acid-13C2, isobutyric acid-D7, valeric acid-D9), pyridine anhydrous, 2-nitro phenylhydrazine (3NPH·HCl), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC·HCl) and were acquired from Sigma-Aldrich (St. Louis, MO, USA). All SCFAs stock solutions were prepared in 50% acetonitrile and stored at -20 oC. LC-MS grade acetonitrile, HPLC grade acetonitrile, HPLC grade methanol, and formic acid were obtained from J.T. Baker. Ultra-pure water (Mili-Q water) was produced by a water purification system (Mili-Q, Millipore, Milford, MA, USA).

2.7.2. Sample preparation

A sample of stools was weighed and homogenized (100mg sample per 400µl of 0.9% NaCl), and the sample was centrifuged for 5 min at 5000 rpm. The supernatant was transferred to a new

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laboratory tube. Sample preparation was as follows: 40µL of sample and 80 µL methanol (containing internal standards) were mixed for 1 min. Next, 20 µL of 3NPH solution and 20 µL of EDC- pyridine solution were added and the mixture was incubated at room temperature for 30 mins. The solution was diluted to 1mL with 15% aqueous acetonitrile, centrifuged and 10 µL of the mixture was injected into the apparatus.

2.7.3. Analyzes

SCFAs quantification was performed using Waters Acquity Ultra Performance Liquid Chromatograph coupled with Waters TQ-S triple-quadrupole mass spectrometer. LC/MS/MS analysis was performed in negative electrospray ionization (ESI)- multiple-reaction monitoring (MRM) mode. The SCFAs were separated using a Waters BEH C18 column (1.7µm, 2.1mm x 50 mm) and a Waters BEH C18 guard column (1.7µm, 2.1mm x 5 mm). A 1mL of formic acid in 1L water was used as mobile phase A, and 1mL of formic acid in acetonitrile was used as mobile phase B. The flow rate of the mobile phase was set at 0.6 mL/min.

The limits of quantification (LOQ) were 10 µM for acetic acid, propionic acid, and butyric acid, 0.1 µM for isovaleric acid, valeric acid, and caproic acid.

2.8. Statistical analyzes

The Shapiro-Wilk test was used for testing departure of analyzed variables from the Gaussian distribution. Since variables showed significant departure from parametric distribution, non-parametric statistics were used. Presented are median (and inter-quartile range), Spearman correlation r, and p-value from the Mann-Whitney test. Statistica v. 10 (StatSoft Inc., Tulsa, USA) was used for statistical calculations. A p-value less than 0.05 was considered significant.

3. Results

There were 77 healthy non-obese participants (31 men and 46 women) enrolled. Mean age, height, weight, and BMI were: 37 years, 72 kg, 173 cm, and 24 kg/m² respectively. There were no significant differences in age between males and females ($p > 0.05$), whereas all body composition and anthropometric indicators were significantly different ($p < 0.05$). No sex-specific differences in HOMA-IR, TC, LDL-C, and CRP values were observed. However, significantly higher TG values and lower HDL-C levels were found in males compared to females (119 vs. 78 mg/dl, < 0.001 and 53 vs. 67 mg/dl, $p < 0.001$ respectively). When it comes to lifestyle factors, levels of physical activity (PA) (moderate PA, as well as vigorous PA, jointly referred to as MVPA) were observed as significantly higher in males in comparison to females (90 vs 57 min/day, $p < 0.05$). The same sex-specific differences were observed in energy, protein, fats, and fiber intake, whereas at the same time, no differences in sleep duration and fecal SCFAs percentage were observed.

The descriptive characteristics of the study population are shown in Table 1.

Table 1. Characteristics of study participants.

	Total n = 77		**Females n = 46**		**Males n = 31**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Basic parameters							
Age (years)	36.75	4.69	36.26	4.46	37.48	5.00	*0.354*
Body weight (kg)	72.07	14.42	62.99	8.25	85.55	10.51	*<0.001*
Height (cm)	172.75	9.69	166.72	6.51	181.69	6.01	*<0.001*
BMI (kg/m²)	23.96	3.12	22.64	2.52	25.91	2.93	*<0.001*
WC (cm)	83.86	11.61	77.60	8.16	93.16	9.60	*<0.001*
Body composition parameters							
VAT (cm²)	118.21	82.94	84.02	51.25	168.94	95.05	*<0.001*
SAT (cm²)	97.73	35.41	88.59	32.90	111.29	35.14	*0.006*

VAT/SAT	1.15	0.58	0.93	0.31	1.49	0.72	*<0.001*
FFM (kg)	51.98	10.40	44.39	3.71	63.25	5.80	*<0.001*
FFM (%)	72.27	5.62	71.05	5.63	74.10	5.18	*0.022*
FM (kg)	20.34	6.52	18.69	5.65	22.78	7.03	*0.011*
FM (%)	27.74	5.54	29.04	5.46	25.81	5.17	*0.016*
TBW (Lt)	36.98	7.86	31.24	3.00	45.50	4.19	*<0.001*
TBW (%)	51.28	3.62	49.93	3.14	53.28	3.40	*<0.001*
Biochemical parameters							
TC (mg/dl)	199.32	29.80	199.19	26.60	199.53	34.46	*0.775*
HDL-C (mg/dl)	61.46	14.55	67.22	14.57	52.90	9.53	*<0.001*
LDL-C (mg/dl)	120.35	23.88	116.37	21.42	126.26	26.38	*0.162*
TG (mg/dl)	94.87	47.72	78.35	26.63	119.38	60.55	*<0.001*
CRP (mg/l)	1.54	2.85	1.20	1.21	2.05	4.23	*0.270*
Fasting blood glucose (mg/dl)	97.55	7.33	96.57	5.79	99.00	9.06	*0.192*
Fasting insulin (μU/ml)	8.18	4.64	7.12	2.88	9.77	6.14	*0.153*
HOMA-IR	2.00	1.24	1.71	0.74	2.44	1.65	*0.111*
Physical activity and sleep parameters							
MPA (min/day)	61.69	31.34	52.53	16.89	75.29	41.71	*0.034*
VPA (min/day)	9.00	15.32	5.06	7.55	14.85	21.21	*0.039*
MVPA (min/day)	70.48	43.72	57.39	19.51	89.92	60.15	*0.037*
TST [hr/night]	7.27	1.27	7.45	1.41	7.01	0.98	*0.136*
Short - chain fatty acids in stool							
C 2:0 (AA) [%]	60.80	6.02	61.62	5.83	59.57	6.19	*0.122*
C 3:0 (PA) [%]	15.67	3.49	15.62	2.97	15.74	4.20	*0.640*
C4:0 i (IBA) [%]	2.49	1.31	2.61	1.33	2.30	1.27	*0.383*
C4:0 n (BA) [%]	14.81	6.08	13.99	5.92	16.02	6.20	*0.161*
C5:0 i (IVA) [%]	2.25	1.35	2.39	1.42	2.06	1.25	*0.345*
C5:0 n (VA) [%]	2.73	0.94	2.65	1.02	2.85	0.82	*0.406*
C6:0 i (ICA) [%]	0.04	0.04	0.04	0.03	0.04	0.04	*0.934*
C6:0 n (CA) [%]	1.22	1.36	1.08	0.97	1.42	1.79	*0.771*
Diet parameters							
Energy [kcal/d]	2040.59	448.92	1803.27	263.01	2413.53	428.68	*<0.001*
Protein [g/d]	85.35	23.90	73.37	16.53	104.18	21.57	*<0.001*
Fats [g/d]	77.83	21.02	70.50	14.85	89.34	24.21	*<0.001*
Carbohydrates [g/d]	242.06	63.76	220.98	39.35	275.20	79.75	*0.001*
Fiber [g/d]	25.12	9.09	23.28	8.84	28.02	8.87	*0.027*

Abbreviations: BMI - body mass index, WC - waist circumference, FFM - fat free mass, FM - fat mass, VAT – visceral adipose tissue, SAT - subcutaneous adipose tissue, TBW - total body water, HOMA-IR -homeostatic model assessment insulin resistance, TC - total cholesterol, TG – triglycerides, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, CRP - c-reactive protein, MPA - moderate physical activity, VPA - vigorous physical activity, MVPA – moderate and vigorous physical activity, TST - total sleep time, C 2:0—acetic acid (AA), C 3:0—propionic acid (PA), C 4:0 i— isobutyric acid (IBA), C 4:0 n butyric acid (BA), C 5:0 i— isovaleric acid (IVA), C 5:0 n—valeric acid (VA), C 6:0 i— isocaproic acid (ICA), C 6:0 n—caproic acid (CA).

3.1. Correlation of lifestyle factors and SCFAs

In the correlation analysis conducted in a group of women, the total concentration of SCFAs showed a significant positive association ($p<0.05$) with total sleep time and fiber intake, while in the case of VAT/SAT ratio, we noticed a negative correlation with a total amount of SCFAs. In addition, a positive correlation was observed between some SCFAs and the percentage of FFM and TBW – mainly isobutyric and isovaleric acids, while both above-mentioned SCFAs were significantly

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negatively correlated (p<0.005) with the percentage of fat mass. In the described correlation analysis, fecal SCFAs showed no association with physical activity and laboratory outcomes (Table 2).

Table 2. Correlations of anthropometric, biochemical, and lifestyle parameters and fecal SCFAs – females (n = 46).

	C 2:0 (AA)	C 3:0 (PA)	C4:0 i (IBA)	C4:0 n (BA)	C5:0 i (IVA)	C5:0 n (VA)	C6:0 i (ICA)	C6:0 n (CA)	Total
Age (yr)	-0.04	-0.06	0.16	-0.01	0.15	0.09	0.07	0.16	-0.03
BMI (kg/m²)	-0.14*	-0.10	-0.34*	-0.15*	-0.33	-0.29	0.04	-0.18	-0.15
WC (cm)	-0.27	-0.17	-0.26	-0.23	-0.22	-0.31*	-0.06	-0.12	-0.27
VAT/SAT	-0.36*	-0.30	-0.07*	-0.39**	0.01	-0.18	-0.11	0.00	-0.36*
FFM (%)	0.11	0.07	0.39**	0.14	0.39**	0.32*	0.00	0.17	0.14
FM (%)	-0.11	-0.07	-0.39**	-0.13	-0.39**	-0.32*	0.01	-0.17	-0.13
TBW (%)	0.09	0.08	0.33*	0.11	0.33*	0.22	0.02	0.04	0.11
TC (mg/dl)	-0.21	-0.15	-0.18	-0.07	-0.15	-0.16	-0.29	-0.05	-0.18
HDL-C (mg/dl)	0.02	-0.02	-0.14	0.19	-0.07	-0.03	-0.16	0.17	0.05
LDL-C (mg/dl)	-0.26	-0.18	-0.12	-0.24	-0.12	-0.16	-0.23	-0.14	-0.25
TG (mg/dl)	0.01	0.03	0.00	0.03	-0.03	-0.05	0.08	-0.13	0.00
CRP (mg/l)	-0.15	-0.08	-0.02	-0.02	-0.02	0.07	0.06	0.18	-0.14
Fasting blood glucose (mg/dl)	-0.04	-0.09	-0.07	-0.10	-0.01	0.00	0.03	0.11	-0.05
Fasting insulin (μU/ml)	-0.13	0.02	0.02	-0.24	0.03	0.02	0.03	-0.03	-0.13
HOMA-IR	-0.13	0.01	0.02	-0.25	0.03	0.02	0.02	-0.02	-0.14
MVPA (min/d)	-0.26	-0.10	-0.07	-0.10	-0.07	-0.24	-0.07	-0.12	-0.21
TST [hr/night)	0.34*	0.33*	-0.09	0.23	-0.12	0.12	0.32*	-0.01	0.33*
Energy [kcal/d]	0.04	0.04	-0.21	0.19	-0.14	-0.03	-0.05	0.08	0.08
Carbohydrates [g/d]	0.16	0.12	-0.28	0.25	-0.29	-0.07	-0.07	0.05	0.25
Protein [g/d]	0.08	0.04	0.03	0.16	0.07	0.10	-0.07	0.12	0.12
Fats [g/d]	0.14	0.12	0.07	0.25	0.12	0.20	0.11	0.13	0.21
Fiber [g/d]	0.36*	0.30*	-0.14	0.45**	-0.15	0.11	0.10	0.03	0.38*

The Spearman correlation coefficient, r_s. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. **Abbreviations:** BMI - body mass index, WC - waist circumference, FFM - fat free mass, FM - fat mass, VAT – visceral adipose tissue, SAT - subcutaneous adipose tissue, TBW - total body water, HOMA-IR -homeostatic model assessment insulin resistance, TC - total cholesterol, TG – triglycerides, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, CRP - c-reactive protein, MVPA – moderate and vigorous physical activity, TST - total sleep time, C 2:0—acetic acid (AA), C 3:0—propionic acid (PA), C 4:0 i— isobutyric acid (IBA), C 4:0 n butyric acid (BA), C 5:0 i— isovaleric acid (IVA), C 5:0 n—valeric acid (VA), C 6:0 i— isocaproic acid (ICA), C 6:0 n—caproic acid (CA).

In a group of males, we found no correlations between fecal SCFAs and body composition/ anthropometric parameters, besides waist circumference (significant negative association with propionic and butyric acid), however, we noticed a significant positive association (p<0.05) with some SCFAs and laboratory outcomes, such as TG, CRP, and fasting blood glucose. In addition, in the group of men, as in the group of women, we observed a significant influence of factors related to diet on the concentration of SCFAs. The most noticeable correlations were indicated in the case of fiber intake (significant positive association) and energy and fat intake (significant negative association). In terms of fiber intake, we notice elevated concentrations of almost all analyzed SCFAs.

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Table 3. Correlations of anthropometric, biochemical, and lifestyle parameters and fecal SCFAs – males (n = 31).

	C 2:0 (AA)	C 3:0 (PA)	C4:0 i (IBA)	C4:0 n (BA)	C5:0 i (IVA)	C5:0 n (VA)	C6:0 i (ICA)	C6:0 n (CA)	Total
Age (yr)	-0.15	-0.11	0.06	-0.05	0.04	0.07	0.05	0.21	-0.14
BMI (kg/m²)	-0.10	-0.23	-0.12	-0.21	-0.16	-0.11	-0.03	0.21	-0.17
WC (cm)	-0.19	-0.36*	-0.07	-0.38*	-0.10	-0.15	-0.12	0.07	-0.32
VAT/SAT	-0.08	-0.17	0.22	-0.22	0.19	0.11	-0.10	0.10	-0.15
FFM (%)	0.06	0.17	0.09	0.16	0.08	0.07	-0.07	-0.12	0.13
FAT (%)	-0.10	-0.18	-0.09	-0.23	-0.10	-0.12	0.05	0.06	-0.18
TBW (%)	-0.06	0.04	0.24	0.08	0.23	0.07	0.04	0.04	0.04
TC (mg/dl)	0.11	0.11	-0.14	0.15	-0.07	-0.01	-0.15	0.09	0.15
HDL-C (mg/dl)	-0.18	-0.06	-0.20	-0.16	-0.15	-0.30	-0.25	-0.19	-0.16
LDL-C (mg/dl)	0.19	0.16	-0.09	0.31	0.01	0.09	-0.05	0.08	0.24
TG (mg/dl)	0.01	-0.06	0.18	0.06	0.18	0.22	0.05	0.38*	0.06
CRP (mg/l)	0.08	0.18	0.28	0.25	0.33	0.37*	0.27	0.13	0.17
Fasting blood glucose (mg/dl)	0.36*	0.35	0.01	0.19	-0.05	0.04	-0.09	-0.35	0.30
Fasting insulin (μU/ml)	0.08	0.03	-0.09	-0.14	-0.17	-0.08	-0.04	-0.20	-0.03
HOMA-IR	0.12	0.06	-0.09	-0.10	-0.17	-0.06	-0.03	-0.21	0.01
MVPA (min/d)	0.33	0.27	-0.02	0.18	-0.10	0.12	0.08	0.17	0.28
TST [hr/night]	-0.35	-0.29	0.03	-0.37	0.09	-0.19	-0.01	0.13	-0.31
Energy [kcal/d]	0.11	0.11	-0.37*	0.09	-0.47*	-0.09	-0.09	0.01	0.06
Protein [g/d]	0.26	0.23	-0.32	0.19	-0.37	-0.07	-0.02	0.02	0.24
Carbohydrates [g/d]	0.14	0.20	-0.09	0.28	-0.19	0.26	0.32	0.35	0.14
Fats [g/d]	0.11	-0.16	-0.55**	-0.05	-0.58**	-0.49**	-0.56**	-0.24	-0.02
Fiber [g/d]	0.61***	0.39*	-0.16	0.64***	-0.16	0.48**	0.12	0.39*	0.62***

The Spearman correlation coefficient, r_s. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. **Abbreviations**: BMI - body mass index, WC - waist circumference, FFM - fat free mass, FM - fat mass, VAT – visceral adipose tissue, SAT - subcutaneous adipose tissue, TBW - total body water, HOMA-IR -homeostatic model assessment insulin resistance, TC - total cholesterol, TG – triglycerides, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, CRP - c-reactive protein, MVPA – moderate and vigorous physical activity, TST - total sleep time, C 2:0—acetic acid (AA), C 3:0—propionic acid (PA), C 4:0 i— isobutyric acid (IBA), C 4:0 n butyric acid (BA), C 5:0 i— isovaleric acid (IVA), C 5:0 n—valeric acid (VA), C 6:0 i— isocaproic acid (ICA), C 6:0 n—caproic acid (CA).

3.2. Fecal SCFAs profile: percentage of SCFAs depending on various fiber intake

Considering that the diet factors have a strong correlation with the concentration of fecal SCFAs, we divided study participants into two groups according to fiber intake, to check whether the percentage profile of SCFAs has changed. These two groups were selected for both women and men enrolled in the study. There were statistically significant differences in the percentage of valeric acid between the female groups divided based on fiber intake. In the group of men enrolled in the study, we noticed that these individuals who reported a higher fiber intake had statistically significant elevated percentages of butyric acid and valeric acid (Tables 4 and 5). The other analyzed SCFAs did not differ significantly depending on fiber intake in both genders.

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Table 4. Differences in fecal SCFAs percentage depending on various fiber intake - females.

	fiber intake ≥ 25g (n = 17)		fiber intake < 25g (n = 27)		
SCFA [%]	**median**	**IQR**	**Median**	**IQR**	**p-value***
C 2:0 (AA) [%]	60.31	8.46	60.35	6.11	*0.376*
C 3:0 (PA) [%]	15.75	3.89	15.06	4.09	*0.599*
C4:0 i (IBA) [%]	2.02	2.54	2.87	1.59	*0.157*
C4:0 n (BA) [%]	17.50	8.02	12.54	8.66	*0.070*
C5:0 i (IVA) [%]	1.66	2.37	2.66	1.82	*0.179*
C5:0 n (VA) [%]	2.17	0.93	2.81	0.71	*0.042*
C6:0 i (ICA) [%]	0.03	0.03	0.03	0.03	*0.703*
C6:0 n (CA) [%]	0.54	1.11	1.11	1.44	*0.390*

* Mann–Whitney test **Abbreviations:** C 2:0—acetic acid (AA), C 3:0—propionic acid (PA), C 4:0 i— isobutyric acid (IBA), C 4:0 n butyric acid (BA), C 5:0 i— isovaleric acid (IVA), C 5:0 n—valeric acid (VA), C 6:0 i— isocaproic acid (ICA), C 6:0 n—caproic acid (CA).

Table 5. Differences in fecal SCFAs percentage divided depending on various fiber intake – males.

	fiber intake ≥ 25g (n = 17)		fiber intake < 25g (n = 11)		
SCFA [%]	**median**	**IQR**	**Median**	**IQR**	**p-value***
C 2:0 (AA) [%]	57,98	7,42	59,25	11,88	*0,175*
C 3:0 (PA) [%]	13,71	4,94	14,26	8,41	*0,487*
C4:0 i (IBA) [%]	1,49	0,85	3,46	1,51	*<0,001*
C4:0 n (BA) [%]	19,22	5,09	11,97	9,39	*<0,001*
C5:0 i (IVA) [%]	1,23	1,14	3,16	1,62	*0,002*
C5:0 n (VA) [%]	2,71	1,08	2,79	1,42	*0,430*
C6:0 i (ICA) [%]	0,03	0,03	0,05	0,07	*0,264*
C6:0 n (CA) [%]	1,60	1,89	0,97	1,18	*0,329*

* Mann–Whitney test **Abbreviations:** C 2:0—acetic acid (AA), C 3:0—propionic acid (PA), C 4:0 i— isobutyric acid (IBA), C 4:0 n butyric acid (BA), C 5:0 i— isovaleric acid (IVA), C 5:0 n—valeric acid (VA), C 6:0 i— isocaproic acid (ICA), C 6:0 n—caproic acid (CA).

4. Discussion

Acetic, propionic, and butyric acids are the most abundant SCFAs present in the colon. Typically, the molar ratio of these SCFAs remains consistent at 60:20:20, respectively, under normal physiological conditions [17]. Our research found that proportions of acetic, propionic, and butyric acid and other SCFAs were similar both in the group of women (62:16:18:3:1, respectively) as well as in a group of men (60:16:18:5:1, respectively), with no sex-specific statistically significant differences.

SCFAs play a vital metabolic role – scientific research has revealed that they mediate the activation of G protein-coupled receptors, including GPR41 and GPR43, and assist in preventing fat accumulation in adipose tissue. SCFAs also enhance the metabolism of unincorporated lipids and glucose in other tissues, resulting in improved insulin sensitivity [18,19]. Moreover, SCFAs facilitate the release of satiety hormones such as glucagon-like peptide-1 and peptide YY, further contributing to their beneficial effects [20,21]. Evidence suggests that fecal SCFAs are negatively correlated with adiposity parameters, including VAT and WC [22–24]. Furthermore, it was indicated that dietary supplementation with acetate, propionate, butyrate, or a combination of these can effectively hinder weight gain caused by a high-fat diet [25]. In our study we also indicate a significant negative correlation between acetic, butyric, and isobutyric acid with BMI, VAT/SAT ratio, and percentage of fat in a group of females. In addition, butyric acid has a significant negative association with WC in males. Besides this, we also showed the same significant negative associations for valeric acid with fat and WC in females, being at the same time in significant positive association with the percentage

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of TBW and FFM. All our findings support the above-mentioned thesis, that SCFAs (in our study especially acetic, butyric, and valeric acid) may protect healthy individuals against overweight and obesity. However, some other authors came to different conclusions, and the positive association between elevated fecal SCFAs and cardiometabolic disease risk factors, obesity, or gut microbiome dysbiosis has been demonstrated in several human studies [26–29]. These findings may be explained by low SCFAs absorption in epithelial cells in the gut and the occurrence of chronic inflammation associated with overweight and obesity. These mechanisms warrant further investigation but indeed it is worth mentioning that the concentration of SCFAs in feces is determined by intestinal bacteria's production of these compounds, their absorption, and expenditure in the gastrointestinal tract [29]. Moreover, SCFAs as an energy source may be linked to the development of overweight or obesity [27]. Besides, some studies have shown that the acetic acid produced by the intestinal microbiota is associated with de novo lipogenesis and stimulation of hepatic cholesterogenesis, which may lead to insulin resistance and increased concentration of serum fasting blood glucose [30]. This dependence was partially confirmed in our research – we indicated the significant relationship between acetic acid and fasting blood glucose in a group of men. However, we did not observe a statistically significant relationship between acetic acid and the composition of the host's lipids. Similar results were obtained in a study by Granado-Serrano et al. [31], where no significant difference in the concentrations of acetic acid in the serum between individuals with hypercholesterolemia and those with normocholesterolemia was founded. Besides the above-mentioned association, the significant positive correlations were also notable in the case of other laboratory test results – such as valeric acid and CRP, as well as caproic acid and TG. All described correlations we observed only in the group of men, which may be explained by the higher prevalence of cardiometabolic risk factors occurrence in men [32–34], confirmed also in our study (e.g., lower concentration of HDL, and higher concentration of TG in comparison to women) (Table 1).

Evidence suggests that SCFAs may affect sleep via gut–brain communications by crossing the blood–brain barrier. They have been shown to increase neurogenesis and improve neuronal homeostasis and function [35]. Moreover, bacterial metabolites and components of the bacterial cell wall are likely to provide important links between the intestinal commensal microbiota and sleep-generating mechanisms in the brain [36]. Emerging evidence suggests that the intestinal microbiota is a source of sleep-promoting signals [36–38]. One study showed that giving butyrate orally or by direct intraportal injection resulted in an approximately 50% rise in non-REM sleep in young mice, which lasted for four hours post-treatment [36]. Another animal study detected elevated concentrations of butyrate in mice that exhibited longer sleep duration and faster onset of sleep [37]. Moreover, in human infants, increased fecal propionate was associated with longer sleep duration [38]. In our study, we also indicated such association in the group of females enrolled in the study - acetic, propionic, isocaproic acids and total SCFAs in stool, were associated with longer total sleep time (Table 2).

The connection between physical activity and microbiota composition can be explained through several mechanisms. These include immune modulation, antioxidant activity, gastrointestinal permeability, and the production of metabolites such as SCFAs [39]. However, in our study, we didn't demonstrate any associations between physical activity and SCFAs. In addition, it is worth mentioning that associations between physical activity and SCFAs have not been extensively studied, the results of existing publications are inconsistent [41–44] and the mechanism by which physical activity modulates SCFAs and vice-versa remains speculative [40].

Changes in diet can lead to positive effects on overall health through SCFAs increased production [45]. Studies have demonstrated that increasing the amount of fiber in the diet contributes to the increased concentration of SCFAs in the gut [46,47], while a high-fat diet with a low dietary fiber content decreases levels of SCFAs [48]. Wan et al. [49] conducted a controlled-feeding trial to compare three dietary patterns with varying proportions of fat and carbohydrates. In a randomized controlled feeding trial Wan et al. [49] compared three dietary patterns differing in carbohydrate and fat proportions. The study included 217 healthy young adults who were monitored for 6 months. The diets tested were a lower-fat diet (20% energy from fat), a moderate-fat diet (30% energy from fat),

and a higher-fat diet (40% energy from fat). The researchers found that the high-fat diet had negative effects on the fecal bacterial metabolites, gut microbiota, and markers of inflammation. Conversely, the lower-fat diet was associated with a more advantageous impact on these biomarkers. Also, the results of our study acknowledged the importance of diet - we noticed the significant negative associations between energy and fat intake and some SCFAs (isobutyric, isovaleric, valeric, and isocaproic acids). This finding may support the thesis that a high-fat and high-energy diet lowers levels of SCFAs in the intestine and the introduction of a low-energy diet may increase the quantity and richness of the microorganisms and SCFAs [49–51]. In terms of fiber intake, we noticed (for both genders) elevated concentrations of acetic, propionic, butyric acid, and SCFAs in total, while in the group of men, in addition, caproic and valeric acid were elevated. These strong correlations reflect the fact that SCFAs are synthesized from dietary fiber and a diet with a number of fiber-rich products elevates the synthesis of SCFAs [46,50]. We also showed this correlation in the percentage distribution analysis of SCFAs - our results confirmed the significantly elevated percentage of butyric acid and valeric acid in the groups of individuals with high fiber intake (≥ 25g) (Table 4 and 5).

Some limitations of the present study need to be kept in mind when interpreting the data. The primary limitation of this research was the small size of the participant sample. To draw more objective conclusions, a study that includes a large group of both men and women would be required. Additionally, supplementing our study with an analysis of SCFAs in the circulation and the microbiome profile of each participant, undoubtedly enrich our findings.

5. Conclusions

Our findings support the thesis that SCFAs may protect healthy individuals against overweight and obesity and consequently have a potential role in modulating cardiometabolic disease risk by interacting with adiposity parameters and diet. However, we obtained inconsistent findings regarding biochemical outcomes in a group of men – a significant positive correlation between some fecal SCFAs and fasting blood glucose, CRP, and TG. These contradictory effects require further investigation.

Analyzing lifestyle factors, we didn't identify any associations between physical activity and SCFAs. At the same time, we concluded that a potential direct link between diet (mainly dietary fiber) and SCFAs - indicated in our study - may at least partly contribute to sleep mechanisms and sleep improvement which was shown in the case of women enrolled in the study (however findings in existing publications are scarce and inconsistent).

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