

ARE CHANGES IN FAECAL SHORT-CHAIN FATTY ACID PROFILES POSSIBLE UNDER THE INFLUENCE OF A VEGETARIAN DIET?

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ABSTRACT

Background. Differences in gut microbiota composition between vegetarians and individuals consuming mixed diets are well documented. Increased dietary fibre promotes bacterial fermentation, producing short-chain fatty acids (SCFA). This study analysed whether a shift to a lacto-ovo-vegetarian diet alters faecal SCFA profiles.

Material and methods. Seventy-five healthy adults were enrolled and divided into two groups: participants who adopted a lacto-ovo-vegetarian diet for one month, and a control group who maintained their usual diet. The study was conducted in 2023–2024 in Poznań, at the Department of Bromatology, Poznań. University of Medical Sciences.

Results. Baseline faecal SCFA concentrations did not differ significantly between groups. After 30 days, the study group (SG) showed significant changes in acetic acid concentrations compared with baseline. Propionic acid concentrations also differed significantly between the study group and the control group (CG) after four weeks. Within the SG, significant changes in propionic acid were observed between baseline, two weeks, and four weeks. No significant differences were observed in butyric acid concentrations.

Conclusions. Although some changes in acetic and propionic acid concentrations were observed, the overall lack of consistent differences in SCFA profiles may be explained by insufficient increases in dietary fibre intake. Fibre intake remained comparable between groups and did not increase in the study group. Since fibre is the primary substrate for SCFA production by intestinal bacteria, its limited consumption likely constrained broader changes in faecal SCFA concentrations.

Keywords: SCFA, vegetarian diet, microbiota, lacto-ovo-vegetarian diet

INTRODUCTION

Interest in plant-based diets has grown markedly in the past decade, driven by evidence of health benefits such as reduced risks of chronic disease, as well as ethical and environmental considerations, including animal welfare, lower carbon emissions, reduced resource use, and more sustainable food production systems

(Kamiński et al., 2020). Vegetarian diets are based primarily on plant foods – fruits, vegetables, legumes, cereals, nuts, and seeds – that are rich sources of dietary fibre (Aidoo et al., 2023).

Dietary fibre, both soluble and insoluble, plays a central role in gut health. Insoluble fibre is fermented

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by the intestinal microbiota to produce three principal short-chain fatty acids (SCFAs): acetic acid, propionic acid, and butyric acid. Among these, acetic acid is present in the greatest amounts in the large intestine and constitutes the largest proportion of SCFAs detected in faeces. The human gut microbiota (GM) is a complex ecosystem composed primarily of bacteria, but also including archaea, viruses, fungi, and protozoa. These microorganisms associate symbiotically with the host, aiding in the digestion of otherwise indigestible foods, regulating immune and metabolic functions, and providing protection against opportunistic pathogens (Pagliai et al., 2020). The GM also communicates with intestinal epithelial and immune cells, influencing immune regulation, metabolic pathways, inflammatory responses, and intestinal barrier function through the production of SCFAs. Growing evidence suggests that SCFAs exert pleiotropic effects on digestive and metabolic health, as well as on eating behaviour. Their faecal concentrations are positively associated with fruit, vegetable, and legume intake. Butyric acid, in particular, plays a dual role in colonocyte metabolism: it serves as the primary energy source for colonocytes, promoting the maturation of healthy cells, while also limiting the growth of cancer cells – a phenomenon known as the “butyrate paradox” (Facchin et al., 2024). Plant-based diets have been shown to significantly increase serum SCFA levels (De Filippis et al., 2016). Furthermore, SCFAs are crucial for microglial function, maturation, and the regulation of blood–brain barrier integrity (Berding et al., 2021) by modulating the expression of tight junction proteins such as claudin-5 and occludin (Nandwana et al., 2022).

Butyric acid and its producing bacteria are beneficial to human health (Zhang et al., 2023). As the primary carbon source for colonocytes, butyric acid is critical for maintaining the health and function of the intestinal epithelium. It contributes to key gut functions, including motility, mucus production, visceral sensitivity, epithelial barrier integrity, immune homeostasis, and regulation of mucosal oxygen gradients (Borycka-Kiciak et al., 2017). Accordingly, dietary fibre and carbohydrates may influence SCFA production and alter the abundance of associated microbes. In summary, high-fibre diets may benefit both gut and overall health.

This study analysed changes in faecal SCFA profiles in participants who shifted from a mixed diet including meat products to a lacto-ovo-vegetarian diet for one month.

METHODS

Study design and participants

A total of 75 healthy adults were enrolled and randomly assigned in a 1:1 ratio using randomizer.org.

Study group (SG) – 40 participants (28 females, 70%; 12 males, 30%; age 18–53 years) adopted a lacto-ovo-vegetarian diet for one month. To ensure correct implementation, each participant received individual training from a dietitian (DR).

Control group (CG) – 35 participants (24 females, 68.5%; 11 males, 31.5%; age 20–50 years) continued their habitual omnivorous diet.

Inclusion criteria were – healthy adults aged 18–60 years, willing to modify their current diet to a lacto-ovo-vegetarian diet for one month.

Exclusion criteria were – pregnancy, severe chronic disease, ongoing or recent antibiotic therapy, or supplementation with vitamins or other compounds that could affect study outcomes.

Participation was voluntary, and all participants were informed that they could withdraw at any time without providing a reason.

Methodology of obtaining biological material

Faecal samples for SCFA concentration analysis were collected at home by participants after receiving prior instruction.

Participants in Group 1 (study group; SG) switched to a lacto-ovo-vegetarian diet for 30 days and provided faecal samples on days 0 (diet initiation; sample 1), 14 (sample 2), and 30 (sample 3), while participants in Group 2 (control group; CG) provided samples at the same time points.

All samples were frozen immediately after collection and stored until analysis. In addition, participants completed a 3-day food diary (two weekdays and one weekend day) to assess fibre intake (g/day). Food diaries were collected before and after the intervention and analysed using Dietetyk 2015 software (Jumar, Poznań, Poland). The frequency of consumption of selected dietary fibre sources was assessed using

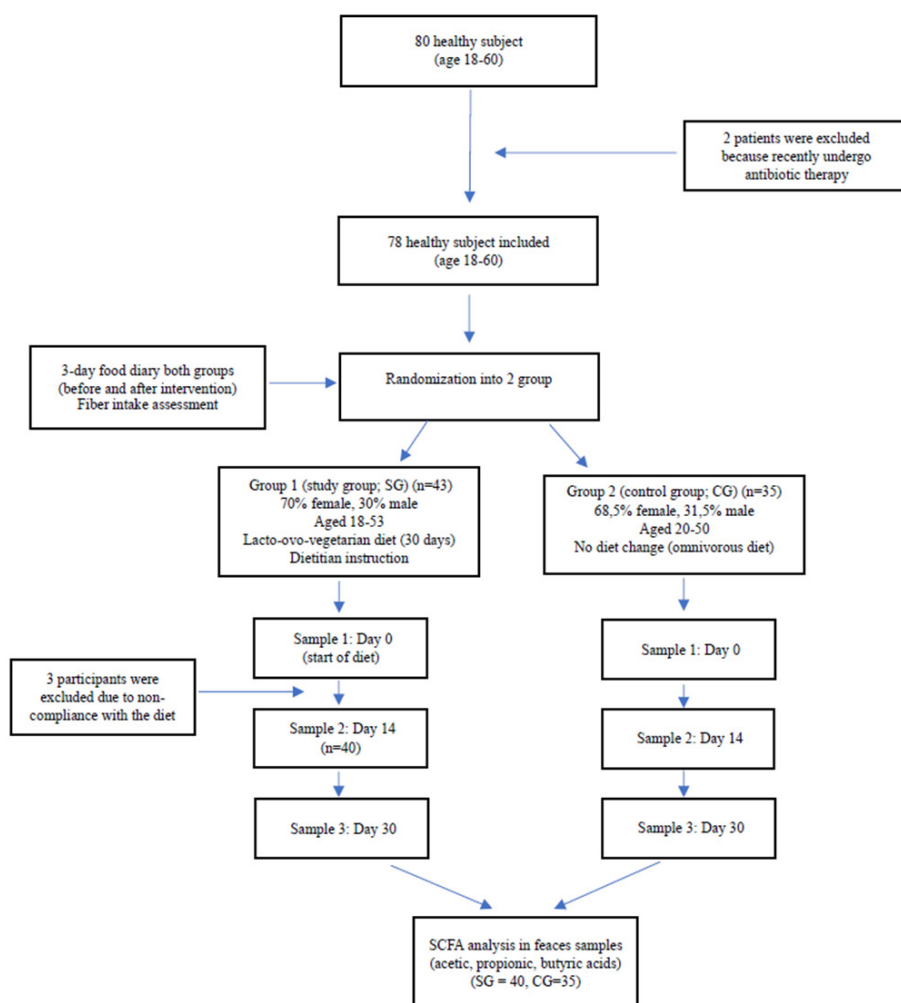


Fig. 1. Participant of study group

the Block questionnaire, originally developed for the NHANES II study (Block, 1982; Block et al., 2000). This questionnaire includes items on the frequency of intake of fruits, fruit juices, salads, potatoes, legumes, white bread, whole grain bread, and other cereal products (Block et al., 2000).

Participants were provided with written recommendations for a lacto-ovo-vegetarian diet. The materials described the principles of this dietary pattern, identified foods to be excluded, and suggested plant-based alternatives to animal products, such as legumes. To support implementation, participants also received a list of sample meals consistent with the recommended diet.

Obtaining short-chain fatty acids from biological material

SCFA concentrations were determined using a modified method of Scortichini et al. (2020). Thawed faecal samples (100 mg) were weighed on an analytical balance, and 250 µl of 50% sulfuric acid was added to each sample. Samples were vortexed for 3 minutes, and 50 µl of IC6 was introduced as the internal standard. Extraction was performed by adding 800 µl of diethyl ether, followed by centrifugation for 5 minutes at 2800 x g. The organic phase from each sample was transferred to vials with an automatic pipette. This extraction was repeated three times, yielding a total of 2.4 ml of the organic phase. Qualitative and quantitative

analyses of SCFAs were performed using a gas chromatograph equipped with a flame ionisation detector (Agilent 7890 series II Agilent Technologies, Santa Clara, CA, USA) and a BPX 70 column (BPX70, 25 m × 0.22 mm ID × 0.25 µm, SGE Analytical Science, Ringwood, Australia). Retention times were used for qualitative analysis, while quantitative analysis was based on peak areas obtained from the chromatograms using MSD ChemStation (Agilent Technologies, Santa Clara, CA, USA).

Statistical methods

SCFA concentrations in faecal samples were expressed as [µmol/g], and fibre intake was expressed as g/day. Descriptive statistics were calculated for all variables. To evaluate the effect of the dietary modification over time on SCFA concentrations, a two-way ANOVA with repeated measures was performed. Extreme outliers were excluded due to the nature of the measured features. When the interaction between dietary modification and time was statistically significant, additional analyses were conducted to examine the nature and direction of the interaction effects. One-way ANOVA was applied to assess the effect of diet at each time point and the effect of time within each group (control and study). Pairwise t-tests were then used to compare mean values between groups, providing further insight into the observed effects. When the assumptions of a two-way repeated measures ANOVA were not fully met, alternative analytical approaches were considered. For non-normally distributed data, differences between groups were assessed using the Wilcoxon rank-sum test, while differences across time points within each group were evaluated using the Wilcoxon signed-rank test for paired samples. A p-value <0.05 was considered statistically significant. The calculations were performed in R software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

The study was approved by the Bioethical Committee of the Poznań University of Medical Sciences, Poznań, Poland (decision no. 394/22) and conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

The anthropometric characteristics of the study participants are presented in Table 1.

Table 1. Anthropometric data of the study and control groups

Parameters	Study group	Control group	P
	Median (1st–3rd quartile)		
N	40	35	–
Age [years]	36.5 (22.5–39.0)	34.5 (21.5–35.5)	ns.
Sex ratio [F/M]	28/12 (70.0%)	24/11 (68.6%)	ns.
BMI [kg/m ²]	25.6 (22.1–30.7)	26.8 (23.6–29.5)	ns.

BMI – body mass index; P-values obtained using the U Mann-Whitney test.

Daily dietary fibre intake at baseline and after one month of dietary intervention was comparable between groups. Notably, despite dietary modification, fibre intake in the study group did not increase (Table 2).

Table 2. Fibre intake of the study and control groups at baseline and after four weeks

Parameters	Study group		Control group		P*
	Median (1st–3rd quartile)				
	Baseline	After 4 weeks	Baseline	After 4 weeks	
Fibre [points]	24.5 (16.7–35.7)	24.9 (20.1–26.6)	23.7 (15.7–28.3)	23.3 (17.0–25.6)	ns.

P-value from the U Mann-Whitney test.

Analysis of food frequency data using the Block questionnaire revealed insufficient fibre intake (< 30 points) in the study group, both before and after the intervention. Prior to the intervention, 58% of participants had insufficient intake (20–29 points), while 42% had poor intake (< 20 points). Following the one-month intervention, 53% remained in the insufficient category and 47% in the poor intake category.

The basic sample statistics for all measured faecal SCFA concentrations in the study group (SG) and control group (CG) are presented in Table 3.

Table 3. Faecal SCFA concentrations (µmol/g) in the study and control groups at baseline and after two and four weeks

Parameters	Study group			Control group		
	Baseline	After 2 weeks	After 4 weeks	Baseline	After 2 weeks	After 4 weeks
	Median (1st–3rd quartile)					
Acetic acid [µmol/g]	30.98 (22.90–37.14)	25.44 (18.69–32.65)	22.97 (15.56–32.28)	29.13 (22.78–32.36)	26.56 (22.29–32.29)	30.31 (22.06–35.27)
Propionic acid [µmol/g]	11.90 (9.39–16.11)	10.58 (7.51–12.59)	9.59* (7.15–12.41)	10.34 (8.16–15.46)	12.16 (8.73–16.07)	12.21* (10.03–15.97)
Butyric acid [µmol/g]	2.79 (1.68–5.98)	2.53 (1.52–6.55)	3.09 (1.68–6.83)	2.68 (1.53–5.38)	2.60 (1.56–5.39)	2.47 (1.86–5.58)

* $P < 0.05$.

Table 4. Summary of two-way repeated measures ANOVA

Sources	<i>dfn</i>	<i>dfd</i>	<i>SSn</i>	<i>SSd</i>	<i>F</i>	<i>p</i>
Intercept	1	33	161888.095	14447.038	369.786	0.000*
Group	1	33	81.506	5180.655	0.519	0.476
Time	2	66	177.096	1983.481	2.946	0.059
Group:time	2	66	311.543	2841.943	3.618	0.032*

* $p < 0.05$, *dfn* – degrees of freedom for the effect; *dfd* – degrees of freedom for the error; *SSn* – sum of squares for the effect; *SSd* – sum of squares for the error; *F* – *F*-statistic; *p* – *p*-value (probability of the data given the null hypothesis).

Changes in acetic acid concentrations due to intervention

A statistically significant two-way interaction was observed between groups (study group [SG] and control group [CG]) and time points (Table 4, Fig. 2).

Analysis revealed a statistically significant effect of time within the study group (one-way ANOVA, $p_{\text{adj}} = 0.004$). Pairwise comparisons further showed a significant difference in acetic acid concentration between baseline and four weeks after the intervention in the study group (Table 5).

Changes in propionic acid concentrations due to intervention

According to the results of the two-way repeated measures ANOVA, there was a statistically significant interaction between dietary modification and time (Table 6, Fig. 3).

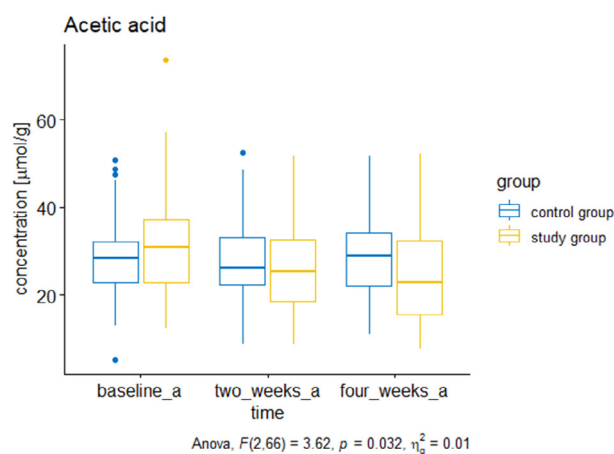


Fig. 2. Quartile distribution of acetic acid concentrations (µmol/g) in the study group and the control group

Table 5. Results of pairwise comparisons

Group	group1	group2	statistic	p.adj
Control	baseline_a	four_weeks_a	-1.03	0.93
Control	baseline_a	two_weeks_a	-0.0527	1
Control	four_weeks_a	two_weeks_a	1.01	0.963
Study	baseline_a	four_weeks_a	3.34	0.005**
Study	baseline_a	two_weeks_a	2.47	0.055
Study	four_weeks_p	two_weeks_p	-1.28	0.63

*p.adj < 0.05.

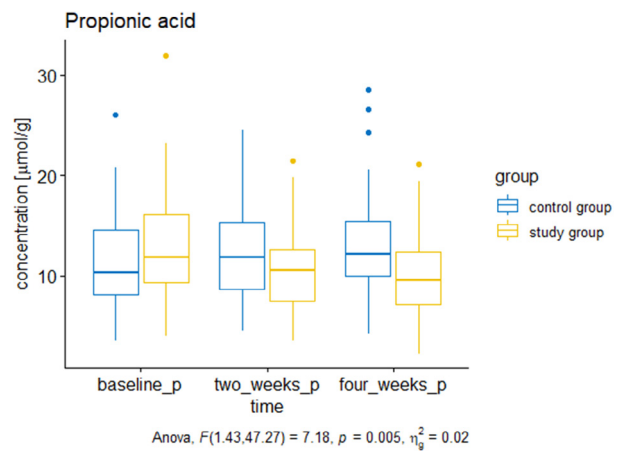


Fig. 3. Quartile distribution of propionic acid concentrations (µmol/g) in the study group and the control group

Table 6. Summary of two-way repeated measures ANOVA

Sources	dfn	dfd	SSn	SSd	F	p
Intercept	1	33	28128.138	2609.833	355.666	0.000*
Group	1	33	80.665	1974.525	1.348	0.254
Time	1.58	52.05	14.066	441.367	1.052	0.342
Group:Time	1.43	47.27	109.866	504.835	7.182	0.005*

* $p < 0.05$, dfn – degrees of freedom for the effect; dfd – degrees of freedom for the error; SSn – sum of squares for the effect; SSd – sum of squares for the error; F – F -statistic; p – p -value (probability of the data given the null hypothesis).

The effect of the diet was evaluated at each time point. One-way ANOVA revealed significant differences in propionic acid concentrations at four weeks following the dietary intervention ($p = 0.022$, Table 7). In addition, pairwise comparisons (Table 8) showed significant differences within the study group

Table 7. Summary of one-way ANOVA

Time	Effect	dfn	dfd	F	p
baseline_p	group	1	33	0.232	0.633
two_weeks_p	group	1	33	2.48	0.124
four_weeks_p	group	1	33	5.79	0.022*

* $p < 0.05$; dfn – degrees of freedom for the effect; dfd – degrees of freedom for the error; F – F -statistic; p – p -value.

between baseline and two weeks after diet initiation, as well as between baseline and four weeks after diet initiation.

Table 8. Results of the pairwise comparisons

Group	group1	group2	statistic	p.adj
control	baseline_p	four_weeks_p	-1.76	0.262
control	baseline_p	two_weeks_p	-1.27	0.642
control	four_weeks_p	two_weeks_p	1.62	0.345
study	baseline_p	four_weeks_p	3.57	0.003**
study	baseline_p	two_weeks_p	2.63	0.037*
study	four_weeks_p	two_weeks_p	-1.76	0.259

*p.adj < 0.05

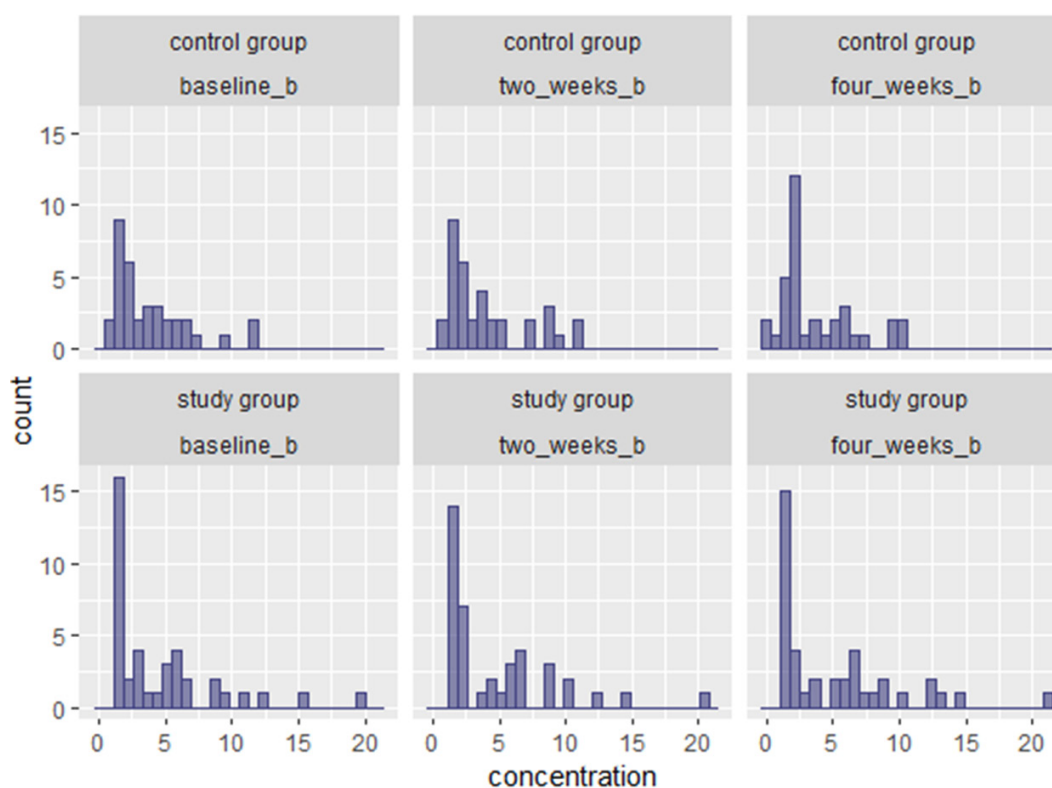


Fig. 4. Histograms showing the distribution of butyric acid concentrations in the study and control groups

Changes in butyric acid concentrations due to intervention

The distribution of the butyric acid concentrations in the study and control groups is presented in Figure 4.

Because the butyric acid concentration data did not meet the assumptions of normality, the Wilcoxon rank-sum test was applied to compare the two groups (Table 9), and the Wilcoxon signed-rank test was used for paired samples (Table 10).

Table 9. Results of the Wilcoxon rank-sum test

Variable	group1	group2	statistic	p
baseline_b	control group	study group	612	0.353
two_weeks_b	control group	study group	650	0.601
four_weeks_b	control group	study group	658	0.659

Table 10. Results of the Wilcoxon signed-rank test on paired samples

Group	group1	group2	statistic	p.adj
control group	baseline_b	four_weeks_b	602	1
control group	baseline_b	two_weeks_b	604	1
control group	four_weeks_b	two_weeks_b	616	1
study group	baseline_b	four_weeks_b	781	1
study group	baseline_b	two_weeks_b	816	1
study group	four_weeks_b	two_weeks_b	852	1

No significant differences in butyric acid concentrations were observed between the study and control groups, nor were any differences detected within groups over time.

DISCUSSION

This study investigated the effect of a 30-day dietary intervention, involving a shift from a mixed diet to a lacto-ovo-vegetarian diet, on the concentrations of acetic, propionic, and butyric acids. After four weeks, propionic acid levels differed significantly between the study group (SG) and the control group (CG). Within the study group, significant changes were observed in propionic acid concentrations between baseline and two weeks, as well as between baseline and four weeks. For acetic acid, concentrations differed significantly between baseline and four weeks. In contrast, butyric acid levels did not differ between groups. These findings align with reports in healthy vegan populations adhering to their diets (Trefflich et al., 2021; Wu et al., 2016). Western populations often show reduced gut microbiota diversity, largely attributed to dietary patterns low in fibre and high in processed foods. In such contexts, SCFA production does not increase proportionately with fibre intake, unlike observations in developing countries (Wu et al., 2016).

Animal studies provide additional insights. Feeding mice a low-fibre diet over multiple generations reduced microbiota diversity, and reintroducing fibre failed to restore it. This suggests that microbiota function can shift irreversibly under long-term environmental pressures, including diet (Sonnenburg et al., 2016). Such mechanisms may explain why SCFA concentrations in our participants remained largely unchanged after one month on a lacto-ovo-vegetarian diet.

The link between diet and gut microbiota is well established (De Filippis et al., 2016; De Filippo et al., 2010). Nutritional interventions increasingly leverage the beneficial effects of diet on the gut microbiome to promote human health (Wu et al., 2021). Higher intake of vegetables and fruits, and thus dietary fibre, is a key contributor to the health benefits of vegetarian diets (Wang et al., 2023). Fibre modulates microbial metabolism and fuels fermentation by intestinal bacteria such as *Roseburia* spp., *Faecalibacterium prausnitzii*, *Ruminococcus bromii* and *Prevotella* spp., resulting in SCFA production (Perler et al., 2023). Cross-sectional studies of vegan and vegetarian populations have reported a greater abundance of *Prevotella* strains,

possibly attributable to higher fibre consumption (Franco-de-Moraes et al., 2017; Ruengsomwong et al., 2016). In the present study, however, SCFA concentrations and fibre intake did not differ significantly after one month on a vegetarian diet, suggesting that fibre alone may not account for increased SCFA production. Further research is needed to clarify the role of additional factors in modulating SCFA outcomes.

De Filippis et al. (2016) observed a significant association between faecal SCFA profiles and nutrient intake in individuals habitually following omnivore, vegetarian, or vegan diets. Higher concentrations of acetic, propionic and butyric acids correlated with greater intake of fruits, vegetables, legumes, and fibre, while elevated valeric and caprylic acid levels were associated with higher intake of animal products rich in protein and saturated fats. Increased adherence to Mediterranean and vegetarian diets corresponded with higher acetic, propionic and butyric acid levels. Among individuals on a mixed diet, closer adherence to the Mediterranean pattern was likewise associated with greater SCFA concentrations, with participants in the highest adherence group showing significantly higher faecal propionic and acetic acid levels than those with the lowest adherence. Positive correlations were also noted between gut microbial composition and SCFA levels: *Firmicutes* and *Bacteroidetes* were associated with SCFA production, and *Prevotella* abundance strongly correlated with faecal SCFA concentrations. Overall, Vegan and vegetarian diets rich in plant-based foods and fibre were associated with higher SCFA production compared with mixed diets (De Filippis et al., 2016).

In a cross-sectional study, Trefflich et al. (2021) compared faecal SCFA and BCFA (branched-chain fatty acids) concentrations, pH, and ammonia levels between vegans and individuals consuming a mixed diet. No significant between-group differences in SCFA or BCFA concentrations were detected. However, several within-group diet trends emerged. Propionate concentrations were higher in individuals consuming a mixed diet, while butyrate concentrations were higher in vegans. In addition, faecal isovalerate, valerate, and isobutyrate levels tended to be lower in vegans compared with those on a mixed diet. Similarly, in our study, no significant differences were observed in acetic, propionic, or butyric acid concentrations. This may

be explained by the absence of increased dietary fibre intake despite the dietary change. Since dietary fibre is a primary substrate for SCFA production by intestinal bacteria (Perler et al., 2023), insufficient fibre intake could account for the lack of measurable changes in faecal SCFA concentrations.

An increasing body of evidence indicates that the composition of the human gut microbiota is highly heterogeneous and shaped by individual variability (Lang et al., 2018). To date, studies examining the impact of vegetarian or vegan diets on gut microbiota have produced inconsistent findings (Trefflich et al., 2020). Given the complexity of the gut microbiota and its interactions with host metabolism, metabolomic approaches are needed to better elucidate the interplay between diet, microbial communities, and metabolite production. In particular, long-term studies are required to clarify the influence of lacto-ovo-vegetarian diets on faecal SCFA profile and concentrations.

This study has some limitations. Participants were not assigned a controlled meal plan, which reduced oversight of actual dietary intake and may have influenced the outcomes. In addition, the relatively short intervention period may have been insufficient to detect significant changes in faecal SCFA levels.

CONCLUSION

Adoption of a lacto-ovo-vegetarian diet in healthy adults did not lead to significant alterations in SCFA faecal concentrations compared with a traditional Western diet (mixed diet). The absence of increased dietary fibre intake, a principal substrate for microbial SCFA production, likely accounts for the lack of measurable changes. Further long-term, controlled studies are needed to clarify the effects of vegetarian diets on SCFA concentrations.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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