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Associations of gut microbiota, dietary intake, and serum short-chain fatty acids with fecal short-chain fatty acids

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In recent years, short-chain fatty acids (SCFAs) have been reported to play an important role in maintaining human health. Fecal SCFA concentrations correlate well with colonic SCFA status and gut microbiota composition. However, the associations with the gut microbiota functional pathway, dietary intake, blood SCFAs, and fecal SCFAs remain uncertain. To clarify these relationships, we collected fecal samples, blood samples, and dietary habit data from 12 healthy adults aged 22–51 years. The relative abundance of several SCFA-producing bacteria, gut microbiota diversity, and functional pathways related to SCFA biosynthesis were positively associated with fecal SCFAs even after adjusting for age and sex. Furthermore, fecal acetate was likely to be positively associated with serum acetate. By contrast, dietary intake was not associated with fecal SCFAs. Overall, the present study highlights the potential usefulness of fecal SCFAs as an indicator of the gut microbiota ecosystem and dynamics of SCFAs in the human body.

Key words: gut microbiota, fecal short-chain fatty acids, serum short-chain fatty acids, dietary intake, human

INTRODUCTION

Short-chain fatty acids (SCFAs) are the products of gut microbial fermentation of host-derived carbohydrates, dietary fiber, protein, and resistant starch [1–3]. The colon is responsible for the absorption of most SCFAs produced by gut microbiota into the body [4, 5]. Acetate, propionate, and butyrate are the major SCFA subtypes and play important roles in maintaining human health by acting as substrates for cholesterol synthesis, *de novo* lipogenesis, and gluconeogenesis in the liver [4, 6, 7] or as primary nutrients for colonocytes [8–10]. Fecal SCFAs concentrations are easily determined and correlate well with SCFA status in the colon [11, 12].

Recent epidemiological studies clarified the association between fecal SCFAs and gut microbiota composition [12–15]. While these previous studies are useful for understanding associations between the gut microbiota and human health in conjunction with SCFAs, they did not account for the functional profiles of the gut microbiota. In addition, consideration of dietary intake may be necessary, as it can affect SCFAs in the colon. Therefore, further research is required to elucidate SCFA metabolism in humans. The aim of the present study was to investigate the associations of gut microbiota composition and functional profiles, as well as dietary intake and fecal SCFAs, in healthy adults. Moreover, we investigated the correlation between fecal SCFAs and serum SCFAs.

MATERIALS AND METHODS

Study participants and sample collection

Participants were recruited among students and employees at our university departments in Sapporo, Hokkaido, Japan, in September 2017. A total of 12 healthy Japanese volunteers (10 males and 2 females) were enrolled in this study. Fasting blood samples were obtained in the morning by cubital venipuncture after overnight fasting from 9:00 p.m. Serum was separated and centrifuged after blood coagulation. Serum samples were stored at −80°C until metabolite analysis. Within 0–7 days of blood collection, participants provided two fresh fecal samples, one which was transferred by the participant into a plastic tube and one collected using a feces collection kit containing guanidine solution (TechnoSuruga Laboratory, Shizuoka, Japan) [16]. Fecal metabolite analysis was performed using the fresh fecal sample soon after collection, while the fecal collection kit sample was refrigerated and

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sent to TechnoSuruga Laboratory Co. Ltd. (Shizuoka, Japan) for fecal bacterial DNA extraction. Dietary intake one week prior to blood collection was assessed using a brief-type self-administered diet history questionnaire (BDHQ) [17]. Data on dietary intake estimated using the BDHQ have been validated in previous publications [17, 18]. Although the BDHQ is designed to ask about dietary habits in the month prior to the day of the questionnaire, we asked participants to answer about their dietary habits for the week prior to the day of blood collection. This study was approved by the Ethics Committee of the Faculty of Medicine, Hokkaido University (17-007), and written informed consent was obtained from all participants.

Measurement of fecal and serum SCFAs

Fresh fecal samples were homogenized with a 10-fold volume of phosphate buffer solution (50 mM Na_2HPO_4/NaH_2PO_4, pH 7.4, and 10% v/v D_2O) containing 0.5 mM 3-trimethylsilypropionatel-2,2,3,3,-d4 (TSP-d4) and 0.04% NaN_3. The mixtures were vortexed for 30 sec and shaken for 15 min at 4°C. The homogenates were centrifuged at 15,000 rpm for 10 min at 4°C. Serum samples were added to the phosphate buffer solution at the same final concentration as the fecal samples. Supernatants (550 µL) were transferred into 5 mm NMR tubes. All NMR data were obtained using a Bruker AVANCE III spectrometer (Bruker Biospin Inc.) at 600 MHz with a TXI z-gradient probe at 25°C. A NOESY pulse sequence with presaturation for water suppression with a relaxation delay of 4 sec and mixing time of 100 ms was used to record ^{1}H NMR spectra of fecal samples. A Carr-Purcell-Meiboom-Gill sequence with a total time filter of 125 ms was used to record ^{1}H NMR spectra of serum samples. Spectra were collected for each sample with 65,536 data points, a spectral width of 16 ppm, and accumulation of 128 scans. All FIDs were zero-filled, and an exponential line-broadening function of 0.2 Hz was applied before the spectra were Fourier transformed. All spectra were manually phase- and baseline-corrected using Delta 5.0.4 (JEOL RESONANCE Inc.). Metabolites were assigned using the Chenomx NMR Suite 8.2 software (Chenomx Inc.). In this report, total SCFAs were defined as the sum of the subtypes of SCFAs, including acetate, butyrate, and propionate.

Fecal bacterial DNA extraction

Bacterial DNA was extracted from fecal samples according to a previously described method [19]. Briefly, fecal solids, lysing buffer, and lysing matrix—including zirconia beads—were added to a BioPulverizer tube and crushed at 5 m/sec for 2 min using a FastPrep-24 Instrument (MP Biomedicals, Irvine, CA, USA). Bacterial DNA was extracted from 200 µL of fecal suspension using an automatic nucleic acid extractor (Magtration System 12gC, Precision System Science, Matsudo, Japan) with a reagent for extracting nucleic acid (MagDea DNA 200, Precision System Science, Matsudo, Japan).

Fecal bacterial 16S ribosomal RNA (rRNA) library preparation and sequencing

16S rRNA library preparation and sequencing were conducted at Morinaga Milk Industry Co., Ltd. (Zama, Japan). The V3-V4 region of bacterial 16S rRNA was amplified and sequenced using an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) according to a previously described method [20]. After removing sequences consistent with data from Genome Reference Consortium human build 37 (GRCh37) or PhiX 174 from the raw Illumina paired-end reads, the 3′ region of each read with a PHRED quality score less than 17 was trimmed. Trimmed reads less than 150 bp in length with an average quality score less than 25 or those lacking paired reads were also removed. Reads that passed the quality filters were combined using the fastq-join script in ea-utils (version 1.1.2-537) [21].

Bioinformatics analysis

All steps from the trimming of paired-end read FASTQ files, which were obtained by 16S rRNA amplicon sequencing, to gut microbiota diversity analysis were automatically performed using QIIME 1.9.1 [22] according to a previously described method (http://doi.org/10.5281/zenodo.1439555). The operational taxonomic units (OTUs) were clustered against the SILVA 128 reference database [23] at 97% similarity using the USEARCH algorithm [24]. The clustered OTUs were classified into five taxonomic rank categories (phylum, order, class, family, and genus) using the SILVA 128 reference database at 97% similarity. Subsequently, three alpha-diversity indices (Chao 1 index, phylogenetic diversity whole tree, and observed OTUs) were calculated. The representative SCFA-producing bacteria were selected based on previous studies [25, 26]. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) 1.0.0 [27] was used to predict the functional gene compositions based on the 16S rRNA gene sequences against the Greengenes [28] database with the default settings (type of functional predictions: KEGG Orthologs).

Statistical analysis

Data on fecal SCFAs are shown as absolute quantitative values (mM), while data on serum SCFAs are shown as relative concentrations because of the use of a Carr–Purcell–Meiboom–Gill sequence. Relative concentrations of serum SCFAs (no unit provided) represent the ratio of serum SCFA values of a given participant relative to the values of each other participant. To avoid confusion about serum SCFA data, all SCFA data were standardized using mean and standard deviation values of fecal and serum SCFAs among the study participants. The standardization yielded a normal distribution with a mean of zero and a standard deviation of one. The Firmicutes to Bacteroidetes ratio (F:B ratio) was determined by dividing the relative abundance of the phylum Firmicutes (%) by the relative abundance of the phylum Bacteroidetes (%). The Shannon diversity index (H') [29], Simpson diversity index (1-D), and inverse Simpson index

(1/D) [30] were calculated using the following equations at the genus level: H' = -Σ*pi*ln*pi* and D = Σ*pi*^2, where *pi* is the relative abundance (%) of *genus i* in the community. Initially, a linear regression model was used to quantify the association of the relative abundance of each bacterium, each functional pathway of the gut microbiota, or intake of each food and nutrient with fecal SCFAs, with age and sex incorporated as covariates. Next, to verify the correlation between fecal SCFAs and SCFAs in the body, a Pearson's correlation coefficient (*r*) was calculated between SCFAs in feces and serum. Furthermore, a similar linear regression model was used to examine the association of fecal SCFAs with serum SCFAs, accounting for age and sex. All statistical analyses were performed using R version 3.4.1. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Characteristics of study participants

All 12 participants (10 males and 2 females) were included in the analysis. Participant age ranged from 22 to 51 years, with a median of 37 years. Four participants collected their feces on the same day as blood collection, and the maximum interval between blood collection and feces collection was 7 days. According to the NMR spectroscopy results, the serum butyrate and propionate values of all the participants were lower than the normal detection limits. Therefore, only serum acetate could be examined among the subtypes of serum SCFAs.

Gut microbiota composition and fecal SCFAs

Table 1 shows the associations of the phylum-level compositions and alpha-diversity indices of the gut microbiota with fecal SCFAs. The relative abundances of phylum Firmicutes, the F:B ratio, Shannon index, Simpson index, and inverse Simpson index were significantly positively associated with fecal butyrate. Table 2 shows the associations of the relative abundances of the representative SCFA-producing bacteria with fecal SCFAs. Of these bacteria, the relative abundances of the representative butyrate-producing bacteria class Clostridia, order Clostridiales, family Ruminococcaceae, genus *Subdoligranulum*, and family Erysipelotrichaceae were significantly positively associated with fecal butyrate. In addition, the relative abundance of the representative acetate-producing bacteria genus *Ruminococcus* was significantly positively associated with fecal total SCFAs and fecal acetate, while that of *Ruminiclostridium* was significantly positively associated with fecal acetate. The relative abundances of the representative propionate-producing bacteria family Porphyromonadaceae, genera *Bacteroides*, and *Ruminococcus* were positively associated with fecal propionate, although the relationships were not statistically significant.

Gut microbiota functional profiles and fecal SCFAs

Table 3 shows the associations of the gut microbiota functional profiles related to the production and metabolism of SCFAs with fecal SCFAs. The functional pathway *fatty acid biosynthesis* was significantly and positively associated with fecal total SCFAs and fecal acetate. This functional pathway was also positively associated with fecal butyrate and fecal propionate, although the relationship was not statistically significant.

Dietary intake and fecal SCFAs

Table 4 shows the associations of dietary intake with fecal SCFAs. Intake of foods containing SCFAs (low fat milk, normal milk, pickled vegetables, soy source, and western confectioneries) and intake of the major sources of SCFAs (protein, animal protein, vegetable protein, carbohydrate,

Table 1. Associations of phylum-level compositions and alpha-diversity indices of the gut microbiota with fecal SCFAs after adjusting for sex and age

	Fecal total SCFAs†		Fecal acetate†		Fecal butyrate†		Fecal propionate†	
	β	95%CI‡	*β*	95%CI‡	*β*	95%CI‡	*β*	95%CI‡
Phylum (%)								
Actinobacteria	−7.88	(−23.30, 7.54)	−7.3	(−22.81, 8.21)	−9.93	(−26.44, 6.59)	−4.86	(−20.92, 11.20)
Bacteroidetes	−2.21	(−10.70, 6.28)	−2.44	(−10.84, 5.96)	−8.04	(−14.99, −1.09)*	4.48	(−3.30, 12.27)
Firmicutes	4.11	(−3.91, 12.12)	4.17	(−3.77, 12.11)	9.73	(4.39, 15.07)*	−2.65	(−10.98, 5.68)
Proteobacteria	−0.20	(−54.23, 53.84)	1.42	(−52.3, 55.14)	29.64	(−24.71, 83.98)	−30.03	(−77.62, 17.55)
Alpha-diversity indices								
F:B ratio	1.3	(−2.15, 4.76)	1.11	(−1.66, 3.87)	0.42	(0.1, 0.75)*	−0.23	(−0.78, 0.32)
Shannon index	1.08	(−0.43, 2.59)	1.1	(−0.39, 2.59)	1.82	(0.6, 3.05)*	−0.18	(−1.90, 1.55)
Simpson index	9.06	(−5.66, 23.77)	9.63	(−4.74, 24.00)	15.13	(1.85, 28.41)*	−3.36	(−19.44, 12.71)
Inverse Simpson index	0.14	(−0.06, 0.34)	0.15	(−0.05, 0.35)	0.25	(0.08, 0.41)*	−0.06	(−0.28, 0.17)
Chao 1 index	0.003	(−0.015, 0.020)	0.002	(−0.015, 0.02)	0.009	(0.01, 0.027)	−0.002	(−0.020, 0.015)
Observed OTUs	0.009	(−0.011, 0.030)	0.009	(−0.011, 0.029)	0.015	(0.006, 0.035)	0.002	(−0.020, 0.023)
PD whole tree	0.17	(−0.23, 0.56)	0.16	(−0.23, 0.55)	0.19	(0.24, 0.62)	0.09	(−0.32, 0.49)

CI: confidence interval; F:B: Firmicutes to Bacteroidetes; OTUs: operational taxonomic units; PD: phylogenetic diversity; SCFAs: short-chain fatty acids. †Values were standardized. ‡Linear regression analyses, adjusted for age and sex. *p<0.05.

Table 2. Associations of the relative abundances of SCFAs-producing bacteria with fecal SCFAs after adjusting for gender and age

	Fecal total SCFAs†		Fecal acetate†		Fecal butyrate†		Fecal propionate†	
	β	95%CI‡	*β*	95%CI‡	*β*	95%CI‡	*β*	95%CI‡
Phylum Actinobacteria								
Bifidobacterium (%)	−8.07	(−23.83, 7.69)	−7.44	(−23.30, 8.43)	−9.24	(−26.47, 7.99)	−5.84	(−22.07, 10.40)
Phylum Bacteroidetes								
Porphyromonadaceae (%)	7.04	(−5.62, 19.71)	7.73	(−4.58, 20.04)	−0.58	(−15.88, 14.73)	5.24	(−7.86, 18.33)
Bacteroides (%)	−1.86	(−7.87, 4.14)	−1.97	(−7.92, 3.98)	−5.83	(−10.72, −0.94)*	2.61	(−3.15, 8.37)
Phylum Firmicutes								
Streptococcus (%)	0.79	(−105.21, 106.79)	−0.59	(−105.99, 104.82)	−18.93	(−134.59, 96.73)	21.67	(−81.81, 125.16)
Clostridia (%)	19.38	(−17.80, 56.57)	16.29	(−13.44, 46.02)	5.15	(1.94, 8.36)*	−2.05	(−8.32, 4.21)
Clostridiales (%)	4.04	(−3.71, 11.79)	4.19	(−3.46, 11.84)	9.03	(3.4, 14.67)*	−2.64	(−10.70, 5.42)
Lachnospiraceae (%)	0.41	(−7.50, 8.31)	0.76	(−7.09, 8.60)	1.59	(−7.02, 10.21)	−2.45	(−10.03, 5.13)
Anaerostipes (%)	1.76	(−63.13, 66.65)	1.78	(−62.75, 66.30)	0.36	(−71.09, 71.81)	1.68	(−62.59, 65.95)
Blautia (%)	24.06	(−6.40, 54.52)	23.64	(−6.79, 54.08)	10.13	(−28.88, 49.15)	22.78	(−7.92, 53.48)
Coprococcus spp. (%)	−6.68	(−56.06, 42.70)	−9.32	(−58.13, 39.50)	20.54	(−31.52, 72.60)	−9.68	(−58.25, 38.89)
Eubacterium hallii (%)	1.82	(−86.04, 89.67)	1.78	(−85.58, 89.15)	−2.33	(−99.03, 94.38)	4	(−82.97, 90.96)
Eubacterium rectale (%)	−1.12	(−26.81, 24.58)	0.38	(−25.19, 25.94)	7.01	(−20.72, 34.73)	−13.90	(−36.71, 8.91)
Roseburia (%)	−53.71	(−218.41, 110.99)	−35.92	(−202.80, 131.00)	−32.74	(−218.4, 152.95)	−127.80	(−260.58, 5.03)
Ruminococcaceae (%)	5.62	(−3.32, 14.56)	5.37	(−3.60, 14.35)	9.83	(2.22, 17.44)*	0.6	(−9.34, 10.53)
Faecalibacterium (%)	2.09	(−14.73, 18.90)	1.32	(−15.45, 18.10)	11.92	(−3.95, 27.79)	−2.48	(−19.10, 14.14)
Ruminiclostridium spp. (%)	71.56	(−1.06, 144.19)	74.06	(3.8, 144.31)*	28.8	(−71.02, 128.62)	50.14	(−32.58, 132.86)
Ruminococcus spp. (%)	19.37	(2.39, 36.36)*	19.29	(2.42, 36.17)*	18.65	(−1.86, 39.17)	9.42	(−12.24, 31.07)
Subdoligranulum (%)	166.64	(−139.71, 472.99)	129.79	(−118.65, 378.24)	36.61	(4.51, 68.71)*	0.24	(−53.57, 54.04)
Erysipelotrichaceae (%)	50.51	(−135.50, 236.53)	38.2	(−112.13, 188.52)	22.09	(4.87, 39.31)*	−9.78	(−39.31, 19.76)

CI: confidence interval; SCFAs: short-chain fatty acids. †Values were standardized. ‡Linear regression analyses, adjusted for age and gender. *p<0.05.

Table 3. Associations of the functional profiles of the gut microbiota with fecal SCFAs adjusting for sex and age

	Fecal total SCFAs†		Fecal acetate†		Fecal butyrate†		Fecal propionate†	
	β	95%CI‡	*β*	95%CI‡	*β*	95%CI‡	*β*	95%CI‡
Carbohydrate digestion and absorption (%)	18.71	(−116.58, 154)	18.57	(−115.97, 153.1)	38.52	(−108.01, 185.06)	−5.62	(−140.38, 129.15)
Carbohydrate metabolism (%)	1.81	(−27.93, 31.56)	2.93	(−26.59, 32.45)	−17.78	(−47.18, 11.62)	9.56	(−18.88, 38.01)
Fatty acid biosynthesis (%)	35.56	(11.63, 59.49)*	35.16	(11.16, 59.15)*	26.22	(−9.21, 61.65)	24.38	(−7.10, 55.87)
Fatty acid metabolism (%)	−19.00	(−85.91, 47.92)	−20.37	(−86.62, 45.88)	−12.47	(−87.38, 62.45)	−6.23	(−74.07, 61.60)
Protein digestion and absorption (%)	−32.33	(−163.11, 98.45)	−35.54	(−165.00, 93.92)	−90.12	(−217.28, 37.03)	44.27	(−82.84, 171.38)
Starch and sucrose metabolism (%)	−1.14	(−11.82, 9.55)	−1.00	(−11.64, 9.63)	−7.27	(−17.48, 2.94)	3.32	(−6.95, 13.60)

CI: confidence interval; SCFAs: short-chain fatty acids. †Values were standardized. ‡Linear regression analyses, adjusted for age and sex. *p<0.05.

total dietary fiber, water-soluble dietary fiber, insoluble dietary fiber, and butyrate) showed no significant associations with fecal SCFAs.

Fecal acetate and serum acetate

Fecal acetate showed a trend toward a positive correlation with serum acetate, although the relationship was not statistically significant (r=0.523, p=0.081; Fig. 1 and Supplementary Fig. 1, illustrated using data on non-standardized fecal and serum acetate values). The possible positive association between fecal and serum acetate was similar even after adjusting for sex and age in the multiple linear regression analysis (β=0.564, 95% CI=−0.123 to 1.251; data not shown).

DISCUSSION

The present study investigated interrelationships among gut microbiota compositions and functional profiles, dietary intake, and fecal and serum SCFAs in 12 healthy adults. The relative abundance of several SCFA-producing bacteria, gut microbiota diversity, and the functional pathway related to SCFAs biosynthesis were positively associated with fecal SCFAs even after adjusting for age and sex. Furthermore, fecal acetate showed a trend toward a positive association with serum acetate. Taken together, these findings provide evidence that fecal SCFAs positively correlate well with the composition of SCFA-producing bacteria, gut microbiota SCFA productivity, and SCFA status in the body.

Table 4. Associations of dietary intake with fecal SCFAs adjusting for sex and age

		Fecal total SCFAs†		Fecal acetate†		Fecal butyrate†		Fecal propionate†	
		β	95%CI‡	*β*	95%CI‡	*β*	95%CI‡	*β*	95%CI‡
Foods									
Low-fat milk	(g/day)	−0.004	(−0.012, 0.004)	−0.003	(−0.011, 0.005)	−0.002	(−0.011, 0.007)	−0.005	(−0.013, 0.002)
Normal milk	(g/day)	0.005	(−0.015, 0.025)	0.004	(−0.016, 0.024)	0.011	(−0.009, 0.031)	0.004	(−0.016, 0.024)
Pickled vegetables	(g/day)	−0.006	(−0.062, 0.051)	−0.006	(−0.063, 0.050)	−0.021	(−0.082, 0.039)	0.013	(−0.043, 0.068)
Soy sauce	(g/day)	−0.075	(−2.374, 2.223)	−0.248	(−2.526, 2.029)	1.095	(−1.273, 3.463)	−0.026	(−2.303, 2.251)
Western confectionery	(g/day)	−0.002	(−0.030, 0.026)	−0.001	(−0.029, 0.027)	−0.012	(−0.041, 0.017)	0.001	(−0.026, 0.029)
Nutrients									
Protein	(g/day)	0.008	(−0.022, 0.038)	0.009	(−0.021, 0.039)	0.016	(−0.015, 0.047)	−0.010	(−0.039, 0.020)
Animal protein	(g/day)	0.020	(−0.022, 0.061)	0.022	(−0.019, 0.062)	0.030	(−0.012, 0.073)	−0.008	(−0.052, 0.035)
Vegetable protein	(g/day)	−0.013	(−0.097, 0.071)	−0.010	(−0.093, 0.074)	0.013	(−0.079, 0.106)	−0.044	(−0.120, 0.032)
Carbohydrates	(g/day)	−0.0004	(−0.006, 0.006)	−0.0002	(−0.006, 0.006)	0.001	(−0.005, 0.008)	−0.003	(−0.008, 0.003)
Total dietary fiber	(g/day)	−0.035	(−0.211, 0.141)	−0.036	(−0.211, 0.139)	−0.016	(−0.211, 0.180)	−0.024	(−0.199, 0.152)
Water-soluble dietary fiber	(g/day)	−0.222	(−0.865, 0.422)	−0.233	(−0.869, 0.404)	−0.012	(−0.747, 0.724)	−0.196	(−0.838, 0.446)
Insoluble dietary fiber	(g/day)	−0.028	(−0.293, 0.237)	−0.028	(−0.291, 0.236)	−0.010	(−0.303, 0.283)	−0.027	(−0.290, 0.235)
Butyrate	(mg/day)	−0.007	(−0.021, 0.008)	−0.007	(−0.021, 0.008)	−0.004	(−0.021, 0.012)	−0.004	(−0.019, 0.011)

CI: confidence interval; SCFAs: short-chain fatty acids. †Values were standardized. ‡Linear regression analyses, adjusted for age and sex.

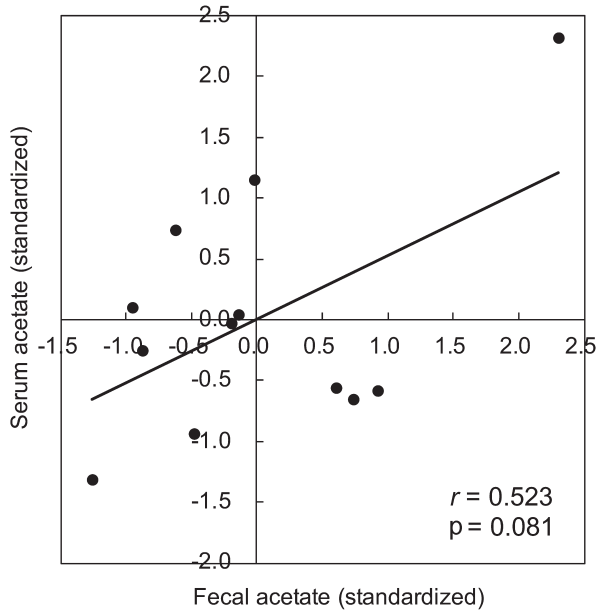

Fig. 1. Correlation between fecal acetate (standardized value) and serum acetate (standardized value). Pearson's correlation coefficients (*r*) and p-values were calculated to assess the correlation of interest.

Previous epidemiological studies reported that the butyrate-producing phylum Firmicutes and F:B ratio are positively associated with fecal butyrate [12, 14], which is consistent with our results. Moreover, we found that gut microbiota species richness and evenness were positively associated with fecal butyrate. Almost all colonic butyrate is metabolized in colonocytes as primary nutrients [8–10]. Our study suggested that the relative abundances of butyrate producers and the diversity of the gut microbiota were associated with the nutritional status of the colonocytes. Likewise, representative acetate-producers and propionate-producers showed a positive association with fecal acetate and propionate, respectively. Fecal acetate and propionate are positively associated with obesity status in humans [12, 14, 31–33]. Colonic acetate and propionate are mainly metabolized in the liver as substrates for cholesterol synthesis and lipogenesis [4, 6, 7]. The functional pathway of the gut microbiota related to SCFA biosynthesis was positively associated with all types of fecal SCFAs, especially with total SCFAs and acetate. Because SCFAs are the gut microbial fermentation products of host-derived dietary fiber [1–3], it seems reasonable that there were positive associations between the SCFA productivity of the gut microbiota and fecal total SCFAs and acetate. To our knowledge, this study is the first to investigate the association of functional profiles of the gut microbiota with fecal SCFAs. Several previous studies examined whether the presence or absence of SCFA-producing bacteria is associated with fecal SCFAs [12–15]. Our study offers new insights, finding that the SCFA production capability of the gut microbiota—but not other functions—affects fecal SCFAs. Overall, fecal SCFAs were positively associated with the presence of producers of each SCFA as well as gut microbiota diversity and the SCFA production capability of the gut microbiota.

A previous study of 570 healthy Japanese adults reported that the mean daily intake of SCFAs was 0.37 g [34], which is drastically lower than the 20 g of SCFAs produced by the human gut microbiota each day [35]. In addition, orally administered SCFAs were reported to be rapidly absorbed in the stomach or small intestine and cleared from circulated blood within 1 hr [36, 37]. Based on these reports, trace amounts of SCFAs in the diet may not reach the large intestine and feces, which supports our finding that intake of SCFA-containing food was not associated with fecal

SCFAs. Conversely, previous *in vitro* studies [38, 39] and human intervention studies [40–42] have shown positive associations between dietary fiber intake and fecal SCFAs, which are inconsistent with our results. Unlike these previous experiments and intervention studies, we investigated the association between dietary fiber intake and fecal SCFAs in a real-life setting, which may have resulted in the inconsistent finding with respect to the effect of dietary fiber intake on fecal SCFAs.

In humans, fecal acetate correlates well with colonic acetate status [11, 12], even though approximately 95% of colonic acetate is rapidly absorbed by the body [43, 44]. The majority of absorbed acetate is metabolized in the liver, with residual acetate appearing in systemic circulation [45]. Based on these conditions, our study showed that fecal acetate was likely to be positively associated with serum acetate. Regarding fecal acetate, a recent report suggested that serum acetate was positively associated with obesity status in humans [46].

We acknowledge that this study is subject to several important limitations. First, the study participants were middle-aged and comprised of predominantly Japanese males. The generalizability of our results is thus limited. Second, we attempted to investigate interrelationships between dietary intake, gut microbiota, and fecal and blood SCFAs on the day of the survey. However, dietary habits were evaluated for the week prior to blood collection, and some participants provided fecal samples up to one week after blood collection. Third, the 16S rRNA gene sequencing approach we used in this study made it possible to calculate only the relative abundance of the microbiota. Unfortunately, no information is available on the absolute abundance of bacteria. However, the gut microbiota is an ecosystem, and it is important to consider the complex bacteria–bacteria interactions. Therefore, it is valuable to evaluate the relative abundance of each SCFA-producing bacterium in the entire microbiota. Fourth, correlations between fecal and serum SCFAs other than acetate were not assessed due to the detection limits for serum samples in NMR spectroscopy. Acetate is the most abundant SCFA in the human body [47] and constitutes about 60% of fecal total SCFAs and over 90% of serum total SCFAs [48, 49]. Therefore, we believe that the positive association between fecal and serum acetate observed in this study will be helpful for understanding the dynamics of SCFAs in the human body.

In conclusion, our study provided evidence that fecal SCFAs positively correlate well with the presence of SCFA-producing bacteria as well as the diversity and SCFA productivity of the gut microbiota in humans. Moreover, fecal acetate was likely positively associated with serum acetate. These findings highlight the potential usefulness of fecal SCFAs as an indicator of the gut microbiota ecosystem and dynamics of SCFAs in the human body. Recently, in the field of biomedical sciences, there has been increasing interest in gut microbiota as a possible factor linked to human health [50]. For example, recent studies suggest that the gut microbiota is linked with obesity via SCFAs [15, 46]. Therefore, the measurement of fecal SCFAs helps investigate the interrelationships among the gut microbiota SCFAs and obesity in humans. We expect that further epidemiological studies in large populations will yield critical insights that will help elucidate the associations between the gut microbiota and human health in conjunction with SCFAs.

Conflict of interest

No potential conflicts of interest were reported by the authors.

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