Fecal short-chain fatty acids and obesity in a community-based Japanese population: the DOSANCO Health Study

Ryodai Yamamura[1]; Koshi Nakamura[2, 3]; Shigekazu Ukawa[4, 3]; Emiko Okada[5, 3]; Takafumi Nakagawa[6]; Akihiro Imae[6]; Tadao Kunihiro[7]; Takashi Kimura[3]; Takumi Hirata[3]; Akiko Tamakoshi[3]

[1] *Division of Biomedical Oncology, Institute for Genetic Medicine, Hokkaido University, N15, W7, Kita-ku, Sapporo 060-0815, Japan*

[2] *Department of Public Health and Hygiene, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan*

[3] *Department of Public Health, Faculty of Medicine, Hokkaido University, N15, W7, Kita-ku, Sapporo 060-8638, Japan*

[4] *Research Unit of Advanced Interdisciplinary Care Science, Graduate School of Human Life Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan*

[5] *Department of Nutritional Epidemiology and Shokuiku, National Institute of Biomedical Innovation, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan*

[6] *The Hokkaido Centre for Family Medicine, 1-18, N41, E15, Higashi-ku, Sapporo 007-0841, Japan*

[7] *Technosuruga Laboratory Co. Ltd., 388-1 Nagasaki, Shimizu-ku, Shizuoka 424-0065, Japan*

Corresponding author: Koshi Nakamura, M.D., Ph.D., Professor, Department of Public Health and Hygiene, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan. Tel: +81-98-895-3331; Fax: +81-98-895-1412;

Email: knakamur@med.u-ryukyu.ac.jp

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Abstract

In Western populations, fecal concentrations of short-chain fatty acids (SCFAs) are positively correlated with the prevalence of obesity. However, gut microbiota involved in the production of SCFA varies between races. Our purpose was to investigate the associations between fecal SCFAs and the prevalence of obesity in a community-based Japanese population. We classified a total of 568 participants aged ≥18 into four quartiles of fecal concentrations of SCFA subtypes (acetate, butyrate, and propionate) and total SCFAs to compare the prevalence of obesity, defined as a body mass index ≥ 25.0 kg/m^2. Using the first quartile SCFA group as a reference, the prevalence ratios of obesity were calculated for each SCFA group through a log-binomial regression model adjusted for major potentially confounding factors including age, sex, exercise habits, total energy intake, and total dietary fiber intake. In the study population, the prevalence of obesity was 35.8%. The prevalence ratios (95% confidence intervals) of obesity in the second, third, and fourth quartile groups of fecal total SCFAs were 1.30 (0.89−1.89), 1.74 (1.23−2.47) and 1.70 (1.19−2.41), respectively, after adjusting for the confounders. Similar positive associations were observed for every subtype. The prevalence ratios (95% confidence intervals) in the fourth quartile groups of fecal acetate, butyrate, and propionate were 1.41 (1.02−1.97), 2.16 (1.49−3.14), and 1.97 (1.35−2.89), respectively, after adjusting for the confounders. In conclusion, our results

demonstrated that fecal SCFA concentrations of every subtype were positively associated with the prevalence of obesity in a community-based Japanese population.

Introduction

Obesity is a major risk factor for various diseases including type 2 diabetes, metabolic syndrome, cardiovascular disease, and some types of cancers [1]. Today, over one fourth of adults have obesity, which is defined as a body mass index (BMI) $\geq$ 30 kg/m^2 in several Western developed countries [2]. In Japan, the prevalence of obesity, commonly defined as a BMI $\geq$ 25 kg/m^2 for East-Asian populations [3], has been increasing over the last several decades [4], affecting 30.7% of male and 21.9% of female adults [5]. Therefore, obesity is a public health burden worldwide.

Short-chain fatty acids (SCFAs) are gut microbial fermentation products mostly of host-derived dietary fiber [6, 7], and approximately 95% of SCFAs in the colon consist of acetate, butyrate, and propionate [6]. Although animal experiments and *in vitro* studies have shown that these SCFAs contribute to improved glucose homeostasis and insulin sensitivity [8], several epidemiological studies have found that fecal concentrations of each subtype of SCFAs and total SCFAs are positively correlated with the prevalence of obesity [9-14]. These epidemiological studies, however, have been conducted in Western populations, and the gut microbiota related to the production of SCFA vary according to racial ethnic group and geographic locations [15-17]. Evidence suggests that the Japanese population has more gut microbiota involved in dietary fiber fermentation and SCFA-production compared with Western populations [17]. In addition, the Japanese population is relatively lean and has fewer bacteria associated with obesity than Western

populations [18]. Since no relevant study has been conducted in Japan, we investigated the association between fecal SCFAs and the prevalence of obesity in a community-based Japanese population.

Methods

Study design and population

This study was conducted as part of the Dynamics of Lifestyle and Neighborhood Community on Health Study (DOSANCO Health Study), a community-based cross-sectional survey that targeted all residents aged ≥3 years, other than those living in nursing homes, in the town of Suttu, Hokkaido, Japan [19]. Overall, of 2638 residents aged ≥3 years living at home, 2100 completed a self-administered questionnaire (response, 79.6%), and 814 underwent an additional detailed survey (response, 30.9%) in 2015. In this study, we used data from 808 participants aged ≥18 years for the analysis that aims to investigate the association between fecal SCFAs and the prevalence of obesity in an adolescent to adult population. Of the 808 participants aged ≥18, 240 were deemed ineligible because of missing data on fecal SCFA concentrations due to the non-provision of fecal samples (n = 229), BMI (n = 4), or habitual exercise (n = 7). The remaining 568 participants aged ≥18 years (277 men and 291 women) were considered eligible and therefore were included in the subsequent data analyses. This study was conducted according to the guidelines laid down in the Declaration of

Helsinki and all procedures involving human subjects were approved by the Ethics Committee of the Faculty of Medicine, Hokkaido University (15-002 and 15-045). Written informed consent was obtained from all participants.

Data collection

Fecal collection kits, refrigerants and a cooler bag were distributed to the participants the day before the anthropometric survey. Participants self-collected fresh fecal samples with the collection kits, packed them into a cooler bag with frozen refrigerants, and brought them to the anthropometric survey location in the morning. Samples were frozen immediately after delivery in a freezer set at -30°C, and then transported to Hokkaido University and stored at -80°C until SCFA measurements were conducted.

Frozen fecal samples were sent to Technosuruga Laboratory Co. Ltd. (Shizuoka, Japan). Fecal SCFAs (acetate, propionate, and butyrate, among others) were extracted from 0.1 g of the feces and measured using a high-performance liquid chromatography (Prominence, SHIMADZU, Kyoto, Japan) as described previously [20]. Fecal concentrations of SCFAs were expressed in mg/g of fecal wet weight. In this report, the total SCFAs was defined as the sum of the subtypes of SCFAs, including acetate, butyrate, and propionate.

Body height and weight was measured, and BMI was calculated as the weight in kg divided by the height in m^2. Obesity was defined as a BMI ≥ 25.0 kg/m2 based on the criteria for obesity recommended by the Japan Society for the Study of Obesity [3]. Data on age, sex, habitual exercise, total energy intake, and total dietary fiber intake were collected via the self-administered questionnaire. Habitual exercise was classified as partaking in ≥10 min of physical exercise at least once a week [19, 21]. Daily total energy intake (kcal/day) and total dietary fiber intake (g/day) were assessed using the brief-type self-administered diet history questionnaire (BDHQ) [22].

Statistical analysis

Initially, we classified the participants into four quartiles according to fecal concentrations of SCFA subtypes (i.e., acetate, butyrate, and propionate) and total SCFAs to compare the prevalence of obesity. We then conducted one-way analysis of variance or the chi-square test to compare each characteristic (i.e., sex, dietary intake, and habitual exercise) in each SCFA group. Next, we calculated prevalence ratios (PRs) of obesity and their corresponding 95% confidence intervals (CIs) for each SCFA group using a log-binomial regression model with the first quartile SCFA group serving as the reference. The models incorporated the following covariates as potential confounding factors: age (years), sex (male or female), habitual exercise (yes or no), total energy intake (kcal/day as a continuous variable), total dietary fiber intake (g/day as a continuous variable),

and the concentrations of the two SCFA subtypes other than the targeted subtype (mg/g wet weight as a continuous variable). Next, we tested the linear associations of each fecal SCFA (mg/g wet-weight as a continuous variable) with BMI (kg/m^2 as a continuous variable). We tested the trend for a linear association using a multiple linear regression model adjusted for the confounding factors. Finally, to explore the association of interest from the opposite direction, we classified the participants into three groups according to their BMI and compared fecal SCFA concentrations among the following three BMI groups: <18.5 (underweight), 18.5－24.9 (normal weight), ≥25.0 kg/m^2 (obesity) [3]. One-way analysis of variance and analysis of covariance were used to compare log-transformed fecal SFCA among the three BMI groups because of skewed distributions of the fecal SFCA data. The analysis of covariance incorporated the same covariates used in the log-binomial regression model. All statistical analyses were performed using R version 3.4.1. All probability values were two-tailed, and the significance level was set at $P < 0.05$.

Results

Characteristics of study participants

The mean age (standard deviation [SD]) of the 568 study participants was 58.4 (16.2) years. The median values (interquartile ranges) for the concentrations of fecal total SCFAs, acetate, butyrate,

and propionate were 5.46 (3.94−7.07), 3.20 (2.3175−4.33), 0.90 (0.58−1.41), and 1.20 (0.9175−1.6125) mg/g wet weight, respectively. The mean (SD) total energy intake and total dietary fiber intake were 1785.0 (610.2) kcal/day and 11.3 (5.5) g/day, respectively. Furthermore, 42.4% (241) of the study participants had an exercise habit. Participants with obesity accounted for 35.7% of the study population (male: 36.8%, female: 34.7%). The mean age was highest in the fourth quartile fecal SCFA concentration group for every subtype of SCFA (Table 1). However, the proportion of female participants did not differ significantly among the four SCFA groups regardless of SCFA subtype (Table 1). The proportion of the participants who habitually exercised decreased with increasing fecal concentrations of acetate, butyrate, and total SCFAs and was the highest in the third quartile group of propionate (Table 1).

Fecal SCFAs and obesity

Among the four quartile groups of fecal total SCFAs, the third quartile group had the highest prevalence of obesity (43.7%), closely followed by the fourth quartile group (42.7%) (Table 2). Compared with the first quartile group, the PRs of obesity were 1.32 (95% CI, 0.91−1.93) for the second quartile group, 1.79 (1.27−2.54) for the third quartile group, and 1.74 (1.23−2.48) for the fourth quartile group after adjusting for age and sex. Furthermore, even after adjusting for additional confounders such as total energy intake, total dietary fiber intake, and exercise habits, the

PRs of obesity for the third and the fourth quartile group remained significant (multivariate-adjusted PR [model 2] 1.74 [95% CI, 1.23–2.47] for the third quartile group, and 1.70 [1.19−2.41] for the fourth quartile group). Similarly, for fecal acetate, butyrate, and propionate, the prevalence of obesity was highest in the third or fourth quartile groups. Similar patterns were observed regarding the adjusted PRs of obesity (models 1 and 2) for each SCFA subtype. In addition, there was a significant positive linear trend for the association between each SCFA subtype and BMI (models 1 and 2). The third and/or the fourth quartile groups also had significantly higher PRs of obesity than the reference group after further adjusting for the fecal concentrations of the two other SCFA subtypes (model 3), although the test for a linear trend was no longer significant. When we had the opposite perspective, all types of fecal SCFAs were significantly higher in the group with higher BMI than in the group with lower BMI, even after adjusting for the same confounders (Table 3).

Discussion

This cross-sectional study investigated the associations between fecal concentrations of SCFAs and obesity in a relatively large community-based population in Japan. Compared with the first quartile group, the prevalence of obesity was higher in the third and the fourth quartile groups of every fecal SCFA subtype even after adjusting for major potentially confounding factors such as age, sex,

146 dietary intake, and exercise habits. Furthermore, there were positive linear associations between
147 BMI and every fecal SCFA subtype. These findings suggest that each fecal SCFA subtype (i.e.,
148 acetate, butyrate, and propionate) is positively associated with the prevalence of obesity in a dose-
149 dependent manner.

150 The results of our study are consistent with the results of previous epidemiological studies
151 conducted in countries other than Japan [9-14]. Five of these, which were conducted in Europe,
152 South America, North America, and South Asia reported that every fecal SCFA subtype was more
153 prevalent in overweight and obese subjects compared to lean and/or normal subjects [9-13], though
154 the definitions of obesity were different from that of our study. One notable relevant study by de la
155 Cuesta-Zuluaga et al. compared the prevalence of obesity (BMI $\geq$ 30 kg/m^2) among Colombian
156 participants grouped according to the tertile of each fecal SCFA [14]. In line with our study, this
157 study revealed that for acetate, propionate, butyrate, and total SCFAs, the group with the highest
158 fecal SCFA concentrations had significantly higher PRs of obesity than the groups with the lowest.
159 Our study showed that fecal SCFAs were positively associated with the prevalence of obesity even
160 in Japanese participants who tend to be less obese, have less bacteria associated with obesity, and
161 more gut microbiota involved in SCFA-production compared with Westerners and some other
162 Asian populations [17, 18]. The average daily energy intake of our study population (1785 kcal)
163 was approximately half of the average daily intake of populations in North American countries

(3760 kcal) and European Union countries (3441 kcal) [23]. Meanwhile, the average daily total dietary fiber consumption was slightly lower in our study population (11.3 g) than in the U.S. population (14.8 g) [24]. Evidence suggests that diet is a crucial factor associated with interactions between gut microbiota and obesity [25]. Nevertheless, our study emphasizes that the positive association between fecal SCFAs and obesity prevalence is universal across racial and regional groups.

The Colombian study by de la Cuesta-Zuluaga et al. used different methods to measure fecal concentrations of SCFAs than our study [14]; therefore, these data were not comparable. We used raw fecal samples, not dried samples, and our pretreatment of SCFA measurement recovered over 96% of all SCFAs [26]. Thus, a simple and consistent method (the organic acid analysis system [SHIMADZU]) was used for measuring fecal SCFAs in our study. The ease and reliability of this method makes it suitable for large-scale epidemiological studies; therefore, our study provided important evidence concerning the association between fecal concentrations of SCFAs and the prevalence of obesity in a community-based population. This is a major strength of our study, since it will be possible to compare our findings with the results of future relevant studies.

Neither the causal relationship nor the mechanisms that would explain the positive associations between fecal SCFAs and the prevalence of obesity are fully understood; however, several hypotheses can be presented. Among the SCFAs produced by the gut microbiota, acetate

and propionate specifically are metabolized as substrates for cholesterol synthesis, *de novo* lipogenesis, adipogenesis and gluconeogenesis in the liver, white adipocytes and brown adipocytes which may lead to fat deposition [27-29]. In addition, SCFAs may serve as a source of energy themselves, providing at least 10% of the energy required for daily life in Western populations [30]. However, there is also some evidence against the potential causality between SCFAs and obesity. Some animal studies have suggested that there are SCFAs that have beneficial effects on body weight via the systemically expressed receptor G protein-coupled receptors [31]. Additionally, several animal experiments [32-34] and an interventional study [35] have found that the oral administration of every SCFA subtype prevents weight gain. However, it may be reasonable to assume that SCFAs have multiple functions in relation to body weight. In addition, SCFAs administered orally and SCFAs produced by the gut microbiota may have different dynamics in the human body.

We acknowledge that this study had several important limitations. First, this study was a cross-sectional study, and therefore it is more difficult to assume causality with regard to the link between increased fecal SCFAs and obesity. Longitudinal studies are needed to clarify this possible causal relationship. Second, our observational study was conducted with 568 residents of a single northern rural community in Japan. Therefore, caution is advised when generalizing the results of this study. Third, we had no data on the use of antibiotics and whole gut transit time (WGTT).

Various antibiotics may affect the colonic production of SCFAs and hence fecal SCFA concentrations by altering the composition of the gut microbiota [36]. In addition, WGTT was found to be one of the determinants of fecal SCFA levels from a human intervention study [37]. However, it is unclear whether antibiotics and WGTT would confound the association between fecal SCFAs and the prevalence of obesity. Finally, we did not directly measure the levels of SCFAs produced in the colon or those circulating in the blood. We assumed that fecal SCFA levels would be equivalent to the levels of SCFAs produced in the colon on the basis that fecal SCFAs are positively associated with the presence of SCFA-producing bacteria and SCFA-productivity of the gut microbiota [12-14, 38, 39]. Meanwhile, a previous report suggested that fecal SCFA levels are positively correlated with serum SCFA levels [12-14, 38, 39]. Despite these study limitations, we believe that our study provides reliable evidence given the sufficient sample size and consideration of potential confounding factors, especially other subtypes of SCFAs.

Conclusions

Our study demonstrated that every subtype of fecal SCFAs was positively associated with the prevalence of obesity in a community-based Japanese population. Our findings suggest that the

positive associations between fecal SCFAs and obesity could be consistent across racial/ethnic groups with different body mass indices and/or gut microbiota compositions.

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Conflict of Interest

None.

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Table 1. Characteristics of the 568 study participants grouped by quartiles of fecal concentrations of total SCFAs and each SCFA subtype

	Fecal total SCFAs, mg/g wet-weight								*P*-value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	1.44 − 3.939		3.940 − 5.460		5.461 − 7.069		7.07 − 16.11		
	(*n* = 140)		(*n* = 143)		(*n* = 142)		(*n* = 143)		
Age, years	56.6	(16.7)	56.1	(15.3)	56.6	(16.3)	64.1	(15.5)	< 0.001
Female, %	73	(52.1)	72	(50.3)	71	(50.0)	75	(52.4)	0.97
Total energy intake, kcal/day	1784.3	(645.7)	1780.9	(605.1)	1758.9	(571.8)	1815.8	(621.2)	0.89
Total dietary fiber intake, g/day	11.7	(5.6)	10.8	(5.6)	10.9	(5.1)	11.8	(5.7)	0.27
Habitual exercise, %	65	(46.4)	59	(41.3)	61	(43.0)	56	(39.2)	0.65
	Fecal acetate, mg/g wet-weight								*P*-value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.62 − 2.3174		2.3175 − 3.1949		3.1950 − 4.329		4.330 − 9.07		
	(*n* = 142)		(*n* = 142)		(*n* = 141)		(*n* = 143)		
Age, years	56.8	(16.8)	55.9	(15.6)	57.2	(16.2)	63.5	(15.5)	< 0.001
Female, %	68	(47.9)	77	(54.2)	72	(51.1)	74	(51.7)	0.76
Total energy intake, kcal/day	1797.2	(644.9)	1787.7	(632.8)	1775.0	(627.8)	1780.1	(535.8)	0.99
Total dietary fiber intake, g/day	11.6	(5.6)	11.0	(5.9)	11.0	(5.4)	11.6	(5.2)	0.59
Habitual exercise, %	71	(50.0)	60	(42.3)	57	(40.4)	53	(37.1)	0.15
Fecal butyrate, mg/g wet-weight	0.5	(0.3)	0.8	(0.3)	1.2	(0.5)	1.9	(1.0)	< 0.001
Fecal propionate, mg/g wet-weight	0.8	(0.3)	1.2	(0.4)	1.4	(0.5)	1.9	(0.8)	< 0.001

(*Continued*)

	Fecal butyrate, mg/g wet-weight								*P*-value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.13 − 0.579		0.580 − 0.8949		0.8950 − 1.409		1.410 − 5.50		
	(*n* = 141)		(*n* = 143)		(*n* = 138)		(*n* = 146)		
Age, years	56.7	(16.4)	56.2	(15.8)	55.7	(16.4)	64.7	(14.7)	< 0.001
Female, %	70	(49.6)	75	(52.4)	73	(52.9)	73	(50.0)	0.93
Total energy intake, kcal/day	1788.5	(658.4)	1714.9	(589.2)	1768.7	(550.2)	1865.8	(632.2)	0.21
Total dietary fiber intake, g/day	11.1	(5.9)	10.5	(5.2)	11.4	(5.5)	12.3	(5.4)	0.06
Habitual exercise, %	61	(43.3)	64	(44.8)	60	(43.5)	56	(38.4)	0.70
Fecal acetate, mg/g wet-weight	2.3	(0.9)	2.9	(0.9)	3.5	(1.0)	5.0	(1.5)	< 0.001
Fecal propionate, mg/g wet-weight	0.9	(0.5)	1.2	(0.4)	1.3	(0.6)	1.9	(0.8)	< 0.001
	Fecal propionate, mg/g wet-weight								*P*-value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.15 − 0.91749		0.9175 − 1.19		1.20 − 1.61249		1.61250 − 4.27		
	(*n* = 142)		(*n* = 138)		(*n* = 146)		(*n* = 142)		
Age, years	56.5	(16.3)	57.2	(15.8)	57.2	(16.2)	62.6	(16.1)	0.01
Female, %	75	(52.8)	68	(49.3)	71	(48.6)	77	(54.2)	0.74
Total energy intake, kcal/day	1797.3	(619.1)	1754.3	(592.1	1769.5	(627.1)	1818.4	(605.2)	0.82
Total dietary fiber intake, g/day	11.7	(5.8)	11.5	(5.7)	10.7	(5.1)	11.5	(5.6)	0.43
Habitual exercise, %	63	(44.4)	58	(42.0)	73	(50.0)	47	(33.1)	0.03
Fecal acetate, mg/g wet-weight	2.3	(0.9)	3.2	(1.3)	3.4	(1.0)	4.9	(1.4)	< 0.001
Fecal butyrate, mg/g wet-weight	0.6	(0.4)	1.0	(0.6)	1.1	(0.5)	1.8	(1.1)	< 0.001

Abbreviations: SCFAs, short-chain fatty acids. Values are presented as mean (standard deviation), or the number (%) of participants in that category.

*One-way analysis of variance, or the chi-square test was used to compare each characteristic in each SCFA group.

Table 2. The prevalence and the prevalence ratios of obesity in participants grouped by quartiles of fecal SCFAs

	Fecal total SCFAs, mg/g wet-weight								*P*-value
	1st quartile		2nd quartile		3rd quartile		4th quartile		for trend‡
	1.44 − 3.939		3.940 − 5.460		5.461 − 7.069		7.07 − 16.11		
	(*n* = 140)		(*n* = 143)		(*n* = 142)		(*n* = 143)		
Obesity, *n*	34		46		62		61		
Prevalence (%)	24.3		32.2		43.7		42.7		
Adjusted PRs (95% CI), model 1†	1.00	(reference)	1.32	(0.91 − 1.93)	1.79	(1.27 − 2.54)	1.74	(1.23 − 2.48)	< 0.001
Adjusted PRs (95% CI), model 2†	1.00	(reference)	1.30	(0.89 − 1.89)	1.74	(1.23 − 2.47)	1.70	(1.19 − 2.41)	< 0.001
	Fecal acetate, mg/g wet-weight								*P*-value
	1st quartile		2nd quartile		3rd quartile		4th quartile		for trend‡
	0.62 − 2.3174		2.3175 − 3.1949		3.1950 − 4.329		4.330 − 9.07		
	(*n* = 142)		(*n* = 142)		(*n* = 141)		(*n* = 143)		
Obesity, *n*	40		43		61		59		
Prevalence (%)	28.2		30.3		43.4		41.3		
Adjusted PRs (95% CI), model 1†	1.00	(reference)	1.08	(0.75 − 1.55)	1.53	(1.11 − 2.12)	1.46	(1.05 − 2.03)	< 0.001
Adjusted PRs (95% CI), model 2†	1.00	(reference)	1.06	(0.74 − 1.52)	1.50	(1.08 − 2.06)	1.41	(1.02 − 1.97)	< 0.001
Adjusted PRs (95% CI), model 3†	1.00	(reference)	1.04	(0.72 − 1.49)	1.41	(1.00 − 1.99)	1.23	(0.80 − 1.88)	0.14

(*Continued*)

	Fecal butyrate, mg/g wet-weight								*P*-value for trend‡
	1st quartile 0.13 − 0.579 (*n* = 141)		2nd quartile 0.580 − 0.8949 (*n* = 143)		3rd quartile 0.8950 − 1.409 (*n* = 138)		4th quartile 1.410 − 5.50 (*n* = 146)		
Obesity, *n*	29		53		56		65		
Prevalence (%)	20.6		37.1		40.6		44.5		
Adjusted PRs (95% CI), model 1†	1.00	(reference)	1.80	(1.22 − 2.65)	1.98	(1.35 − 2.91)	2.15	(1.48 − 3.13)	< 0.001
Adjusted PRs (95% CI), model 2†	1.00	(reference)	1.76	(1.20 − 2.59)	2.00	(1.37 − 2.92)	2.16	(1.49 − 3.14)	< 0.001
Adjusted PRs (95% CI), model 3†	1.00	(reference)	1.75	(1.19 − 2.59)	1.97	(1.32 − 2.92)	2.11	(1.34 − 3.31)	0.20
	Fecal propionate, mg/g wet-weight								*P*-value for trend‡
	1st quartile 0.15 − 0.91749 (*n* = 142)		2nd quartile 0.9175 − 1.19 (*n* = 138)		3rd quartile 1.20 − 1.61249 (*n* = 146)		4th quartile 1.61250 − 4.27 (*n* = 142)		
Obesity, *n*	29		52		63		59		
Prevalence (%)	20.4		37.7		43.2		41.5		
Adjusted PRs (95% CI), model 1†	1.00	(reference)	1.84	(1.25 − 2.72)	2.10	(1.45 − 3.06)	2.03	(1.38 − 2.97)	0.02
Adjusted PRs (95% CI), model 2†	1.00	(reference)	1.83	(1.24 − 2.70)	2.07	(1.42 − 3.00)	1.97	(1.35 − 2.89)	0.03
Adjusted PRs (95% CI), model 3†	1.00	(reference)	1.76	(1.18 − 2.61)	1.95	(1.33 − 2.87)	1.68	(1.07 − 2.62)	0.70

Abbreviations: CI, confidence interval; PRs, prevalence ratios; SCFAs, short-chain fatty acids. Obesity was defined as a body mass index ≥25 kg/m^2. †Three different log-binomial regression models were used to calculate PRs (95% CI) with the first quartile group serving as the reference group: model 1 was adjusted for age and sex; model 2 was adjusted for age, sex, total energy intake, total dietary fiber intake, and exercise habits; and model 3 was adjusted for the same covariates used in Model 2 in addition to the concentrations of the two SCFA subtypes other than the target subtype. ‡Trends were tested using the multiple linear regression model, where the objective variable was body mass index (kg/m^2) and the independent variables were the fecal concentrations of each SCFA subtype (mg/g wet weight) adjusted for covariates.

Table 3. Fecal concentration of total SCFAs and each SCFA subtype in participants grouped by body mass index

	Body mass index, kg/m^2						*P*-value[§]
	<18.5 (underweight) (*n* = 31)		18.5 − 24.9 (normal weight) (*n* = 334)		≥25.0 (obesity) (*n* = 203)		
ln(fecal total SCFAs), mg/g wet-weight							
Crude mean (SD)	1.55	(0.48)	1.63	(0.45)	1.77	(0.41)	< 0.001
Adjusted LSM (SE), model 1[¶]	1.54	(0.08)	1.63	(0.02)	1.77	(0.03)	< 0.001
Adjusted LSM (SE), model 2[¶]	1.53	(0.08)	1.63	(0.02)	1.76	(0.03)	< 0.001
ln(fecal acetate), mg/g wet-weight							
Crude mean (SD)	1.01	(0.45)	1.11	(0.45)	1.22	(0.42)	0.003
Adjusted LSM (SE), model 1[¶]	1.01	(0.08)	1.11	(0.02)	1.22	(0.03)	0.003
Adjusted LSM (SE), model 2[¶]	0.99	(0.08)	1.11	(0.02)	1.21	(0.03)	0.003
Adjusted LSM (SE), model 3[¶]	1.09	(0.05)	1.14	(0.02)	1.14	(0.02)	< 0.001
ln(fecal butyrate), mg/g wet-weight							
Crude mean (SD)	-0.30	(0.75)	-0.20	(0.69)	0.03	(0.63)	< 0.001
Adjusted LSM (SE), model 1[¶]	-0.31	(0.12)	-0.19	(0.04)	0.02	(0.05)	< 0.001
Adjusted LSM (SE), model 2[¶]	-0.32	(0.12)	-0.20	(0.04)	0.02	(0.05)	< 0.001
Adjusted LSM (SE), model 3[¶]	-0.15	(0.08)	-0.15	(0.03)	-0.07	(0.03)	< 0.001

(*Continued*)

ln(fecal propionate), mg/g wet-weight							
Crude mean (SD)	0.06	(0.62)	0.12	(0.52)	0.26	(0.44)	0.004
Adjusted LSM (SE), model 1¶	0.05	(0.09)	0.13	(0.03)	0.26	(0.04)	0.004
Adjusted LSM (SE), model 2¶	0.05	(0.09)	0.13	(0.03)	0.25	(0.04)	0.004
Adjusted LSM (SE), model 3¶	0.17	(0.07)	0.16	(0.02)	0.19	(0.03)	< 0.001

Abbreviations: CI, confidence interval; LSM, least square mean; SCFAs, short-chain fatty acids; SD, standard deviation; SE, standard error. §One-way analysis of variance, or analysis of covariance (¶models 1-3) was used to compare fecal SCFA concentrations in each body mass index group: model 1 was adjusted for age and sex; model 2 was adjusted for the same covariates used in model 1 in addition to total energy intake, total dietary fiber intake, and exercise habits; model 3 was adjusted for the same covariates used in model 2 in addition to the concentrations of two subtypes of SCFAs other than the target subtype.