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

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1 **Abstract**

2 In Western populations, fecal concentrations of short-chain fatty acids (SCFAs) are positively
3 correlated with the prevalence of obesity. However, gut microbiota involved in the production of
4 SCFA varies between races. Our purpose was to investigate the associations between fecal SCFAs
5 and the prevalence of obesity in a community-based Japanese population. We classified a total of
6 568 participants aged ≥ 18 into four quartiles of fecal concentrations of SCFA subtypes (acetate,
7 butyrate, and propionate) and total SCFAs to compare the prevalence of obesity, defined as a body
8 mass index ≥ 25.0 kg/m². Using the first quartile SCFA group as a reference, the prevalence ratios
9 of obesity were calculated for each SCFA group through a log-binomial regression model adjusted
10 for major potentially confounding factors including age, sex, exercise habits, total energy intake,
11 and total dietary fiber intake. In the study population, the prevalence of obesity was 35.8%. The
12 prevalence ratios (95% confidence intervals) of obesity in the second, third, and fourth quartile
13 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),
14 respectively, after adjusting for the confounders. Similar positive associations were observed for
15 every subtype. The prevalence ratios (95% confidence intervals) in the fourth quartile groups of
16 fecal acetate, butyrate, and propionate were 1.41 (1.02–1.97), 2.16 (1.49–3.14), and 1.97
17 (1.35–2.89), respectively, after adjusting for the confounders. In conclusion, our results

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

20

21 **Introduction**

22 Obesity is a major risk factor for various diseases including type 2 diabetes, metabolic syndrome,
23 cardiovascular disease, and some types of cancers [1]. Today, over one fourth of adults have
24 obesity, which is defined as a body mass index (BMI) ≥ 30 kg/m² in several Western developed
25 countries [2]. In Japan, the prevalence of obesity, commonly defined as a BMI ≥ 25 kg/m² for East-
26 Asian populations [3], has been increasing over the last several decades [4], affecting 30.7% of
27 male and 21.9% of female adults [5]. Therefore, obesity is a public health burden worldwide.

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

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

42

43 **Methods**

44 *Study design and population*

45 This study was conducted as part of the Dynamics of Lifestyle and Neighborhood Community on
46 Health Study (DOSANCO Health Study), a community-based cross-sectional survey that targeted
47 all residents aged ≥ 3 years, other than those living in nursing homes, in the town of Suttu,
48 Hokkaido, Japan [19]. Overall, of 2638 residents aged ≥ 3 years living at home, 2100 completed a
49 self-administered questionnaire (response, 79.6%), and 814 underwent an additional detailed survey
50 (response, 30.9%) in 2015. In this study, we used data from 808 participants aged ≥ 18 years for the
51 analysis that aims to investigate the association between fecal SCFAs and the prevalence of obesity
52 in an adolescent to adult population. Of the 808 participants aged ≥ 18 , 240 were deemed ineligible
53 because of missing data on fecal SCFA concentrations due to the non-provision of fecal samples (n
54 = 229), BMI ($n = 4$), or habitual exercise ($n = 7$). The remaining 568 participants aged ≥ 18 years
55 (277 men and 291 women) were considered eligible and therefore were included in the subsequent
56 data analyses. This study was conducted according to the guidelines laid down in the Declaration of

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

60

61 ***Data collection***

62 Fecal collection kits, refrigerants and a cooler bag were distributed to the participants the day before
63 the anthropometric survey. Participants self-collected fresh fecal samples with the collection kits,
64 packed them into a cooler bag with frozen refrigerants, and brought them to the anthropometric
65 survey location in the morning. Samples were frozen immediately after delivery in a freezer set at -
66 30°C, and then transported to Hokkaido University and stored at -80°C until SCFA measurements
67 were conducted.

68 Frozen fecal samples were sent to Technosuruga Laboratory Co. Ltd. (Shizuoka, Japan).

69 Fecal SCFAs (acetate, propionate, and butyrate, among others) were extracted from 0.1 g of the
70 feces and measured using a high-performance liquid chromatography (Prominence, SHIMADZU,
71 Kyoto, Japan) as described previously [20]. Fecal concentrations of SCFAs were expressed in mg/g
72 of fecal wet weight. In this report, the total SCFAs was defined as the sum of the subtypes of
73 SCFAs, including acetate, butyrate, and propionate.

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

81

82 ***Statistical analysis***

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

92 and the concentrations of the two SCFA subtypes other than the targeted subtype (mg/g wet weight
93 as a continuous variable). Next, we tested the linear associations of each fecal SCFA (mg/g wet-
94 weight as a continuous variable) with BMI (kg/m² as a continuous variable). We tested the trend for
95 a linear association using a multiple linear regression model adjusted for the confounding factors.
96 Finally, to explore the association of interest from the opposite direction, we classified the
97 participants into three groups according to their BMI and compared fecal SCFA concentrations
98 among the following three BMI groups: <18.5 (underweight), 18.5 – 24.9 (normal weight), ≥25.0
99 kg/m² (obesity) [3]. One-way analysis of variance and analysis of covariance were used to compare
100 log-transformed fecal SFCA among the three BMI groups because of skewed distributions of the
101 fecal SFCA data. The analysis of covariance incorporated the same covariates used in the log-
102 binomial regression model. All statistical analyses were performed using R version 3.4.1. All
103 probability values were two-tailed, and the significance level was set at $P < 0.05$.

104

105

106 **Results**

107 *Characteristics of study participants*

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

110 and propionate were 5.46 (3.94–7.07), 3.20 (2.3175–4.33), 0.90 (0.58–1.41), and 1.20
111 (0.9175–1.6125) mg/g wet weight, respectively. The mean (SD) total energy intake and total
112 dietary fiber intake were 1785.0 (610.2) kcal/day and 11.3 (5.5) g/day, respectively. Furthermore,
113 42.4% (241) of the study participants had an exercise habit. Participants with obesity accounted for
114 35.7% of the study population (male: 36.8%, female: 34.7%). The mean age was highest in the
115 fourth quartile fecal SCFA concentration group for every subtype of SCFA (Table 1). However, the
116 proportion of female participants did not differ significantly among the four SCFA groups
117 regardless of SCFA subtype (Table 1). The proportion of the participants who habitually exercised
118 decreased with increasing fecal concentrations of acetate, butyrate, and total SCFAs and was the
119 highest in the third quartile group of propionate (Table 1).

120

121 ***Fecal SCFAs and obesity***

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

128 PRs of obesity for the third and the fourth quartile group remained significant (multivariate-adjusted
129 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
130 fourth quartile group). Similarly, for fecal acetate, butyrate, and propionate, the prevalence of
131 obesity was highest in the third or fourth quartile groups. Similar patterns were observed regarding
132 the adjusted PRs of obesity (models 1 and 2) for each SCFA subtype. In addition, there was a
133 significant positive linear trend for the association between each SCFA subtype and BMI (models 1
134 and 2). The third and/or the fourth quartile groups also had significantly higher PRs of obesity than
135 the reference group after further adjusting for the fecal concentrations of the two other SCFA
136 subtypes (model 3), although the test for a linear trend was no longer significant. When we had the
137 opposite perspective, all types of fecal SCFAs were significantly higher in the group with higher
138 BMI than in the group with lower BMI, even after adjusting for the same confounders (Table 3).

139

140

141 **Discussion**

142 This cross-sectional study investigated the associations between fecal concentrations of SCFAs and
143 obesity in a relatively large community-based population in Japan. Compared with the first quartile
144 group, the prevalence of obesity was higher in the third and the fourth quartile groups of every fecal
145 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 ($BMI \geq 30 \text{ kg/m}^2$) 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

164 (3760 kcal) and European Union countries (3441 kcal) [23]. Meanwhile, the average daily total
165 dietary fiber consumption was slightly lower in our study population (11.3 g) than in the U.S.
166 population (14.8 g) [24]. Evidence suggests that diet is a crucial factor associated with interactions
167 between gut microbiota and obesity [25]. Nevertheless, our study emphasizes that the positive
168 association between fecal SCFAs and obesity prevalence is universal across racial and regional
169 groups.

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

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

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

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

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

212

213

214 **Conclusions**

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

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

219

220

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224

225

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233

234

235 **Conflict of Interest**

236 None.

237

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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								<i>P</i> -value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	1.44 – 3.939 (<i>n</i> = 140)		3.940 – 5.460 (<i>n</i> = 143)		5.461 – 7.069 (<i>n</i> = 142)		7.07 – 16.11 (<i>n</i> = 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								<i>P</i> -value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.62 – 2.3174 (<i>n</i> = 142)		2.3175 – 3.1949 (<i>n</i> = 142)		3.1950 – 4.329 (<i>n</i> = 141)		4.330 – 9.07 (<i>n</i> = 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

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	Fecal butyrate, mg/g wet-weight								<i>P</i> -value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.13 – 0.579 (<i>n</i> = 141)		0.580 – 0.8949 (<i>n</i> = 143)		0.8950 – 1.409 (<i>n</i> = 138)		1.410 – 5.50 (<i>n</i> = 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								<i>P</i> -value*
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.15 – 0.91749 (<i>n</i> = 142)		0.9175 – 1.19 (<i>n</i> = 138)		1.20 – 1.61249 (<i>n</i> = 146)		1.61250 – 4.27 (<i>n</i> = 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								<i>P</i> -value for trend‡
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	1.44 – 3.939 (<i>n</i> = 140)		3.940 – 5.460 (<i>n</i> = 143)		5.461 – 7.069 (<i>n</i> = 142)		7.07 – 16.11 (<i>n</i> = 143)		
Obesity, <i>n</i>	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								<i>P</i> -value for trend‡
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.62 – 2.3174 (<i>n</i> = 142)		2.3175 – 3.1949 (<i>n</i> = 142)		3.1950 – 4.329 (<i>n</i> = 141)		4.330 – 9.07 (<i>n</i> = 143)		
Obesity, <i>n</i>	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

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	Fecal butyrate, mg/g wet-weight								P-value for trend [‡]
	1st quartile		2nd quartile		3rd quartile		4th quartile		
	0.13 – 0.579 (n = 141)		0.580 – 0.8949 (n = 143)		0.8950 – 1.409 (n = 138)		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		2nd quartile		3rd quartile		4th quartile		
	0.15 – 0.91749 (n = 142)		0.9175 – 1.19 (n = 138)		1.20 – 1.61249 (n = 146)		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/m². [†]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/m²) 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						<i>P</i> -value [§]
	<18.5		18.5 – 24.9		≥25.0		
	(underweight)		(normal weight)		(obesity)		
	<i>n</i> = 31		<i>n</i> = 334		<i>n</i> = 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

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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.