Title	Fecal short-chain fatty acids and obesity in a community-based Japanese population : The DOSANCO Health Study
Author(s)	Yamamura, Ryodai; Nakamura, Koshi; Ukawa, Shigekazu et al.
Citation	Obesity Research & Clinical Practice, 15(4), 345-350 https://doi.org/10.1016/j.orcp.2021.06.003
Issue Date	2021-07
Doc URL	https://hdl.handle.net/2115/86444
Rights	© 2021. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	RE_Manuscript_Yamamura_ObesResClinPract_210526.pdf

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

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/m^2. 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

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) ≥ 30 kg/m^2 in several Western developed

countries [2]. In Japan, the prevalence of obesity, commonly defined as a BMI ≥ 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

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

4

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 m^2. Obesity was defined as a BMI ≥ 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 ≥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),

6

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/m^2 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/m^2 (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,

9

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

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

233

234

14

235   **Conflict of Interest**

236   None.

237

238   **References**

239   [1] Pi-Sunyer X. The medical risks of obesity. Postgrad Med. 2009;121:21-33.

240   [2] Organisation for Economic Co-operation and Development. Obesity Update 2017.

241   https://www.oecd.org/health/obesity-update.htm [accessed May 17th 2021].

242   [3] Matsuzawa Y, Inoue S, Ikeda Y, Sakata T, Saito Y, Sato Y, et al. A new criteria of judgment of

243   obesity and diagnosis of pathological obesity. Journal of Japan Society for the Study of Obesity.

244   2000;6:18-28.

245   [4] Nagai M, Ohkubo T, Murakami Y, Takashima N, Kadota A, Miyagawa N, et al. Secular trends of

246   the impact of overweight and obesity on hypertension in Japan, 1980–2010. Hypertens Res.

247   2015;38:790.

248   [5] Ministry of Health, Labour and Welfare. National Health and Nutrition Survey. 2018.

249   [6] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota

250   metabolic interactions. Science. 2012;336:1262-1267.

251   [7] Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M. The role of Gut Microbiota in

252   the development of obesity and Diabetes. Lipids Health Dis. 2016;15:108.

253   [8] Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin

254   sensitivity. Nature Reviews Endocrinology. 2015;11:577-591.

255   [9] Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, et al. Microbiota and SCFA in lean

16

256   and overweight healthy subjects. Obesity (Silver Spring). 2010;18:190-195.

257   [10] Patil DP, Dhotre DP, Chavan SG, Sultan A, Jain DS, Lanjekar VB, et al. Molecular analysis of

258   gut microbiota in obesity among Indian individuals. J Biosci. 2012;37:647-657.

259   [11] Teixeira TF, Grzeskowiak L, Franceschini SC, Bressan J, Ferreira CL, Peluzio MC. Higher level

260   of faecal SCFA in women correlates with metabolic syndrome risk factors. Br J Nutr.

261   2013;109:914-919.

262   [12] Rahat-Rozenbloom S, Fernandes J, Gloor GB, Wolever TM. Evidence for greater production of

263   colonic short-chain fatty acids in overweight than lean humans. Int J Obes (Lond).

264   2014;38:1525-1531.

265   [13] Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, Comelli EM. Adiposity, gut microbiota

266   and faecal short chain fatty acids are linked in adult humans. Nutr Diabetes. 2014;4:e121.

267   [14] de la Cuesta-Zuluaga J, Mueller N, Álvarez-Quintero R, Velásquez-Mejía E, Sierra J, Corrales-

268   Agudelo V, et al. Higher fecal short-chain fatty acid levels are associated with gut microbiome

269   dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. Nutrients. 2019;11:51.

270   [15] Hester CM, Jala VR, Langille MG, Umar S, Greiner KA, Haribabu B. Fecal microbes, short

271   chain fatty acids, and colorectal cancer across racial/ethnic groups. World J Gastroenterol.

272   2015;21:2759-2769.

273   [16] Clemente JC, Pehrsson EC, Blaser MJ, Sandhu K, Gao Z, Wang B, et al. The microbiome of

274   uncontacted Amerindians. Sci Adv. 2015;1.

275   [17] Nishijima S, Suda W, Oshima K, Kim SW, Hirose Y, Morita H, et al. The gut microbiome of

276   healthy Japanese and its microbial and functional uniqueness. DNA Res. 2016;23:125-133.

277   [18] Andoh A, Nishida A, Takahashi K, Inatomi O, Imaeda H, Bamba S, et al. Comparison of the gut

278   microbial community between obese and lean peoples using 16S gene sequencing in a Japanese

279   population. J Clin Biochem Nutr. 2016;59:65-70.

280   [19] Nakamura K, Hui S-P, Ukawa S, Okada E, Nakagawa T, Okabe H, et al. Serum 25-

281   hydroxyvitamin D3 levels and poor sleep quality in a Japanese population: the DOSANCO

282   Health Study. Sleep Med. 2019;57:135-140.

283   [20] Saji N, Murotani K, Hisada T, Kunihiro T, Tsuduki T, Sugimoto T, et al. Relationship between

284   dementia and gut microbiome-associated metabolites: a cross-sectional study in Japan. Sci Rep.

285   2020;10:1-11.

286   [21] World Health Organization. Global Physical Activity Questionnaire (GPAQ) Analysis Guide.

287   https://www.who.int/ncds/surveillance/steps/resources/GPAQ_Analysis_Guide.pdf   [accessed

288   May 17th 2021].

289   [22] Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, et al. Comparison of relative

290   validity of food group intakes estimated by comprehensive and brief-type self-administered diet

291   history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr.

292   2011;14:1200-1211.

293   [23]   Food   and   Agriculture   Organization   of   the   United   Nations.   FAOSTAT.

294   http://www.fao.org/faostat/en/ [accessed May 17th 2021].

295   [24] King DE, Mainous AG, Lambourne CA. Trends in Dietary Fiber Intake in the United States,

296   1999-2008. J Acad Nutr Diet. 2012;112:642-648.

297   [25] Kim B, Choi H-N, Yim J-E. Effect of Diet on the Gut Microbiota Associated with Obesity. J

298   Obes Metab Syndr. 2019;28:216-224.

299   [26] Aoe S, Nakamura F, Fujiwara S. Effect of wheat bran on fecal butyrate-producing bacteria and

300   wheat bran combined with barley on bacteroides abundance in Japanese healthy adults. Nutrients.

301   2018;10:1980.

302   [27] Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and

303   short chain fatty acids. J Clin Gastroenterol. 2006;40:235-243.

304   [28] Jocken JWE, González Hernández MA, Hoebers NTH, van der Beek CM, Essers YPG, Blaak

305   EE, et al. Short-Chain Fatty Acids Differentially Affect Intracellular Lipolysis in a Human White

306   Adipocyte Model. Front Endocrinol (Lausanne). 2017;8:372.

307   [29] Hu J, Kyrou I, Tan BK, Dimitriadis GK, Ramanjaneya M, Tripathi G, et al. Short-Chain Fatty

308   Acid Acetate Stimulates Adipogenesis and Mitochondrial Biogenesis via GPR43 in Brown

309   Adipocytes. Endocrinology. 2016;157:1881-1894.

310   [30] Brahe LK, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and

311   obesity-related metabolic diseases? Obes Rev. 2013;14:950-959.

312   [31] Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, et al. The gut microbiota

313   suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat

314   Commun. 2013;4:1829.

315   [32] Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves insulin

316   sensitivity and increases energy expenditure in mice. Diabetes. 2009;58:1509-1517.

317   [33] Lin HV, Frassetto A, Kowalik EJ, Jr., Nawrocki AR, Lu MM, Kosinski JR, et al. Butyrate and

318   propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid

319   receptor 3-independent mechanisms. PLoS One. 2012;7:e35240.

320   [34] Lu Y, Fan C, Li P, Lu Y, Chang X, Qi K. Short Chain Fatty Acids Prevent High-fat-diet-induced

321   Obesity in Mice by Regulating G Protein-coupled Receptors and Gut Microbiota. Sci Rep.

322   2016;6:37589.

323   [35] Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SE, et al. Effects

324   of targeted delivery of propionate to the human colon on appetite regulation, body weight

325   maintenance and adiposity in overweight adults. Gut. 2015;64:1744-1754.

326   [36] Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, et al. Antibiotics in early life alter the

327   murine colonic microbiome and adiposity. Nature. 2012;488:621-626.

328 [37] Lewis SJ, Heaton KW. Increasing butyrate concentration in the distal colon by accelerating

329   intestinal transit. Gut. 1997;41:245-251.

330 [38] Fernandes J, Wang A, Su W, Rozenbloom SR, Taibi A, Comelli EM, et al. Age, dietary fiber,

331   breath methane, and fecal short chain fatty acids are interrelated in Archaea-positive humans. J

332   Nutr. 2013;143:1269-1275.

333 [39] Yamamura R, Nakamura K, Kitada N, Aizawa T, Shimizu Y, Nakamura K, et al. Associations of

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

335   acids. Bioscience of Microbiota, Food and Health. 2020;39:11-17.

336

337

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 1.44 − 3.939 (n = 140)	2nd quartile 3.940 − 5.460 (n = 143)	3rd quartile 5.461 − 7.069 (n = 142)	4th quartile 7.07 − 16.11 (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 0.62 − 2.3174 (n = 142)	2nd quartile 2.3175 − 3.1949 (n = 142)	3rd quartile 3.1950 − 4.329 (n = 141)	4th quartile 4.330 − 9.07 (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

	Fecal butyrate, mg/g wet-weight				*P*-value[*]
	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)	
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 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)	
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

	Fecal butyrate, mg/g wet-weight				*P*-value
	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)	for trend[‡]
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
	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)	for trend[‡]
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

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.