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ORIGINAL ARTICLE

CHANGES IN GUT MICROBIOTA COMPOSITION WITH AGING IN OBESE ADULTS

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Abstract It has been reported that an association between gut microbiota and obesity, and changes in the composition of gut microbiota have been reported with aging. The aim of this study is clarify the relationship between obesity and gut microbiota as a function of age. Furthermore, the impact of lifestyle factors (alcoholic drink and smoking) on gut microbiota were discussed. Fecal samples from 1082 healthy Japanese adults aged 19–90 years who participated in the Iwaki Health Promotion Project in 2014 were analyzed by using 16S rDNA gene-targeted sequencing. The participants were stratified into six age groups: ≤39, 40–49, 50–59, 60–69, 70–79 and ≥80. The participants with a body mass index (BMI) of <25 kg/m^2 were classified as non-obese, and those with a BMI of ≥25 kg/m^2 were classified as obese. Changes in the composition of gut microbiota with age were different between the obese and non-obese groups. Bacterial diversity decreased with age at the phylum level, however, this diversity was not observed at the class level. The abundances of certain gut microbiota, such as *Bacteroidetes* and *Firmicutes*, were different between the obese and non-obese groups. There were no significant differences in alcoholic and smoking habits. Bacterial diversity was different with age between the obese and non-obese group. The composition of *Bacteroidetes* and *Firmicutes* changed with increasing age in the obese group.

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Key words: aging; general population; gut microbiota; lifestyle; obesity.

Background

Obesity has become a serious public health issue worldwide. Approximately 266 million men and 375 million women in the world were obese in 2014[1)]. The prevalence of obesity in developing and developed countries will greatly increase in the future. This is an urgent and crucial concern because obesity is associated with many medical complications and increased incidence of disease. For example, previous studies have suggested that obesity is one of the risk factors for developing cancers of the esophagus, colon, kidney, breast, endometrium, and cervix[2)]. Various causative factors, including genetics, contribute to obesity[3)]; however, little is known about a solution for obesity, except for calorie control and exercise.

The human gut microbiota consists of trillions of bacteria that play important roles in the human body, such as affecting metabolism and producing a wide range of enzymes, hormones, and vitamins[4)]. In recent years, some studies have shown an association between gut microbiota and obesity[5-9)]. It was found that obese individuals and animals have a higher proportion of the phylum *Firmicutes* and a lower proportion of the phylum *Bacteroidetes* in their guts[10-12)]. However, other studies have contradicted these findings[13-15)]. Gut microbiome transplantation was

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investigated as a possible effective therapy for the treatment of obesity[16, 17]. Moreover, changes in the composition of gut microbiota have been reported with aging[18]. An initial change in gut microbiota composition occurs at birth by inheriting the mother's gut microbiota[19-21]. After that, although the composition of gut microbiota is considered to be mostly stable in adults, changes in diet might influence bacterial composition[21]. Obesity and aging have a potential influence on the composition of gut microbiota, and to our knowledge, there are few reports regarding this association among the general population.

To avoid an epidemic of severe obesity, various efforts have been made, such as implementation of anti-obesity policies[22]. The global obesity problem could be solved with a better understanding of how gut microbiota is associated with obesity; gut microbiota has the potential for increasing body weight reduction. Therefore, in this study, we aimed to clarify the relationship between obesity and gut microbiota as a function of age.

Methods

Participants

Participants of this study were 1082 healthy Japanese adults aged 19–90 years (410 men and 672 women) who participated in the Iwaki Health Promotion Project in 2014. The exclusion criteria for our study were (1) a medical history of colorectal cancer, (2) presence of diabetes mellitus, (3) and current use of antibiotics. According to the Japan Society for the Study of Obesity, participants with a body mass index (BMI) of <25 kg/m^2 were classified as non-obese, and those with a BMI of ≥25 kg/m^2 were classified as obese. Participants were stratified into six age groups: ≤39, 40–49, 50–59, 60–69, 70–79 and ≥80.

Lifestyle characteristics

Demographic data (age, sex), alcohol habits (none or previous, current), smoking habits (none or previous, current), and medical history were obtained from self-reported questionnaires and interviews. The height and weight of the participants were measured, and BMI was calculated.

Clinical characteristics

Blood samples were collected in the morning from the peripheral veins of the participants under fasting conditions. Serum level was determined using LSI Medience Corp. , Tokyo, Japan. The following clinical characteristics were measured: triglyceride, HDL-cholesterol, LDL-cholesterol, glucose, and HbA1c levels. HbA1c (%) is expressed as a National Glycohemoglobin Standardization Program value.

DNA samples

Fresh fecal samples were transferred into tubes by the participants and immediately enclosed in plastic bags. Each fecal sample was crushed using the FastPrep FP100A Instrument (MP Biomedicals, USA) with zirconia beads. Each DNA sample was adjusted to a concentration of 10 ng/μL using the ND-1000 (NanDrop Technologies, USA).

Sequencing and data processing

The V3–V4 region of the bacterial 16S rDNA gene was amplified with PCR using the Rotor-Gene Q quantitative thermal cycler (Qiagen, Germany) and primers Pro341F/Pro805R. The amplified DNA was quantified using the Illumina MiSeq sequencing system with Illumina MiSeq Reagent Kit, version 2 (Illumina, San Diego, CA, USA).

Taxonomic analysis

Sequences were analyzed using Metagenome@ KIN analysis software (World Fusion, Japan)

Table 1 Characteristics of study participants.

		BMI < 25 (kg/m^2) n = 847	BMI ≥ 25 (kg/m^2) n = 235	P value
Sex	Men	298 (35.2)	112 (47.7)	<0.001
	Women	549 (64.8)	123 (52.3)	
Age (years)	≤ 39	191 (22.6)	36 (15.3)	0.02
	40–49	137 (16.2)	41 (17.4)	
	50–59	165 (19.5)	37 (15.7)	
	60–69	227 (26.8)	71 (30.2)	
	70–79	106 (12.5)	38 (16.2)	
	≥ 80	21 (2.5)	12 (5.1)	
BMI (kg/m^2); Median (IQR)		21.7 (3.2)	26.7 (2.8)	
Drinking habit	Non-drinker	487 (57.5)	135 (57.4)	0.99
	Drinker	360 (42.5)	100 (42.6)	
Smoking habit	Non-smoker	709 (83.7)	193 (82.1)	0.57
	Smoker	138 (16.3)	42 (17.9)	

n (%)

Table 2 Biochemical characteristics of study participants.

	Non-obese n = 847	Obese n = 235	P value
Triglyceride (mg/dL)	72.0 (45.0)	102.0 (72.0)	<0.001
HDL-cholesterol (mg/dL)	66.0 (22.0)	55.0 (17.0)	<0.001
LDL-cholesterol (mg/dL)	113.0 (38.0)	122.0 (39.0)	0.004
Glucose (mg/dL)	78.0 (13.0)	83.0 (13.0)	<0.001
HbA1c (NGSP, %)	5.6 (0.4)	5.8 (0.5)	<0.001

Median (IQR)

based on data from bacterial genera with a 97% similarity cut-off from the TechnoSuruga Lab Microbial Identification database DB-BA 9.0 (TechnoSuruga Laboratory, Japan). The analysis was performed according to Takahashi, et al[36].

Statistical analysis

The abundance of gut microbiota was calculated as the ratio to total abundance. Differences between the non-obese and obese groups were determined using the Wilcoxon signed-rank test, Chi-square test and Kruskal–Wallis test using Stata 13 statistical software (StataCorp LLP, College Station, TX, USA). Intergroup differences were analyzed using the Steel test with JMP 13 statistical software (SAS Institute Inc. Cary, NC, USA).

Ethics approval and consent to participate

The methods and purposes of this study were thoroughly explained to the participants, and written informed consents were obtained from all of them prior to the investigation. The Iwaki Health Promotion Project and this study were approved by the Ethics Committee of Hirosaki University Graduate School of Medicine.

Results

Participant characteristics

Participant characteristics are shown in Tables 1 and 2. A total of 847 non-obese (BMI < 25 kg/m^2) and 235 obese (BMI ≥ 25 kg/m^2) participants were enrolled in this study. The rate of obesity was higher in men than in women. Drinking and smoking habits were not significantly different between the two groups.

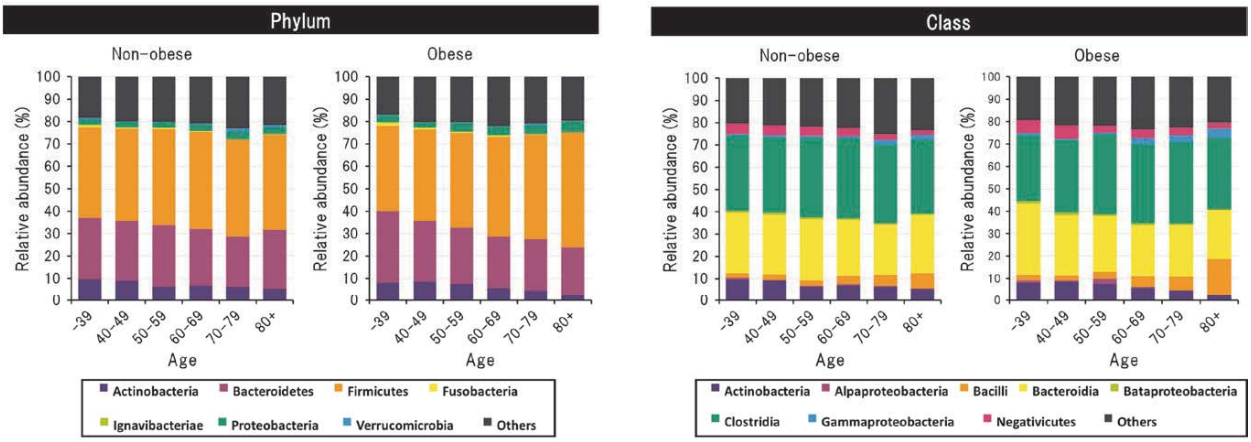

Figure 1 Age-related sequential difference in gut microbiota composition.

Clinical characteristics (Table 2) showed that triglyceride, LDL-cholesterol, glucose, and HbA1c levels were significantly higher and HDL-cholesterol level was significantly lower in the obese group than in the non-obese group.

Overview of gut microbiota composition in each age group

The phylum and class level composition of gut microbiota are shown in Figure 1. The relative abundance of *Actinobacteria* gradually decreased with increasing age among both the obese and non-obese groups. In the obese group, the relative abundance of *Bacteroidetes* decreased and that of *Firmicutes* gradually increased with age in the obese group.

At the class level, the abundance of *Actinobacteria* gradually decreased with aging in both the obese and non-obese groups, whereas the relative abundances of *Bacilli* gradually increased with age among the obese and non-obese groups.

Differences in bacterial diversity and composition for each taxonomic rank

The median diversity results (Shannon–Wiener index) are shown in Figure 2. At the phylum level, bacterial diversity tended to decrease with increasing age in the obese and non-obese groups. At the class level, no significant changes were observed in the obese group. For both class and order, bacterial diversity in the non-obese group was significantly higher in the ≤ 39 years age group than in the 40–49, 50–59, and 60–69 years age groups. At the family, genus, and species levels, bacterial diversity in the obese group was significantly higher in the ≥ 80 years age group than in the ≤ 39 years age group.

Differences in gut microbiota composition at the phylum level

Figure 3 shows the proportional abundances of gut microbiota stratified by age group at the phylum level (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Ignavibacteria*). The abundance of *Actinobacteria* and *Ignavibacteriae* significantly decreased with age in the obese and non-obese groups. The abundance of *Bacteroidetes* decreased, whereas that of *Firmicutes* significantly increased with age in the obese group; however, there were no differences in the proportional abundances in the non-obese group.

Differences in gut microbiota composition induced by drinking habit

Table 3 shows differences of the proportional abundances of gut microbiota (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Ignavibacteriae*) star-

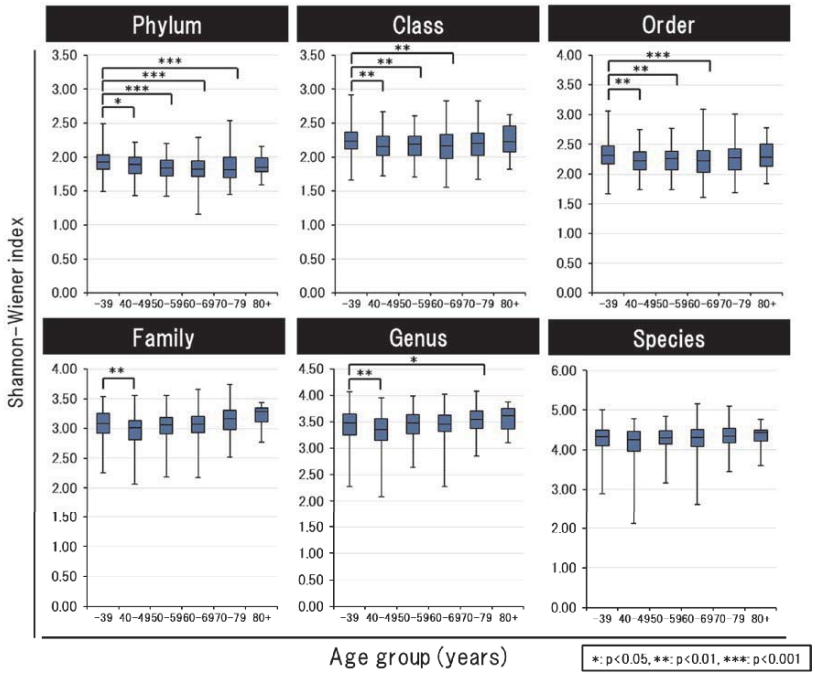

Figure 2a Comparison of bacterial diversity (non-obese).

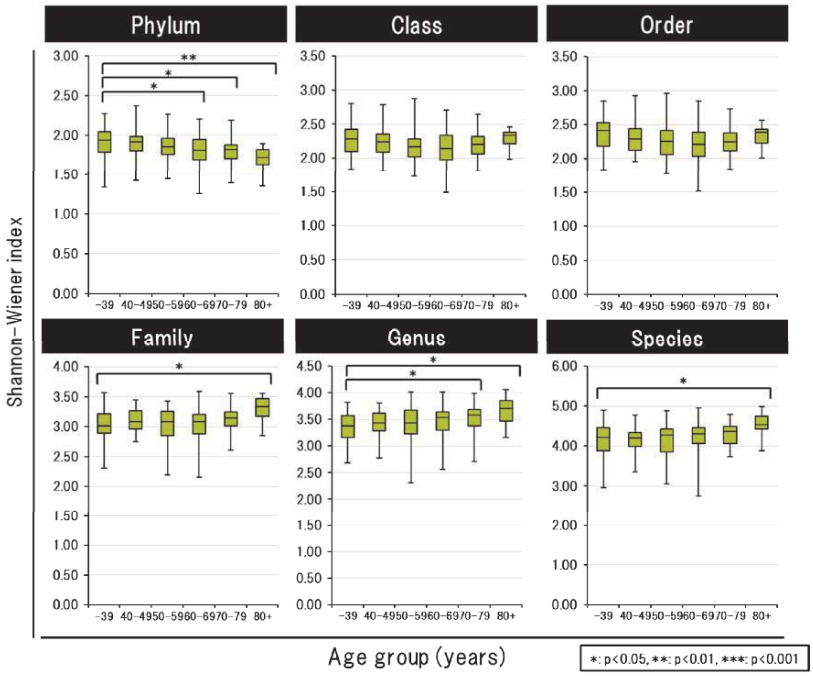

Figure 2b Comparison of bacterial diversity (obese).

tified by drinking habits. There were no significant differences between non-drinkers and drinkers among non-obese and obese groups.

Differences in gut microbiota composition induced by smoking habit

Table 4 shows differences of the proportional abundances of gut microbiota (*Actinobacteria*,

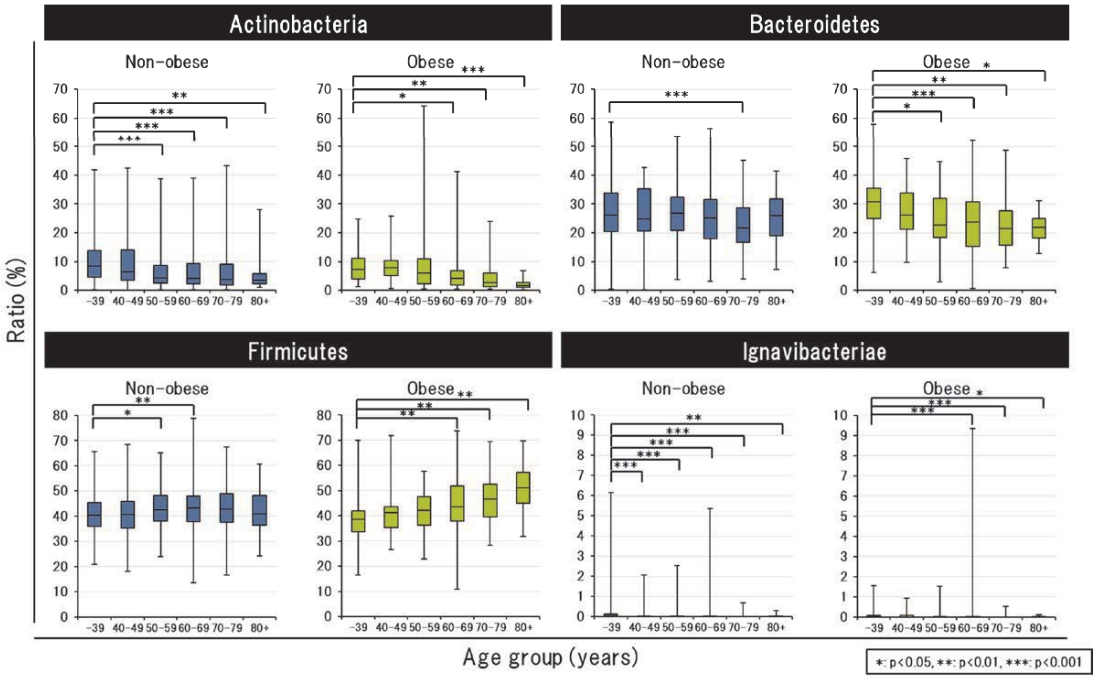

Figure 3 Changes in proportional gut microbiota abundance with age at the phylum level.

Bacteroidetes, Firmicutes, Ignavibacteriae) stratified by smoking habits. There were no significant differences between non-smokers and smokers among non-obese and obese groups.

Discussion

In the present study, the composition of gut microbiota significantly changed with age[18, 23]; several studies have previously demonstrated the relationship between gut microbiota and obesity[5-9, 24]. Our results showed that changes in gut microbiota composition with age were different between the obese and non-obese groups. Bacterial diversity decreased with age at the phylum level, however, this difference was not observed at the class level. The abundances of certain gut microbiota, such as *Bacteroidetes* and *Firmicutes*, were different between the obese and non-obese groups. Other gut microbiota, such as *Actinobacteria* and *Ignavibacteriae*, tended to decrease with increasing age in both groups. However, there were no significant differences in alcoholic and smoking habits.

Gut microbiota composition at the phylum level showed apparent changes in the abundances of *Bacteroidetes* and *Firmicutes* with increasing age in the obese group. However, the balance between *Bacteroidetes* and *Firmicutes* in the non-obese group was stable across all age groups. Our results suggest the possibility of an effective intervention regarding gut microbiota for older obese individuals who need to reduce body weight. Several methods have been reported to increase *Bacteroidetes* and decrease *Firmicutes* in the gut. For example, a habitual diet rich in fiber is associated with a higher proportion of *Prevotella* spp. , whereas a diet rich in animal fat and protein is related to a higher proportion of *Bacteroides* spp[25]. Many older individuals have greater difficulty with physical exercise because of increased rates of physical disabilities[26, 27]; therefore, a diet favorable for increasing *Bacteroidetes* and decreasing *Firmicutes* would benefit the elderly by helping to decrease and maintain a desirable body weight.

Table 3 Differences in gut microbiota composition induced by drinking habit.

	Age (years)	Non-obese		P value	Obese		P value
		Non-drinker (n=487)	Drinker (n=360)		Non-drinker (n=135)	Drinker (n=100)	
Actinobacteria (%)	≤ 39	9.0 (8.2)	8.5 (9.9)	0.72	8.1 (6.7)	5.5 (7.2)	0.26
	40-49	7.3 (10.5)	6.0 (7.2)	0.13	7.8 (4.0)	8.3 (7.2)	0.96
	50-59	3.6 (6.3)	4.7 (5.6)	0.77	8.3 (8.4)	4.7 (7.9)	0.19
	60-69	4.2 (7.7)	4.0 (6.4)	0.55	3.8 (5.9)	4.2 (4.6)	0.84
	70-79	4.1 (9.0)	3.1 (3.5)	0.37	2.9 (5.6)	1.9 (2.8)	0.36
	≥ 80	3.2 (2.6)	11.7 (11.5)	0.01	1.9 (1.5)	1.2 (1.0)	0.41
Bacteroidetes (%)	≤ 39	26.7 (10.7)	25.5 (14.8)	0.44	30.6 (10.5)	31.5 (9.7)	0.70
	40-49	23.3 (13.9)	28.1 (14.8)	0.22	26.1 (10.8)	26.7 (13.3)	0.22
	50-59	25.4 (11.6)	29.1 (10.6)	0.01	21.7 (8.0)	24.9 (19.1)	0.27
	60-69	24.6 (13.6)	26.1 (14.4)	0.35	17.8 (15.1)	24.6 (12.5)	0.16
	70-79	21.4 (10.2)	24.2 (12.4)	0.32	20.0 (9.6)	26.7 (13.1)	0.12
	≥ 80	28.6 (11.2)	19.5 (4.0)	0.16	21.7 (4.8)	21.9 (7.5)	0.78
Firmicutes (%)	≤ 39	40.5 (6.8)	40.1 (10.9)	0.97	37.5 (8.0)	39.7 (10.5)	0.13
	40-49	41.0 (9.8)	40.5 (10.7)	0.93	41.9 (8.2)	38.5 (9.1)	0.05
	50-59	42.6 (10.4)	42.5 (10.5)	0.38	44.4 (9.5)	39.8 (13.5)	0.05
	60-69	43.3 (10.1)	43.2 (9.8)	0.98	45.7 (14.3)	42.8 (10.1)	0.79
	70-79	43.0 (11.4)	41.6 (10.7)	0.54	49.2 (12.5)	41.9 (10.7)	0.35
	≥ 80	40.8 (12.8)	42.9 (8.8)	0.68	51.3 (11.8)	51.1 (10.6)	0.93
Ignavibacteriae (%)	≤ 39	0.0 (0.1)	0.0 (0.2)	0.38	0.0 (0.0)	0.0 (0.1)	0.52
	40-49	0.0 (0.0)	0.0 (0.0)	0.96	0.0 (0.0)	0.0 (0.1)	0.31
	50-59	0.0 (0.0)	0.0 (0.0)	0.73	0.0 (0.0)	0.0 (0.0)	0.18
	60-69	0.0 (0.0)	0.0 (0.0)	0.70	0.0 (0.0)	0.0 (0.0)	0.54
	70-79	0.0 (0.0)	0.0 (0.0)	0.50	0.0 (0.0)	0.0 (0.0)	0.85
	≥ 80	0.0 (0.0)	0.0 (0.1)	0.96	0.0 (0.0)	0.0 (0.0)	0.90

Median (IQR)

In addition, our results indicate that gut microbiota is associated with serum glucose levels. The obese group in our study had higher blood glucose levels than the non-obese group, and serum glucose levels increased with age in both groups (Table 5). Previous studies have reported that higher blood glucose levels are associated with a reduced relative proportion of *Bacteroides* spp. among the elderly[28]. Although the impact of serum glucose levels is unclear in our results, it is a possible confounding factor for the association between obesity and gut microbiota.

The relationship between *Bacteroidetes* and *Firmicutes* is controversial. Recently, several studies have observed obese individuals with higher abundances of *Firmicutes* and lower abundances of *Bacteroidetes* than non-obese individuals[10-12]. On the other hand, there are several studies that have reported higher abundances of *Bacteroidetes* in obese individuals than in non-obese individuals[13-15]. Gut microbiota diversity is caused by various factors, such as the use of antibiotics, aging, dietary habits, lifestyle, and medical history[29, 30], thus making understanding of the dynamics of these organisms perplexing. Our cross-sectional study design did not allow us to determine why differences occurred in bacterial diversity with increasing age between the obese and non-obese groups. In future studies, we plan to investigate annual changes in gut microbiota in the same participants.

Lifestyle factors on gut microbiota have been investigated. Recent investigations have demonstrated that smoking influenced on gut microbiota composition of *Bacteroidetes-Prevotella* in individuals with Crohn's Disease and healthy individuals[31]. Moreover, there are several studies

Table 4 Differences in gut microbiota composition induced by smoking habit.

	Age (years)	Non-obese		P value	Obese		P value
		Non-smoker (n=709)	Smoker (n=138)		Non-smoker (n=193)	Smoker (n=42)	
Actinobacteria (%)	≤ 39	8.7 (9.2)	8.5 (9.2)	0.92	7.1 (6.4)	11.0 (7.3)	0.17
	40-49	5.6 (10.5)	8.4 (9.3)	0.52	6.7 (4.4)	8.6 (7.4)	0.13
	50-59	4.4 (5.9)	4.1 (6.8)	0.65	5.2 (7.7)	8.9 (9.4)	0.43
	60-69	4.1 (6.8)	3.5 (7.6)	0.82	4.1 (5.0)	5.2 (3.1)	0.40
	70-79	3.7 (7.3)	3.0 (3.6)	0.63	2.8 (5.2)	-	-
	≥ 80	3.5 (3.5)	-	-	1.6 (1.5)	-	-
Bacteroidetes (%)	≤ 39	26.8 (11.8)	23.1 (17.4)	0.29	30.6 (10.1)	31.5 (11.7)	0.67
	40-49	23.9 (13.5)	29.9 (15.1)	0.10	29.9 (12.9)	25.9 (11.5)	0.04
	50-59	27.0 (11.5)	26.4 (11.2)	0.57	20.5 (14.6)	24.9 (12.4)	0.14
	60-69	25.1 (13.4)	26.6 (15.6)	0.39	23.5 (15.2)	28.4 (8.8)	0.24
	70-79	21.3 (11.0)	31.3 (4.1)	0.01	21.2 (11.7)	-	-
	≥ 80	26.6 (13.8)	-	-	21.8 (6.8)	-	-
Firmicutes (%)	≤ 39	40.6 (9.2)	38.8 (11.0)	0.50	38.8 (8.2)	36.0 (7.6)	0.67
	40-49	41.1 (11.9)	39.7 (5.9)	0.41	40.3 (6.3)	42.6 (10.4)	0.19
	50-59	43.1 (10.1)	41.1 (7.8)	0.16	43.5 (11.1)	40.5 (8.8)	0.69
	60-69	43.2 (10.1)	43.5 (10.1)	0.81	43.5 (14.7)	43.7 (6.4)	0.53
	70-79	42.5 (12.0)	43.0 (2.3)	0.93	47.7 (13.3)	-	-
	≥ 80	40.8 (12.8)	-	-	51.2 (12.3)	-	-
Ignavibacteriae (%)	≤ 39	0.0 (0.1)	0.1 (0.2)	0.09	0.0 (0.0)	0.0 (0.2)	0.58
	40-49	0.0 (0.0)	0.0 (0.0)	0.75	0.0 (0.1)	0.0 (0.0)	0.53
	50-59	0.0 (0.0)	0.0 (0.0)	0.80	0.0 (0.0)	0.0 (0.0)	0.47
	60-69	0.0 (0.0)	0.0 (0.0)	0.80	0.0 (0.0)	0.0 (0.0)	0.16
	70-79	0.0 (0.0)	0.0 (0.0)	0.20	0.0 (0.0)	-	-
	≥ 80	0.0 (0.0)	-	-	0.0 (0.0)	-	-

Median (IQR)

Table 5 Age-specific biochemical characteristics of study participants.

		Age group (years)						P value
		≤ 39	40–49	50–59	60–69	70–79	≥ 80	
Non-obese	BMI (kg/m^2)	20.5 (3.5)	21.0 (3.7)	21.6 (3.0)	22.0 (2.3)	22.1 (2.6)	21.3 (3.2)	<0.001
	Triglyceride (mg/dL)	62.0 (47.5)	73.0 (44.0)	76.0 (49.0)	74.0 (40.0)	79.5 (49.3)	74.0 (31.0)	0.001
	HDL-cholesterol (mg/dL)	65.0 (21.0)	68.0 (24.0)	69.0 (22.0)	66.0 (20.0)	63.0 (17.0)	61.0 (19)	0.07
	LDL-cholesterol (mg/dL)	95.0 (37.0)	107.0 (34.0)	127.0 (37.0)	120.0 (35.5)	116.5 (26.0)	98.0 (47.0)	<0.001
	Glucose (mg/dL)	72.0 (9.0)	75.0 (9.0)	80.0 (12.0)	82.0 (15.0)	84.0 (13.8)	88.0 (11.0)	<0.001
	HbA1c (NGSP, %)	5.4 (0.3)	5.6 (0.3)	5.7 (0.4)	5.7 (0.3)	5.8 (0.4)	5.8 (0.5)	<0.001
Obese	BMI (kg/m^2)	27.3 (5.7)	27.4 (3.2)	26.2 (1.3)	26.6 (2.5)	27.1 (2.3)	25.8 (2.8)	0.26
	Triglyceride (mg/dL)	126.5 (103.8)	98.0 (103.0)	107.0 (71.0)	95.0 (57.5)	89.5 (47.5)	114.5 (75.0)	0.38
	HDL-cholesterol (mg/dL)	50.0 (17.5)	54.0 (12.0)	54.0 (15.0)	56.0 (16.5)	57.0 (21.0)	51.0 (19.3)	0.38
	LDL-cholesterol (mg/dL)	117.0 (34.3)	124.0 (35.0)	127.0 (37.0)	120.0 (37.0)	118.0 (38.0)	119.0 (24.8)	0.38
	Glucose (mg/dL)	76.0 (12.0)	78.0 (9.0)	82.0 (10.0)	87.0 (15.5)	86.5 (19.8)	92.0 (13.0)	<0.001
	HbA1c (NGSP, %)	5.6 (0.3)	5.7 (0.3)	5.7 (0.4)	5.9 (0.6)	6.0 (0.6)	6.2 (0.6)	<0.001

Median (IQR)

which suggested that alcohol intake influenced on gut microbiota[32, 33]. Smoking and alcoholic drinks are risk factors of colorectal cancer[34], and some previous studies pointed out that gut microbiota might associate with the development of colorectal cancer[35]. To determine the impact of gut microbiota on colorectal diseases, investigations estimating influences of lifestyle, such as alcohol intake and smoking, on incidence of colorectal diseases are needed.

There is another limitation to this study. Bacterial genes in fecal matter include genes

from both gut microflora and bacteria ingested by the mouth with foods. At the class level, the composition and abundance of *Bacilli* were high in the ≥ 80 years age group because the elderly individuals in this study had diets rich in pickled vegetables. However, the abundances of *Bacteroidia* and *Clostridia* are not greatly affected by external bacteria from various foods.

Conclusions

In conclusion, our study showed that bacterial diversity was different with age between the obese and non-obese group. The composition of *Bacteroidetes* and *Firmicutes* changed with increasing age in the obese group. Our findings could be helpful for developing a weight intervention for obese individuals by increasing the composition of certain gut bacteria.

Competing interests

The authors declare no conflicts of interest associated with this manuscript.

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