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The Relationship between Habitual Dietary Intake and Gut Microbiota in Young Japanese Women

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Summary Recent studies have shown that dietary content affects the health of the host by changing the gut microbiota. However, little is known about the association of microbiota composition with habitual diet in Japanese people. Here, we aimed to clarify the relationship between the fecal microbiota and habitual dietary intake of micronutrients, macronutrients and food groups in healthy young Japanese women. Analysis of fecal microbiota was performed by the terminal restriction fragment length polymorphism (T-RFLP) method, and a dietary survey was conducted over three consecutive days using a weighed food record method. T-RFLP pattern analysis divided the subjects into two clusters, where cluster A group had a high relative abundance of *Bacteroides* and *Clostridium* cluster IV, and cluster B group had a high relative abundance of *Bifidobacterium* and *Lactobacillales*. Cluster A group also had lower intakes of iron and vitamin K and higher intakes of mushrooms and snacks than cluster B group. Analysis of Spearman rank correlations found several significant relationships between fecal microbiota and intake of nutrients and food groups. *Bifidobacterium* was correlated with iron intake, and *Clostridium* cluster XI was negatively correlated with intakes of cholesterol and eggs. These results suggest that dietary habits may strongly affect *Bifidobacterium*, *Bacteroides* and *Clostridium* abundance in the gut microbiota of young Japanese women. This is the first study to show relationships between fecal microbiota and habitual dietary intake in Japanese people. Accumulation of results from similar studies will help to elucidate the relationships between dietary intake and diseases in Japanese people.

Key Words habitual dietary intake, gut microbiota, young Japanese women, cross-sectional study

A great variety of bacteria live in the human intestinal tract, populating a complicated microbial ecosystem (gut microbiota). Total bacterial cell numbers in 1 g of human feces is estimated at ~10^{11}–10^{12}, and the dominant bacterial phyla of the human gut microbiota are the *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* with a lower relative abundance of *Verrucomicrobia* and *Proteobacteria* (*1*, *2*). The composition of gut microbiota has been shown to vary with clinical conditions such as type 2 diabetes, inflammatory bowel disease, nonalcoholic fatty disease, and allergy (*3–6*).

Previous research suggests that gut microbiota composition may also be affected by dietary intake. For example, subjects who consumed a high-fat diet showed a significantly reduced abundance of fecal *Bifidobacteria* than those who consumed a low-fat diet (*7*). Some studies have shown that obese people have an increased abundance of *Firmicutes* in the gut microbiota compared with lean people (*8*, *9*). The mouse gut microbiota is also responsive to increased energy intake, with an increased abundance of *Firmicutes* and reduced abundance of *Bacteroidetes* (*10–12*). In addition, a number of research groups have reported that the supplementation with prebiotics and/or probiotics tends to increase the abundance of *Bifidobacterium* in humans and animals (*13–16*).

Cross-sectional studies have shown a correlation between dietary patterns and gut microbiota composition in humans. De Filippo et al. detected a major difference in the fecal microbiota between 15 healthy European children who consumed a high animal fat and high-protein diet (microbiota dominated by *Firmicutes*), and 14 healthy African children who consumed a high-fiber diet (microbiota dominated by *Bacteroidetes*) (*17*). Lin et al. reported that the gut microbiota of Bangladeshi children had higher bacterial diversity and an increased abundance of *Prevotella* relative to that of American children (*18*). Moreover, Zimmer et al. compared fecal samples between vegetarians, vegans, and control subjects, and found that the abundance of *Bacteroides* and *Bifidobacterium* was significantly lower in vegetarians and vegans than in control subjects (*19*).

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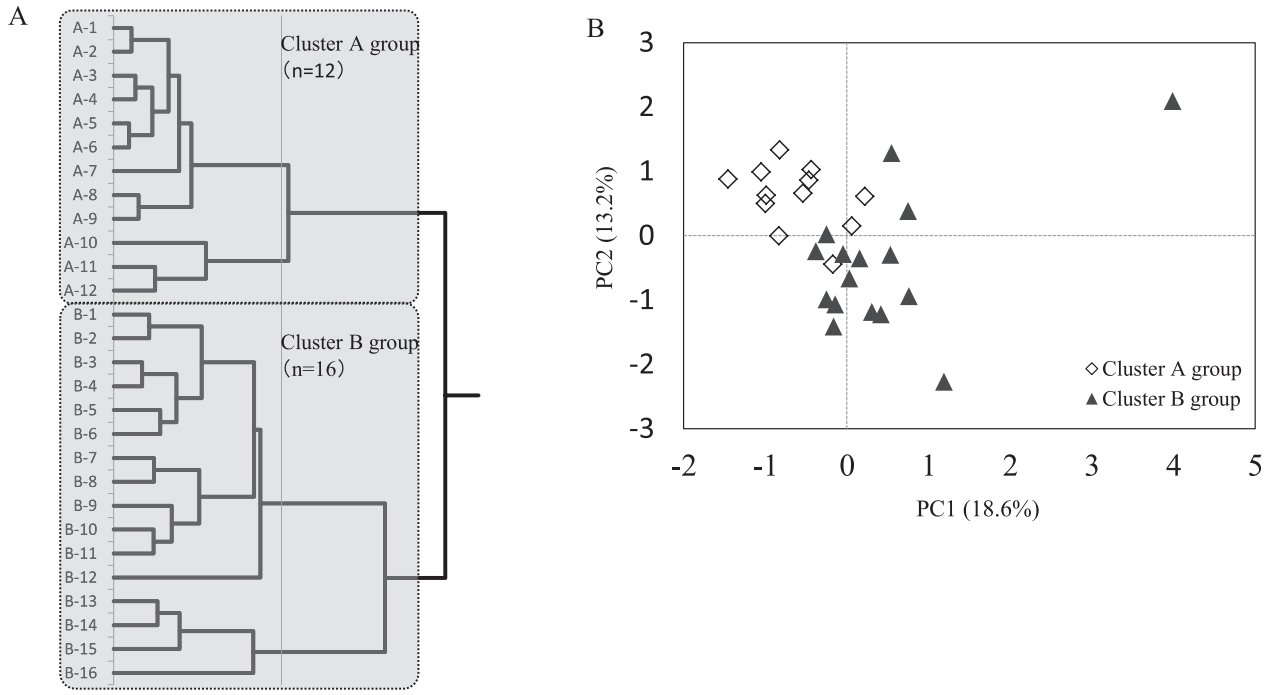

Fig. 1. Analysis of fecal bacterial communities from all subjects. Terminal restriction fragment length polymorphism (T-RFLP) profiles of fecal microbiota from 28 samples were analyzed by cluster analysis using Ward's method and used to construct a dendrogram (A). Plot of principal component analysis (PCA) scores from T-RFLP data (B).

Overall, these studies indicate that the Western diet of high fat, high protein, and low fiber is associated with an increase in the *Firmicutes*/*Bacteroidetes* ratio. However, associations of nutritional factors such as micronutrients, macronutrients, and food groups with gut microbiota composition are still poorly understood (*20*). Moreover, to the best of our knowledge, no research has yet been conducted on the relationship between dietary intake and gut microbiota in young Japanese women. These women have low protein and dietary fiber intake and show diversity in their dietary intake with four main patterns previously identified (*21*). Recent reports have shown a correlation between dietary patterns and constipation (*22*), so the gut microbiota of young Japanese women could be associated with dietary intake. The aim of this study was to examine the relationship between habitual diet and fecal microbiota in healthy young Japanese women, using the terminal restriction fragment length polymorphism (T-RFLP) method for microbiota analysis.

MATERIALS AND METHODS

Study subjects. This cross-sectional study was conducted at a women's college in Kanagawa, Japan, between August 2014 and July 2015. Twenty-eight healthy, normal-weight young Japanese women aged 20–22 y participated in the study. The study was performed in compliance with the principles of the Declaration of Helsinki and subjects gave their written informed consent to participate. All aspects of the study were approved by the Ethics Committee of the Sagami Women's University (No. 1423).

Dietary assessment. The dietary survey covered three consecutive days' intake using a weighed food record method. The subjects measured all of the food they ate and drank (excluding dietary supplements) over this period with a scale and recorded it on record paper. Food records were checked by trained dietitians in consultation with the subjects after the 3-d period. The intakes of energy, nutrients (macronutrients and micronutrients), and food groups were analyzed using Excel Eiyo-kun software (Version 7.0, Kenpaku Co., Ltd., Tokyo, Japan), which is based on the Standard Tables of Food Composition in Japan (*23*). The intake of nutrients and food groups was calculated per 1,000 kcal of energy intake.

Fecal microbiota analysis (T-RFLP method). Fecal samples were collected once in fecal collection tubes containing a guanidine thiocyanate solution (Techno Suruga Laboratory Co., Ltd., Japan) during the dietary survey period. Analysis of fecal microbiota by the T-RFLP method was outsourced to Techno Suruga Laboratory Co., Ltd. and performed according to a protocol described by previous reports (*24*). The abundance of each terminal restriction fragment (T-RF) was calculated by first dividing it into 29 operational taxonomic units (OTUs) by the method described in Nagashima's reports (*25*, *26*). Each OTU was quantified as a percent of the total OTU area, expressed as a percent of the area under the curve (%AUC).

Statistical analysis. Statistical analysis was done by SPSS Statistical for Windows software version 22.0 (IBM, Tokyo, Japan). All data are expressed as mean±standard deviation (SD). A cluster analysis classified the T-RFLP profiles of fecal microbiota from 28 samples into simi-

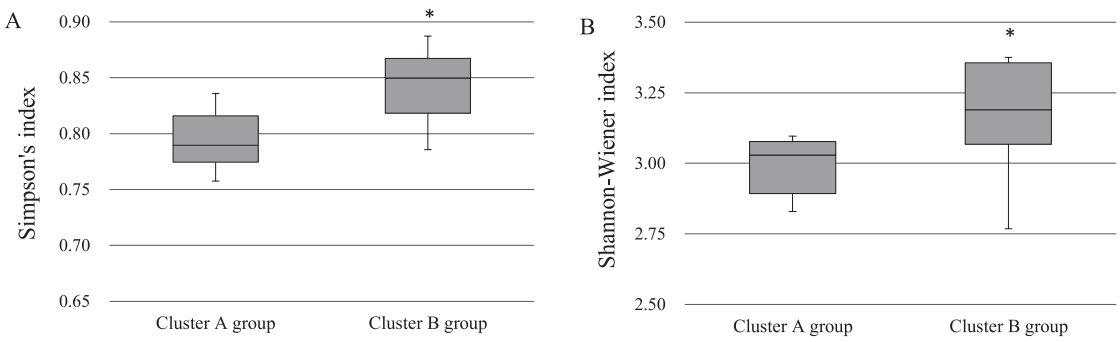

Fig. 2. Box plot comparing diversity of terminal restriction fragment length polymorphism (T-RFLP) profiles in fecal microbiota between cluster A and cluster B groups. Calculations of the Simpson (A) and Shannon-Wiener (B) diversity indexes for the cluster A and cluster B groups. * $p<0.05$.

Table 1. Comparison of fecal microbiota by terminal restriction fragment length polymorphism (T-RFLP) between the cluster A and cluster B groups.

Bacteria (%)	All subjects (n=28)	Cluster A group (n=12)	Cluster B group (n=16)
Bifidobacterium	12.0±8.1	4.8±3.5	17.5±5.9**
Lactobacillales	4.2±2.8	2.9±1.8	5.2±3.0*
Bacteroides	37.2±10.8	45.3±4.8	31.1±10.1**
Clostridium cluster IV	9.9±5.9	12.4±4.7	7.9±6.2*
Clostridium cluster XI	1.1±2.1	0.4±0.6	1.6±2.6
Clostridium subcluster XIVa	24.6±7.6	23.8±7.9	25.7±7.7
Clostridium cluster XVIII	1.8±1.6	2.5±1.8	1.2±1.2*
Others	9.3±4.7	8.0±2.0	10.3±5.8
Prevotella	0.0±0.0	0.0±0.0	0.0±0.0

Values are means±standard deviation.
* $p<0.05$, ** $p<0.01$ versus cluster A group determined by unpaired t-test or Mann-Whitney test.

lar groups using Ward's method and constructed a dendrogram. A principal component analysis was also performed based on the T-RFLP profiles of fecal samples. Microbial diversity was calculated using the Simpson and Shannon-Wiener indexes of diversity to assess microbial community members' evenness and richness (*27*). The distributions of the variables were investigated with the Shapiro Wilk test. Parametric data were analyzed using the unpaired t-test and nonparametric data were analyzed using the Mann-Whitney U-test. The Spearman rank correlation test was used to test the associations between fecal microbiota and nutritional intakes or food group intakes. Differences were considered significant at $p<0.05$.

RESULTS

Cluster analysis of the T-RFLP profiles of fecal samples divided the subjects into two groups (cluster A and cluster B groups) (Fig. 1A). Twelve of the 28 subjects were included in the cluster A group and the remaining 16 subjects were included in the cluster B group. In addition, principal component analysis results showed that the microbial communities were different between the cluster A and cluster B groups (Fig. 1B). The mean diversity of the cluster A and cluster B groups is shown in Fig. 2. The Simpson and Shannon-Wiener indexes were significantly higher in the cluster B group than the cluster A group ($p<0.05$).

We examined the differences in fecal microbiota composition between the cluster A and cluster B groups. The results of a comparison of fecal microbiota between the cluster A and cluster B groups are shown in Table 1. The relative abundance of *Bifidobacterium* and *Lactobacillales* was significantly lower in the cluster A group than the cluster B group ($p<0.01$ and $p<0.05$, respectively). The relative abundance of *Bacteroides*, *Clostridium* cluster IV, and *Clostridium* cluster XVIII was significantly higher in the cluster A group than the cluster B group ($p<0.01$, $p<0.05$, and $p<0.05$, respectively).

We conducted a dietary survey to record the energy, nutrient, and food group intakes of the cluster A and cluster B groups. The results of a comparison of nutritional intakes between the cluster A and cluster B groups are shown in Table 2. Intakes of iron and vitamin K were significantly higher in the cluster B group than the cluster A group ($p<0.05$ and $p<0.01$, respectively). The results of a comparison of food group intakes between the cluster A and cluster B groups are shown in Table 3. Intakes of mushrooms and snacks were significantly higher in the cluster A group than the cluster B group

Table 2. Comparison of nutritional intakes between the cluster A and cluster B groups.

	Cluster A group	Cluster B group
Energy (kcal)	1,746±322	1,599±341
Protein (g/1,000 kcal)	38±5	37±3
Fat (g/1,000 kcal)	32±6	32±7
Cholesterol (mg/1,000 kcal)	197±58	185±66
Carbohydrate (g/1,000 kcal)	137±12	138±15
Total dietary fiber (g/1,000 kcal)	6.9±2.0	7.4±2.0
Water-soluble fiber (g/1,000 kcal)	1.6±0.5	1.6±0.4
Water-insoluble fiber (g/1,000 kcal)	5.0±1.5	5.1±1.4
Salt (g/1,000 kcal)	4.3±1.0	5.1±1.5
Potassium (mg/1,000 kcal)	1,323±282	1,356±243
Calcium (mg/1,000 kcal)	279±82	294±72
Magnesium (mg/1,000 kcal)	123±18	146±42
Phosphorus (mg/1,000 kcal)	575±99	561±60
Iron (mg/1,000 kcal)	3.5±0.5	4.7±1.9*
Zinc (mg/1,000 kcal)	4.3±0.6	4.4±0.8
Copper (mg/1,000 kcal)	0.6±0.1	0.6±0.2
Manganese (mg/1,000 kcal)	1.5±0.5	2.1±1.5
Retinol equivalents (μg/1,000 kcal)	363±309	254±82
Vitamin D (μg/1,000 kcal)	3.4±2.4	3.7±2.7
α-Tocopherol (mg/1,000 kcal)	3.2±1.1	3.8±2.2
Vitamin K (μg/1,000 kcal)	76±29	128±32**
Vitamin B_1 (mg/1,000 kcal)	0.5±0.1	0.5±0.1
Vitamin B_2 (mg/1,000 kcal)	0.6±0.1	0.7±0.1
Niacin (mg/1,000 kcal)	17±4	16±2
Vitamin B_6 (mg/1,000 kcal)	0.6±0.2	0.6±0.2
Vitamin B_{12} (μg/1,000 kcal)	3.3±1.9	3.2±3.1
Folic acid (μg/1,000 kcal)	159±50	192±54
Pantothenic acid (mg/1,000 kcal)	3.4±0.6	3.3±0.5
Biotin (μg/1,000 kcal)	18±7	19±5
Vitamin C (mg/1,000 kcal)	53±18	58±27

Values are means±standard deviation
* $p<0.05$, ** $p<0.01$ versus cluster A group determined by unpaired *t*-test or Mann-Whitney test.

Table 3. Comparison of food group intakes between the cluster A and cluster B groups.

	Cluster A group	Cluster B group
Cereals (rice, noodles, etc) (g/1,000 kcal)	215±50	226±59
Potatoes (g/1,000 kcal)	21±19	16±12
Green-yellow vegetables (g/1,000 kcal)	61±43	46±36
Other vegetables (g/1,000 kcal)	74±31	88±40
Mushrooms (g/1,000 kcal)	13±14	5±6*
Seaweed (g/1,000 kcal)±	±15	3±6
Beans (g/1,000 kcal)	14±12	27±38
Fish (g/1,000 kcal)	32±19	26±16
Meat (g/1,000 kcal)	49±21	44±25
Eggs (g/1,000 kcal)	21±17	23±18
Milk/dairy products (g/1,000 kcal)	94±61	69±45
Fruits (g/1,000 kcal)	45±33	31±37
Sugar/sweets (g/1,000 kcal)	3±3	5±4
Snacks (g/1,000 kcal)	29±24	12±14*
Beverages (g/1,000 kcal)	270±216	255±207
Nuts (g/1,000 kcal)	1±4	2±3
Oil (g/1,000 kcal)	5±2±	±4
Seasonings/spices (g/1,000 kcal)	25±13	27±11

Values are means±standard deviation.
* $p<0.05$ versus cluster A group determined by unpaired *t*-test or Mann-Whitney test.

($p<0.05$).

We examined the relationship between fecal microbiota and nutritional and food group intakes. Tables 4 and 5 show the Spearman's rank correlation coefficients between fecal microbiota and nutrition and food group intakes, respectively. The relative abundance of *Bifidobacterium* was significantly correlated with the intakes of magnesium, iron and vitamin K ($R=0.45$, $p<0.05$; $R=0.53$, $p<0.01$; and $R=0.69$, $p<0.01$, respectively). The relative abundance of *Bacteroides* was significantly negatively correlated with the intakes of vitamin K and folic acid ($R=-0.46$, $p<0.01$ and $R=-0.42$, $p<0.05$, respectively). The relative abundance of *Clostridium* cluster XI was significantly negatively correlated with the intakes of cholesterol, vitamin B_2, and biotin ($R=-0.60$, $p<0.01$; $R=-0.38$, $p<0.05$; and $R=-0.59$, $p<0.01$, respectively). The relative abundance of *Clostridium* cluster IV was significantly correlated with the intake of mushrooms ($R=0.38$, $p<0.05$). The relative abundance of *Lactobacillales* was significantly negatively correlated with the intake of green-yellow vegetables ($R=-0.38$, $p<0.05$), and *Clostridium* cluster XI was significantly negatively correlated with the intake of eggs ($R=-0.67$, $p<0.01$).

DISCUSSION

Interestingly, the relative abundance of *Clostridium* cluster XI was significantly negatively correlated with the intakes of cholesterol and eggs. Cavallini et al. also reported that *Clostridium* spp. was negatively correlated with total cholesterol in serum (*28*). *Clostridium* species increase the expression of regulatory T cells, which regulate the immune response in the large intestine, and may thus help to maintain the gut barrier function (*29*). Moreover, short chain fatty acids such as acetate and butyrate produced from *Clostridium* cluster IV were reportedly increased in non-obese people compared with obese people (*30*). Short chain fatty acids are a major energy source in colonic epithelial cells and enhance intestinal peristalsis. Therefore, habitual low cholesterol diets might lead to increased short chain fatty acid-producing bacteria, and thereby suppress the onset of constipation, diarrhea, and colitis.

Furthermore, we observed that the relative abundance of *Clostridium* cluster IV was significantly correlated with mushroom intake. Mushrooms are known to contain many prebiotics such as fructooligosaccharides, β-glucan, and inulin (*31*). These prebiotics act as

Table 4. Associations between fecal microbiota and nutritional intakes.

	Bifidobacterium	*Lactobacillales*	*Bacteroides*	*Clostridium* cluster IV	*Clostridium* cluster XI	*Clostridium* subcluster XIVa	*Clostridium* cluster XVIII	Others
	CC	CC	CC	CC	CC	CC	CC	CC
Energy (kcal)	−0.29	−0.24	0.28	−0.02	0.18	0.05	−0.13	0.04
Protein (g/1,000 kcal)	0.01	0.05	0.04	0.20	−0.30	−0.10	0.20	−0.10
Fat (g/1,000 kcal)	−0.08	−0.26	0.06	0.04	0.18	0.16	−0.29	0.12
Cholesterol (mg/1,000 kcal)	0.11	−0.14	0.05	−0.02	−0.60**	−0.20	0.05	−0.13
Carbohydrate (g/1,000 kcal)	0.09	0.14	−0.04	−0.05	−0.17	−0.15	0.25	−0.19
Total dietary fiber (g/1,000 kcal)	0.13	−0.06	−0.18	0.27	0.09	0.17	−0.15	−0.15
Water-soluble fiber (g/1,000 kcal)	0.05	0.08	−0.24	0.32	0.12	0.08	−0.17	−0.06
Water-insoluble fiber (g/1,000 kcal)	0.06	−0.11	−0.12	0.22	−0.01	0.19	−0.12	−0.02
Salt (g/1,000 kcal)	0.28	0.23	−0.13	−0.16	0.08	−0.15	−0.10	0.21
Potassium (mg/1,000 kcal)	0.20	0.06	−0.11	0.05	−0.11	−0.06	0.10	−0.03
Calcium (mg/1,000 kcal)	0.25	0.10	−0.30	0.17	0.05	0.14	0.00	−0.16
Magnesium (mg/1,000 kcal)	0.45*	0.01	−0.24	−0.03	0.09	0.07	−0.11	−0.27
Phosphorus (mg/1,000 kcal)	0.12	−0.02	−0.08	0.25	−0.14	−0.02	0.11	−0.19
Iron (mg/1,000 kcal)	0.53**	−0.01	−0.37	−0.10	−0.25	0.05	−0.21	−0.26
Zinc (mg/1,000 kcal)	0.18	−0.31	0.18	−0.10	−0.16	−0.02	0.11	−0.40*
Copper (mg/1,000 kcal)	0.29	−0.11	−0.15	−0.20	−0.05	0.14	0.04	−0.30
Manganese (mg/1,000 kcal)	0.18	0.06	−0.25	−0.09	−0.17	−0.02	0.22	−0.15
Iodine (μg/1,000 kcal)	0.12	−0.05	0.23	0.00	0.21	−0.22	0.03	−0.10
Retinol equivalents (μg/1,000 kcal)	−0.09	−0.22	−0.08	0.29	−0.23	0.32	0.05	−0.15
Vitamin D (μg/1,000 kcal)	0.14	−0.14	−0.25	0.13	−0.26	0.26	−0.09	−0.25
α-Tocopherol (mg/1,000 kcal)	0.07	−0.28	−0.07	0.18	−0.03	0.32	−0.15	−0.05
Vitamin K (μg/1,000 kcal)	0.69**	0.22	−0.46**	−0.30	−0.14	−0.09	−0.28	0.21
Vitamin B_1 (mg/1,000 kcal)	−0.23	−0.16	0.01	0.37	−0.01	0.16	−0.07	−0.07
Vitamin B_2 (mg/1,000 kcal)	0.22	0.17	−0.30	−0.04	−0.38*	−0.08	0.26	−0.01
Niacin (mg/1,000 kcal)	−0.10	−0.09	0.04	0.24	−0.23	−0.03	0.12	0.01
Vitamin B_6 (mg/1,000 kcal)	−0.04	−0.09	−0.07	0.19	−0.28	0.09	−0.01	0.04
Vitamin B_{12} (μg/1,000 kcal)	0.08	−0.15	0.06	0.02	−0.22	0.00	0.04	−0.40*
Folic acid (μg/1,000 kcal)	0.35	0.16	−0.42*	0.02	−0.21	−0.08	0.27	0.16
Pantothenic acid (mg/1,000 kcal)	0.00	0.06	−0.16	0.28	−0.32	−0.06	0.14	−0.08
Biotin (μg/1,000 kcal)	0.32	0.10	−0.29	0.01	−0.59**	−0.11	0.00	−0.06
Vitamin C (mg/1,000 kcal)	0.10	−0.12	−0.04	0.02	−0.31	−0.06	0.37	0.10

CC, correlation coefficient.
* $p<0.05$, ** $p<0.01$.

Table 5. Associations between fecal microbiota and food group intakes.

	Bifidobacterium	*Lactobacillales*	*Bacteroides*	*Clostridium* cluster IV	*Clostridium* cluster XI	*Clostridium* subcluster XIVa	*Clostridium* cluster XVIII	Others
	CC	CC	CC	CC	CC	CC	CC	CC
Cereals (rice, noodles, etc) (g/1,000 kcal)	0.24	0.14	−0.08	−0.13	−0.18	−0.21	0.20	−0.17
Potatoes (g/1,000 kcal)	−0.28	−0.22	−0.01	0.18	−0.01	0.19	−0.20	−0.12
Green-yellow vegetables (g/1,000 kcal)	−0.18	−0.38*	0.12	0.35	−0.30	0.28	0.08	−0.26
Other vegetables (g/1,000 kcal)	0.07	0.17	−0.18	−0.12	−0.31	0.16	0.11	0.35
Mushrooms (g/1,000 kcal)	−0.31	−0.12	0.15	0.38*	−0.17	0.07	0.14	0.03
Seaweed (g/1,000 kcal)	0.14	−0.03	0.09	0.02	0.21	−0.19	0.06	−0.21
Beans (g/1,000 kcal)	0.30	0.08	−0.04	−0.17	0.24	−0.12	−0.07	−0.14
Fish (g/1,000 kcal)	−0.09	−0.02	0.09	0.00	−0.04	0.16	0.02	−0.08
Meat (g/1,000 kcal)	−0.09	−0.11	0.26	−0.07	−0.19	−0.17	0.12	0.02
Eggs (g/1,000 kcal)	0.15	−0.10	−0.15	−0.04	−0.67**	−0.07	0.05	−0.04
Milk/dairy products (g/1,000 kcal)	−0.13	0.05	0.00	0.13	0.12	0.12	0.27	−0.10
Fruits (g/1,000 kcal)	−0.16	−0.26	0.29	−0.09	−0.31	0.11	−0.01	−0.01
Sugar/sweets (g/1,000 kcal)	0.09	0.36	−0.17	−0.19	0.22	−0.06	−0.01	0.34
Snacks (g/1,000 kcal)	−0.25	−0.11	0.25	0.27	0.12	−0.12	−0.01	−0.19
Beverages (g/1,000 kcal)	−0.12	0.06	−0.22	0.16	−0.15	0.09	−0.14	−0.04
Nuts (g/1,000 kcal)	0.09	−0.25	0.11	−0.31	−0.25	0.27	0.15	−0.01
Oil (g/1,000 kcal)	0.08	0.00	0.17	−0.16	0.01	−0.05	−0.12	0.07
Seasonings/spices (g/1,000 kcal)	0.09	0.06	0.00	−0.08	−0.04	−0.02	−0.18	0.16

CC, correlation coefficient.
* $p<0.05$, ** $p<0.01$.

sources of nutrition themselves in the large intestine, increasing the number of beneficial bacteria including *Bifidobacterium* and *Lactobacillus* (*32–34*). In addition, Chang et al. reported that dietary supplementation with an extract from the *Ganoderma lucidum* mushroom increased the relative abundance of *Clostridium* cluster IV, XIVa, and XVIII in the gut microbiota of mice fed a high fat diet (*35*). Therefore, the high mushroom intake of the cluster A group may have contributed to the high relative abundance of *Bacteroides*, *Clostridium* cluster IV, and XVIII in their fecal microbiota.

The intake of iron was significantly higher in the cluster B group than in the cluster A group, and the number of *Bifidobacterium* was significantly correlated with iron intake. Iron is known to be involved in the growth of microorganisms. For example, *Lactobacillus* spp. have a growth requirement for iron in their culture medium (*36*), and iron deficiency in young women has been associated with low levels of *Lactobacillus* in the feces (*37*). However, a recent study reported that mice fed a high-iron diet had lower abundances of *Bifidobacteriaceae* and *Lactobacillaceae* than mice fed a normal-iron or iron-deficient diet (*38*). In addition, the pathogenic bacteria *Salmonella* and *Escherichia coli* were increased in infants with iron fortification (*39*). Although the mechanisms remain unclear, these studies show imply that iron intake influences gut microbiota composition. The results obtained in this paper likely implicate iron intake in the relative abundance of *Bifidobacterium*.

Kasai et al. analyzed the gut microbiota in non-obese Japanese subjects ($n=23$; 11 men and 12 women) aged 45.6 ± 9.6 y using T-RFLP analysis, and found that the estimated abundance of *Bifidobacterium*, *Lactobacillales*, *Bacteroides*, and *Clostridium* group (IV, XI, XIVa, and XVIII) was 8%, 5%, 41%, and 32%, respectively (*40*). These results are similar to our findings of fecal microbiota composition in all young Japanese women. According to Kao et al., the fecal microbiota composition differs between American, Indian, and Jamaican women, with results showing a greater abundance of *Prevotella* in Indian women than in American or Jamaican women (*41*). However, we could not find *Prevotella* in any of our subjects. *Prevotella* has been found to be a minor component of Japanese gut microbiota (*42*), and is considered to be difficult to detect by the T-RFLP method. While some studies suggest that the gender of the host has an effect on the gut microbiota (*43*), other studies suggest that gender has no effect (*44*). For example, previous studies have reported that the *Bacteroides-Prevotella* groups are more abundant in the gut microbiota of males than females from European locations (*45*). Further studies are needed to determine the relationship between gender and gut microbiota composition in Japanese people.

A metagenomic study has demonstrated that human gut microbiota can be classified into three enterotype clusters, namely *Bacteroides*, *Prevotella*, and *Ruminococcus* (*46*). Wu et al. found two of these enterotype clusters, and reported that the *Bacteroides* enterotype was associated with protein and animal fat intake, and

that the *Prevotella* enterotype was associated with carbohydrate intake in healthy people (*20*). Conversely, our results showed no relationship between the major energy source and bacterial abundance for any bacterial group. Determination of gut microbiota based on the 16S rRNA gene analysis method showed a greater abundance of *Bifidobacterium* in Japanese subjects than in subjects from other countries, especially those from the USA, China, and Korea, who showed a relatively low abundance (<2%) of *Bifidobacterium* (*42*, *47*). Likewise, our data showed a high abundance of *Bifidobacterium* in all of our Japanese subjects (average approximately 12%). *Bifidobacterium* is considered to be the most important member of the gut microbiota and improves the intestinal health of the host (*48*). Previous research has suggested that the consumption of fermented foods increases the abundance of *Bifidobacterium* and decreases that of *Clostridium* (*49*). Moreover, the intake of fermented foods, such as natto, was related to gut microbiota composition, particularly an increased relative abundance of *Bifidobacterium* (*50*). We infer that the high relative abundance of *Bifidobacterium* observed in Japanese people may result from the habitual consumption of traditional Japanese fermented foods (e.g. natto, miso, and pickles).

The present study had several limitations. First, it involved a limited group with a small number of subjects. The fact that dietary fiber and dairy products were not identified as significant variables for the fecal microbiota may be because of this small sample size. In addition, multivariate analysis for extracting independent nutrients and food groups that affect the composition of the fecal microbiota could not be performed. Second, the period of dietary survey was three consecutive days using a weighed food record method. The weighed food record method can provide quantitatively accurate information on food consumed during the recording period; however, women may underreport their usual diets for this period (*51*). Therefore, an examination of long-term dietary surveys using 7-d diet records or the food frequency questionnaire would be preferable for ascertaining the habitual diet. Third, we did not measure stress markers such as salivary cortisol or immunoglobulin A. Gut condition is associated with physical and mental stress (*52*), and salivary cortisol and immunoglobulin A are known to be elevated by stress (*53*, *54*). Takatsuka et al. reported that *Candida* and *Pseudomonas* in the intestines of neutropenic patients with hematological disorders increased after the Hanshin Awaji earthquake (*55*). In future studies, we need to investigate dietary intake and fecal microbiota along with salivary stress markers. Fourth, we investigated the fecal microbiota of healthy young Japanese women using the T-RFLP method. This method can rapidly analyze microbial community structure and dynamics, and is suitable for the examination of many samples (*56*). However, each T-RF peak is derived from more than one kind of bacteria, so it has the disadvantage that it cannot detect all bacterial species. The advancement in next-generation sequencing technologies in recent years has meant that complex intestinal microbial communities at the taxonomic species-level can now be exhaustively analyzed. The resolution of T-RFLP is inferior to metagenomic analysis by next-generation sequencing.

In conclusion, the composition of the gut microbiota including *Bifidobacterium*, *Bacteroides*, and *Clostridium* group abundance may be strongly affected by dietary habits. This is the first study to show relationships between fecal microbiota and habitual dietary intake in Japanese people. Larger sample sizes are required to clarify the relationship between habitual diet and the composition of gut microbiota.

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