

## Japanese Diet Score Is Associated with Gut Microbiota Composition in Young Japanese Adults

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**Summary** The Japanese diet (JD) is accepted as a healthy dietary pattern, which has protective effects against cancer and cardiovascular diseases. However, it remains unclear whether there is any association between the JD and fecal microbiota composition. This cross-sectional study examined the relationship between JD scores and fecal microbiota in young Japanese adults, through the analysis of fecal microbiota using the terminal restriction fragment length polymorphism (T-RFLP) method. JD scores were calculated with regard to nine food groups (rice, miso soup, pickles, green and yellow vegetables, seaweeds, fish, green tea, meat, and coffee) based on a brief self-administered diet-history questionnaire. JD total scores were categorized as low (score 0–3) or high (score 4–8). The high-scoring JD group exhibited a significantly higher relative abundance of *Bacteroides* than the low JD group, whereas the low JD group exhibited a significantly higher relative abundance of *Prevotella* than the high JD group. With regard to food group intakes, a higher consumption of rice was associated with a significantly lower relative abundance of *Prevotella*, whereas a higher consumption of green tea was associated with a significantly higher relative abundance of *Bifidobacterium*. To the best of our knowledge, this is the first study demonstrating an association between JD score and fecal microbiota and may allow the prediction of changes in fecal microbiota due to changes in the JD.

**Key Words** fecal microbiota, diet patterns, BDHQ, young adults, T-RFLP

Over the past decades, several epidemiological studies have identified that an organism genetic background and geographical location are associated with its gut microbiota composition. A metagenomic study found patterns in the fecal microbiota of American, European, and Japanese populations, and reported three enterotype clusters, enriched in *Bacteroides*, *Prevotella*, and *Ruminococcus*, respectively (1). These enterotypes are influenced by habitual diets, in particular, enterotypes including *Bacteroides* and *Prevotella* are strongly linked to protein and carbohydrate intake by healthy people (2). Furthermore, previous studies have reported that the changing composition of the human gut microbiota is affected by various lifestyle factors, such as smoking, exercise, and alcohol consumption (3–5).

Some typical diets, including Western and Mediterranean diets, are also considered to be major modulators of the human gut microbiota. A recent study has shown that the Western diet, which typically includes excessive consumption of fat, animal proteins, and sugars, is associated with high Firmicutes/Bacteroidetes ratios (6). It is well known that high Firmicutes/Bacteroidetes ratios lead to an increase in obesity (7). Moreover, in a study comparing the gut microbiota of people who consumed a high fat diet with that of people who consumed a low-

fat diet, the abundance of *Bifidobacteria* was found to be significantly reduced in high fat diet consumers (8). In contrast, the Mediterranean diet involves the consumption of large amounts of fruits, vegetables, whole grains, nuts, beans, and olive oil, and small amounts of red meat. The Mediterranean diet is enriched in beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* (9). A previous study reported that the Mediterranean diet increased fecal short-chain fatty acid levels, which has a direct anti-inflammatory effect in the gut (10). Furthermore, red wine is frequently drunk as part of the Mediterranean diet, and daily consumption of red wine for 20 d in healthy males induces an increase in the abundance of *Bacteroides* and *Bifidobacterium*, which are associated with reductions in biochemical markers such as triglycerides, cholesterol, and blood pressure (11). Other studies have also linked gut microbiota and the Mediterranean diet (12). Thus, the Mediterranean diet beneficially affects the gut microbiota, whereas the Western diet harms the gut microbiota, leading to the development of lifestyle-related diseases.

In recent years, the Japanese diet (JD) has drawn attention as a distinct diet. In order to evaluate the habitual Japanese diet intake, JD scores have been used as a major approach in nutritional epidemiology research (13, 14). The JD score is an index indicating that food groups such as vegetables, fruits, fish, and

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pickles, are characterized by JD patterns. Recent studies indicate that higher JD scores prevent chronic diseases, such as cancer and cardiovascular disorders (13). Moreover, the intake of individual food groups, including vegetables and fruits, is associated with a lower risk of type 2 diabetes (15). Although the JD contributes health benefits for the host, the composition of gut microbiota remains poorly understood (16). Furthermore, Japanese gut microbiota have been demonstrated to be different to other non-JD countries (17, 18), and we have also researched the fecal microbiota composition of young Japanese women (19), but reasons for such compositional differences are not yet clarified. The purpose of this cross-sectional study was to examine relationships between JD scores, the intake of food groups characterized by Japanese dietary patterns, and human fecal microbiota composition, using the terminal restriction fragment length polymorphism (T-RFLP) method for analysis of fecal microbiota.

## MATERIALS AND METHODS

**Study subjects.** This study conducted a survey among sixty (23 men and 37 women) healthy young university student subjects aged 18 to 22 y old in Japan. The study period was from August 2017 to July 2018, and was performed in compliance with the principles of the Declaration of Helsinki; subjects also provided written informed consent before initiation of the study. All aspects of the study were approved by the Ethics Committee of Aichi Shukutoku University (No. 2017-2).

**Fecal microbiota analysis (T-RFLP method).** Fecal samples were collected in collection tubes containing guanidine thiocyanate solution (Techno Suruga Laboratory Co., Ltd., Shizuoka, Japan). DNA extraction from fecal samples, and analysis of fecal microbiota by the T-RFLP method, were outsourced to Techno Suruga Laboratory Co., and performed according to a protocol described in a previous report (20). The abundance of each terminal restriction fragment (T-RF) was calculated by first dividing them into 29 operational taxonomic units (OTUs) by the method described in reports by Nagashima and colleagues (21, 22). Each OTU was quantified as a percent of the total OTU area, expressed as a percentage of the area under the curve (% AUC).

**Dietary assessment.** For the dietary intake assessment, a brief self-administered diet-history questionnaire (BDHQ) was conducted so as to obtain information regarding food intake quantities and the consumption frequency per meal over the previous month. BDHQ is a food intake frequency survey method that evaluates the contents of the daily diets of Japanese people and is based on questions regarding 56 food and beverage items. The energy and nutrient compositions of foodstuffs reported in BDHQ were estimated based on Standard Tables of Food Composition in Japan (23). The validity of the BDHQ has already been demonstrated by Kobayashi et al. (24). The consumption of nutrients and food groups were calculated per 1,000 kcal of energy intake. All subjects took the dietary survey during the week of fecal sampling.

**Japanese diet score.** The JD score has been previously evaluated based on the results of the semi-quantitative food frequency questionnaire and BDHQ (14, 25). To assess dietary patterns, our JD scores were calculated with reference to a previous study using BDHQ (25). Briefly, JD scores were evaluated based on nine food groups (rice, miso soup, seaweeds, pickles, green and yellow vegetables, fish, green tea, meat (beef and pork), and coffee). For each of the rice, miso soup, seaweeds, pickles, green and yellow vegetables, fish, and green tea groups one point was given to subjects whose intake was more than or equal to the sex-specific median. For the meat and coffee groups one point was given to subjects whose intake was below the sex-specific median. The total scores ranged from 0 to 9, with higher scores indicating greater adherence to the JD. The cutoff was a score of 3 points. We established that values from 0 to 3 points were categorized as the low JD group, whereas values between 4 and 8 points were categorized as the high JD group. There were no individuals with a score of 9 points. Subjects were additionally divided into tertiles of food group intakes, and fecal microbiota compositions were compared.

**Statistical analysis.** Statistical analysis was performed using SPSS Statistical Analysis software for Windows, version 23.0 (IBM, Tokyo, Japan). All data are expressed as mean  $\pm$  standard error (SE). Examination of normality was investigated using the Shapiro-Wilk test. Parametric data were analyzed using non-paired Student's *t*-test, whereas nonparametric data were analyzed using the Mann-Whitney *U* test. The relation between food group intakes and fecal microbiota was analyzed by Jonckheere-Terpstra test.  $p < 0.05$  was considered statistically significant. Principal component analysis was performed based on the T-RFLP profiles of fecal samples. Microbial diversity was calculated using the Shannon-Wiener index of diversity to assess evenness and richness of microbial community members (26).

## RESULTS

The 60 subjects comprised 22 (10 men and 12 women) in the low JD group and 38 (13 men and 25 women) in the high JD group. According to the data calculated from the BDHQ, the energy intake and macronutrient intakes were not significantly different between the two groups (Table 1). For nine food groups, the intake of rice, miso soup, pickles, seaweeds, and fish, were significantly higher in the high JD group than in the low JD group ( $p < 0.05$ ) (Table 2). On the other hand, the intake of coffee was significantly lower in the high JD group than the low JD group ( $p < 0.05$ ).

Figure 1 is a graphical representation of the fecal microbiota diversity and communities, based on the T-RFLP profiles in the low JD and high JD groups. The Shannon-Wiener index was not significantly different between the two groups. Similarly, there was no clear separation of fecal microbiota communities for the two groups using PCA analysis. The PCA indicated that two components explained 42.2% of the variance. As can be

Table 1. Energy and macronutrient intakes for the low and high JD groups.

	Low JD group (n=22)	High JD group (n=38)
Energy (kcal)	1,864±176	2,113±116
Protein (% of energy)	14.2±1.1	14.5±0.4
Fat (% of energy)	31.3±1.7	28.0±0.9
Carbohydrate (% of energy)	54.5±2.4	57.5±1.3
Water soluble fiber (g/1,000 kcal)	1.4±0.1	1.6±0.1
Water insoluble fiber (g/1,000 kcal)	3.7±0.3	4.4±0.2
Total dietary fiber (g/1,000 kcal)	5.2±0.4	6.2±0.3
Salt (g/1,000 kcal)	5.2±0.2	5.1±0.2

Values are means±SE.

Table 2. Intake of food groups characterized by Japanese dietary patterns for the low and high JD groups.

	Low JD group	High JD group
Rice (g/1,000 kcal)	127±18	175±12*
Miso soup (g/1,000 kcal)	50±14	69±8*
Pickles (g/1,000 kcal)	2.5±0.9	4.8±0.8*
Green and yellow vegetables (g/1,000 kcal)	61±12	67±5
Seaweeds (g/1,000 kcal)	3.1±0.9	7.6±1.3*
Fish (g/1,000 kcal)	24±5	36±3*
Green tea (g/1,000 kcal)	117±35	127±21
Meat (g/1,000 kcal)	50±8	41±3
Coffee (g/1,000 kcal)	70±19	20±5*

Values are means±SE.

\* $p<0.05$  versus low JD group determined by unpaired  $t$ -test, or Mann-Whitney  $U$  test.

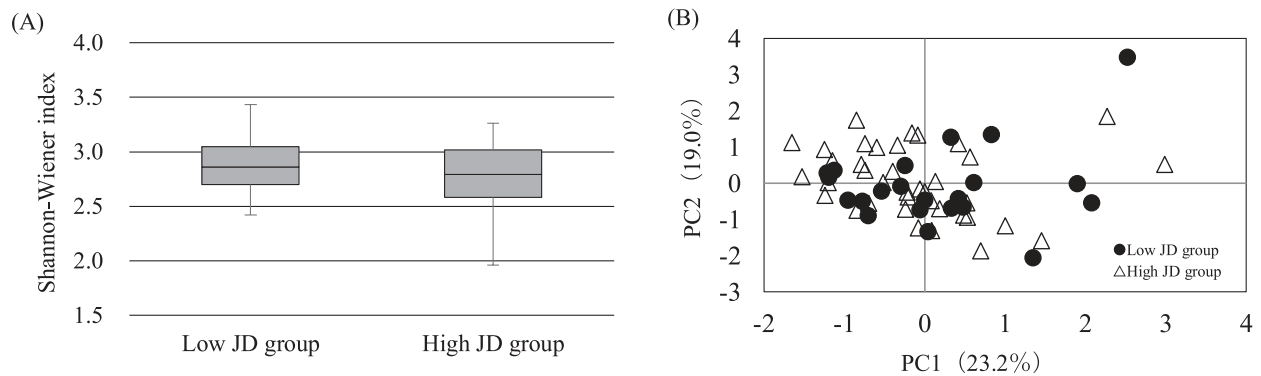


Fig. 1. Fecal microbiota diversity of terminal restriction fragment length polymorphism (T-RFLP) profiles for the low and high JD groups analyzed by Shannon-Wiener index (A) and principal component analysis (B).

seen from these results, there was no difference in terms of bacterial diversity between the two groups.

The results of a comparison of fecal microbiota compositions based on T-RFLP profiles, between the low JD and high JD groups, are shown in Table 3. The relative abundance of *Bacteroides* was significantly lower in the low JD group than the high JD group ( $p<0.05$ ). The relative abundance of *Prevotella* was significantly higher in the low JD group than the high JD group ( $p<0.05$ ).

Figure 2 presents a comparison of fecal microbiota according to differences in food group intakes. Increasing rice and seaweeds intake were associated with sig-

nificant decreases in the relative abundance of *Prevotella* and *Clostridium* cluster IX, respectively ( $p<0.05$ ). The relative abundance of *Bifidobacterium* had a positive trend of association with the intake of green tea ( $p<0.05$ ).

## DISCUSSION

We conducted a cross-sectional study to investigate associations between JD scores and fecal microbiota using T-RFLP analysis. Our results showed that fecal microbiota, particularly the relative abundance of *Bacteroides* and *Prevotella*, were different between low and

high JD groups. Moreover, the intake of food groups characterized by Japanese dietary patterns, such as rice, green tea, and seaweeds, resulted in changes to the fecal microbiota. Thus, we observed that differences in JD scores might have the potential to lead to modifications in the gut microbiota composition.

The Mediterranean diet is characterized by a high intake of fish, vegetables, and seaweeds and a low intake of meat, which is consistent with the JD (27). In several studies, the Mediterranean diet has been associated with an increase in the relative abundance of *Bacteroides* in the gut microbiota (9). Similar associations were detected in our study, in which the high JD group, which consumed high levels of green and yellow vegetables, seaweeds, and fish and a low level of meat, had a significantly higher relative abundance of *Bacteroides*, compared to the low JD group. Carrothers et al. reported that the relative abundance of *Bacteroides* is negatively associated with protein intake in lactating women (28). In addition, several studies showed the

association between vegetarian dietary patterns and fecal microbiota composition (29, 30). The results of these studies indicated vegetarian have an increased abundance of *Bacteroides* in the fecal microbiota compared with omnivorous. Therefore, the intake of various food groups including meat and vegetables may contribute to the high relative abundance of *Bacteroides* in the fecal microbiota. However, *Bacteroides* has been linked to long-term dietary patterns of Western diets containing high amounts animal protein (2). Further studies are needed to determine the relationship between *Bacteroides* and JD.

In this study, the fecal microbiota of the high JD group showed a significantly lower relative abundance of *Prevotella*, compared with the low JD group. We also found that subjects who consumed high quantities of rice exhibited a reduced relative abundance of *Prevotella*. However, several previous cross-sectional studies have shown that *Prevotella*-rich enterotypes are associated with high-carbohydrate diets in healthy people (2). Egyptian and rural African children following a long-term high carbohydrate and plant-based diet have a relatively high abundance of *Prevotella* in their gut (31, 32). Non-digestible carbohydrates, such as dietary fiber and resistant starch, have been suggested as being the major promoters of *Prevotella* abundance (33). Furthermore, *Prevotella* is an established biomarker of diet and lifestyle (34). Previous research suggested a major difference in the gut microbiota composition between Japanese and other populations, in particular, in terms of higher proportions of *Bifidobacterium* and lower proportions of *Prevotella* in the gut microbiota (17). Our previous research had also revealed very similar analytical results using the T-RFLP method in young Japanese women (19). The intake of rice, which is a staple foodstuff in the JD, may be involved in the composition of gut microbiota in Japanese people, but further research is necessary in order to understand these complicated relationships.

Interestingly, green tea intake showed a significant positive trend of association with the relative abundance of *Bifidobacterium*. Previous studies have demonstrated that dietary polyphenols, such as tea catechin, affect the

Table 3. Comparison of fecal microbiota by terminal restriction fragment length polymorphism (T-RFLP) analysis between the low and high JD groups.

Bacteria	Low JD group (%)	High JD group (%)
<i>Bifidobacterium</i>	10.4±2.0	9.9±1.3
<i>Lactobacillales</i>	4.8±1.3	3.7±0.6
<i>Bacteroides</i>	42.6±3.0	50.3±1.5*
<i>Prevotella</i>	7.7±3.2	0.1±0.1*
<i>Clostridium</i> cluster IV	6.5±0.8	6.9±0.7
<i>Clostridium</i> subcluster XIVa	13.9±0.9	15.5±1.0
<i>Clostridium</i> cluster IX	5.3±1.2	4.9±0.9
<i>Clostridium</i> cluster XI	0.4±0.2	0.3±0.1
<i>Clostridium</i> cluster XVIII	1.8±0.3	1.5±0.3
Others	6.5±0.8	6.8±0.6

Values are means±SE.

\* $p < 0.05$  versus low JD group determined by unpaired *t*-test or Mann-Whitney *U* test.

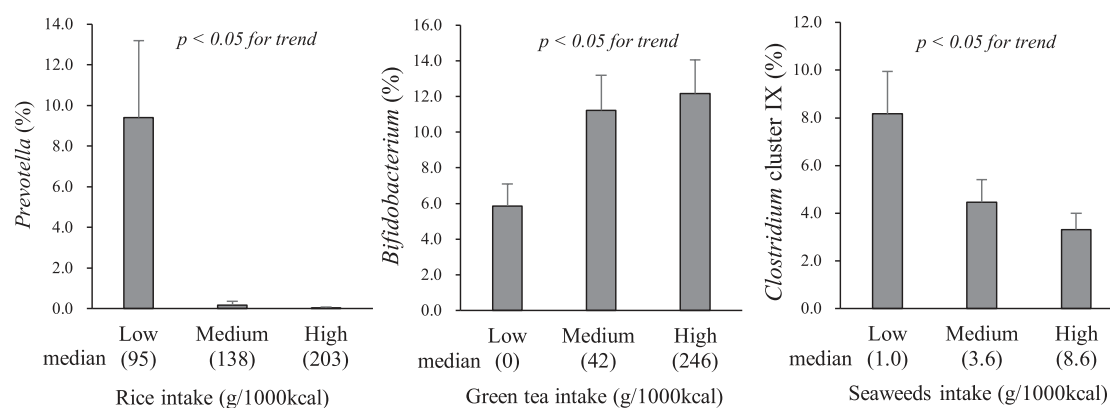


Fig. 2. Comparison of fecal microbiota by terminal restriction fragment length polymorphism according to differences in the intake of food groups characterized by Japanese dietary patterns. Subjects were classified into low, medium, and high tertiles. The difference was statistically significant among the three groups ( $p$  trend  $< 0.05$ , Jonckheere-Terpstra test).



composition of gut microbiota. The influence of green tea on human fecal microbiota was investigated by Jin et al. (35). After 10 d of consuming 1,000 mL green tea, healthy subjects exhibited an increased level of *Bifidobacterium* species. Furthermore, previous in vitro investigations using a batch-culture in a human large intestine model, indicate that (+)-catechin significantly enhances growth of *Bifidobacterium* (36). Green tea may enhance the abundance of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, by reducing the proportion of Gram positive bacteria, including *Clostridium* (37). Our results support previous studies based on short-term trials of green tea polyphenolic supplements and suggests that long-term green tea consumption may influence the gut microbiota composition.

The Tohoku University of Medicine and the Faculty of Agriculture groups have reported that JD has potential health benefits that include the prevention of metabolic syndrome and reduced risk of obesity (38–40). This research team has also indicated that JD affects the gut microbiota, in particular the 1975 JD, can reduce the prevalence of Bacteroidetes and increase the prevalence of Firmicutes (16). Another study reported an association of the combination of JD intake and exercise with an increase in the *Bacteroides* and *Clostridium* (41). In this study, although there were differences in *Prevotella* and *Bacteroides* between the low JD and high JD groups, no changes were observed at the phylum level. To complement our findings, we are now investigating the association with JD and fecal microbiota composition.

There are certain limitations in the present study. First, the T-RFLP method does not give a complete picture of the microbiota, because the roles of many bacterial species are unclear. T-RFLP may be inferior to other methods, such as next-generation sequencing. Second, this study involved a limited cohort with a small number of subjects. As a result, due to deviation of subject number in the groups, it was impossible to compare the fecal microbiota composition by dividing the JD scores into tertiles. Larger sample sizes may be required to clarify the association between the JD score and fecal microbiota. Finally, given the cross-sectional design of our study, further studies using different methods, such as longitudinal research, are needed to enhance investigation in this area.

In conclusion, our findings revealed that the JD may be associated with fecal microbiota composition, in particular, the involvement of *Bacteroides* and *Prevotella*. To the best of our knowledge, this is the first study in which the JD score has been shown to lead to changes in the gut microbiota composition. We will continue to increase the number of samples for this analysis and expect the relationship between the JD and gut microbiota to be clarified further.

#### Disclosure of state of COI

We have no conflicts of interest to declare.

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