

The Nested Study on the Intestinal Microbiota in GENKI Study with Special Reference to the Effect of Brown Rice Eating

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Abstract

Purpose: Brown rice eaters are healthy and have good bowel movement, which suggests a good intestinal environment and good composition of microbiota. However, knowledge about the normal variation of the intestinal microbiota of these people still remain limited.

Subjects and Methods: A nested study on the intestinal microbiota was carried out among the GENKI study participants. Metagenomic analysis of 109 samples were carried out with a questionnaire asking about dietary changes from a baseline survey.

Results and Discussion: 109 healthy subjects (18 males, 90 females, unknown 1) provided stools for analysis. The average age was 50.1 ± 15.1 years old in males and 55.8 ± 13.8 years old in females. Average body mass index (BMI) of males was 20.9 ± 2.3 kg/m² and 20.2 ± 2.2 kg/m² in females. Common bacterial profiles at phylum level were *Fermicutes* $44.3 \pm 9.9\%$, *Bacteroides* $20.7 \pm 8.8\%$, *Actinobacteria* $8.3 \pm 6.3\%$, *Proteobacteria* $1.7 \pm 2.7\%$ and *Verrucobacteria* $1.2 \pm 4.2\%$ (max 39.4%). At the genus level, *Bacteroides* (12.7%), *Blautia* (8.3%), *Faecalibacterium* (7.9%), *Bifidobacterium* (6.3%), *Prevotella* (5.3%), *Eubacterium* (4.9%), *Ruminococcus* (3.8%), *Fusicatenibacter* (2.6%), *Collinsella* (1.9%), *Streptococcus* (2.4%), *Subdoligranulum* (2.1%), *Anaerostipes* (1.7%), *Akkermansia* (1.2%), and *Roseburia* (1.7%) occupied more than 1%. The difference between the brown rice and white rice eaters by microbiota profile was high butyrate producing bacteria and low fusobacterium. High prevalence of *Faecalibacterium prausnitzii* (5.28%) suggested the benefit on butyrate production, and *Blautia wexlerae* (3.67%) suggested the control on intestinal immunity.

Conclusion: Habitual rice eaters showed *Faecalibacterium* and *Blautia* dominant profile of intestinal microbiota. Brown rice eaters showed additional benefits by high prevalence of *Fermicutes* and low prevalence of *Fusobacterium*. These profiles could lead to integrated healthy life.

Keywords

Human microbiota, Butyrate, Brown rice eater, Nested study, Epidemiology

Introduction

Genmai (brown rice) diet of Sagen Ishizuka and Kenzo Futaki is revived with

the *Shoku-iku* (eating education) Act in Japan [1], but there is a claim that scientific evidence is still insufficient.

We had conducted two large scale population-based cohort studies, Japan Public Health Center Study and Saku Control Obesity Program from early 1990, but there were less than 100 people who had eaten brown rice regularly, so it was an insufficient number for statistical analysis at that time [2, 3]. There are many healthy people who take a brown rice based flexible vegetarian diet in the group such as the Japan Society of Integrative Medicine, Japan CI Association, the Japan Macrobiotic Association, and the AOB KEIO Group [4, 5]. Most of them have the same root of Sagen Ishizuka.

We started the *GENKI* study in 2016, in which the abbreviation is “*Genmai* Evidence of Nutrition for *Kenko* (health) Innovation”. It was an epidemiological prospective cohort study to clarify who was eating *genmai* and their health status in relationship to their lifestyle. The baseline data was published in *J OCD* previously [6].

We found that *genmai* (brown rice) eaters were healthy, not obese and had good bowel condition. We considered the intestinal microbiota would play a key role, and planned to perform a nested study on intestinal microbiota among the participants of the *GENKI* study. The second survey was performed one year later, and 350 participants answered the questionnaire and 109 people accepted to join in the study on intestinal microbiota. The present study could present the characteristics of intestinal microbiota profile to support the healthy life of *genmai* eaters.

Ethical issue

The research plan was approved by the Ethics Review Board of the Life Science Promoting Association (No. 003 in 2015). At that time, participants were not specified, and were told that it was not used for purposes other than research. Furthermore, they were free to withdraw at any time during the study. Confidential information was stored separately, and the analysis was done by the anonymous data.

Methods

Between 2016 and 2017, the Research Division of the Life Science Promoting Association conducted a questionnaire survey mainly on the people who had eaten brown rice and their families (*GENKI* study I). In this survey, the purpose of the research was documented to all subjects, such as members of organizations that recommend the brown rice diet, and agreement was obtained from all participants.

1222 people from all over the country participated and reported on the results in terms of overall health and benefits of rice diet, with special reference toward brown (unpolished) rice. A questionnaire survey was conducted for persons who participated in *GENKI* Study I for the second time one year later, and 350 respondents were obtained. 109 of them agreed to participate in the intestinal bacterial examination and submitted feces. The 109 subjects (male: 18; 50.1 ± 15.1 years old, female: 91, 55.8 ± 13.8 years old) were analyzed in this study.

Intestinal bacteria

The survey was carried out from November 2017 to January 2018. 109 people provided stool samples in the tubes and a short questionnaire to confirm dietary change and health status from their baseline data. Fresh feces were collected 3 points of the stools. Fecal samples (approximately 50-100 mg) were sent to Techno Suruga Laboratory, Shizuoka, for sequence amplicon analysis. [7-9]. The stools were suspended in a tube containing 100 mM Tris-HCl buffer with EDTA, 4 M guanidine thiocyanate and 0.001% bromothymol blue. An 0.8 ml of the suspension was homogenized with zirconia beads by FastPrep24 Instrument (MP Biomedicals, Santa Ana, CA), and DNA was extracted from the suspension using an extractor (Precision System Science, Chiba, Japan). MagDEA DNA 200 (GC) (Precision System Science) was used for automatic nucleic acid extraction, as previously described. [10]

NGS analysis of microbial community structure in stool was carried out using a MiSeq (Illumina, San Diego, CA), as previously described [7, 8]. The V3-V4 region of 16S rDNA was amplified using the mixture of forward primer and reverse primer. The forward primer was Pro341 F 5'- ATGATACGGCGACCACCGAGATCTACACXXXXXXXX-ACACTCTTTCCCTACACGAC-GCTCTTC-CGATCTCCTACGGGNBGCASCAG-3'. and the reverse primer was Pro806R 5'-CAAGCAGAAGACGGCATA-GAGATZZZZZZZGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTACNVGGGTATCTA-ATCC-3', where Xs and Zs represent the sample-specific 8-bp barcode sequences; Xs were CTCTCTAT, TATCCTCT, GTAAGGAG, ACTGCATA, AAGGAGTA, CTAAGCCT, CGTCTAAT, TCTCTCCG, TCGACTAG, TTCTAGCT, CCTAGAGT, GCGTAAGA, CTATTAAG, AAGGCTAT, GAGCCTTA and TTATGCGA, and the sequences of Zs were TCGCCTTA, CTAGTACG, TTCTGCCT, GCT-CAGGA, AGGAGTCC, CATGCCTA, GTAGAGAG, CAGCCTCG, TGCCTCTT, TCCTCTAC, TCATGAGC, CCTGAGAT, TAGCGAGT and GTAGCTCC. The PCR was done by the GeneAmp PCR system 9700 (ABI, Foster City, CA). The PCR reaction and preparation of amplicon pool were performed by the method of Takahashi et al. [9]

The more than 30,000 determined 16S rDNA sequences of each sample were subjected to homology search using Metagenome@KIN software (World Fusion Co., Ltd., Tokyo, Japan) against the Techno-Suruga Lab Microbial Identification Database DB-BA10.0 (Techno-Suruga Laboratory), [9].

Statistical analysis

The data of the questionnaire was stored in the Excel database, and transferred to SPSS [11]. Parametric analysis for continuous variables and non-parametric analysis for categorical data were carried out. Data were examined using an unpaired t-test or the two-sided Mann-Whitney test and Fisher's X² test for categorical variables. Correlation analysis was carried out between each profile of microbiota at the phylum level, genus level, and species level, if necessary. Chief component analysis was done to detect the group of coexistence and/or interaction. Bacterial profile more than 0.1% in average or max profile more than 1.0% were selected

for analysis. Most tables show mean, s.d., median and max, because the distribution of bacilli was very irregular, especially the population was small and prevalence was deviated.

The complete dataset for analysis was available for 109 people. The IBM SPSS software Ver. 24 was used for calculation, and P values less than 0.1 were marked and $p < 0.05$ was considered significant. The statistical significance was shown as * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$.

Results

Anthropometric data

Number of subjects by age category is shown in (Table 1) BMI of both sex was $20.9 \pm 2.3 \text{ kg/m}^2$ and $20.2 \pm 2.2 \text{ kg/m}^2$, respectively. Thin person in aged women is noteworthy (Table 1).

Table 1: Rice category and sex-, age-category. In males brown rice eater is 11/18 (61%) and 54/90 (60% in females). There was no significant difference.

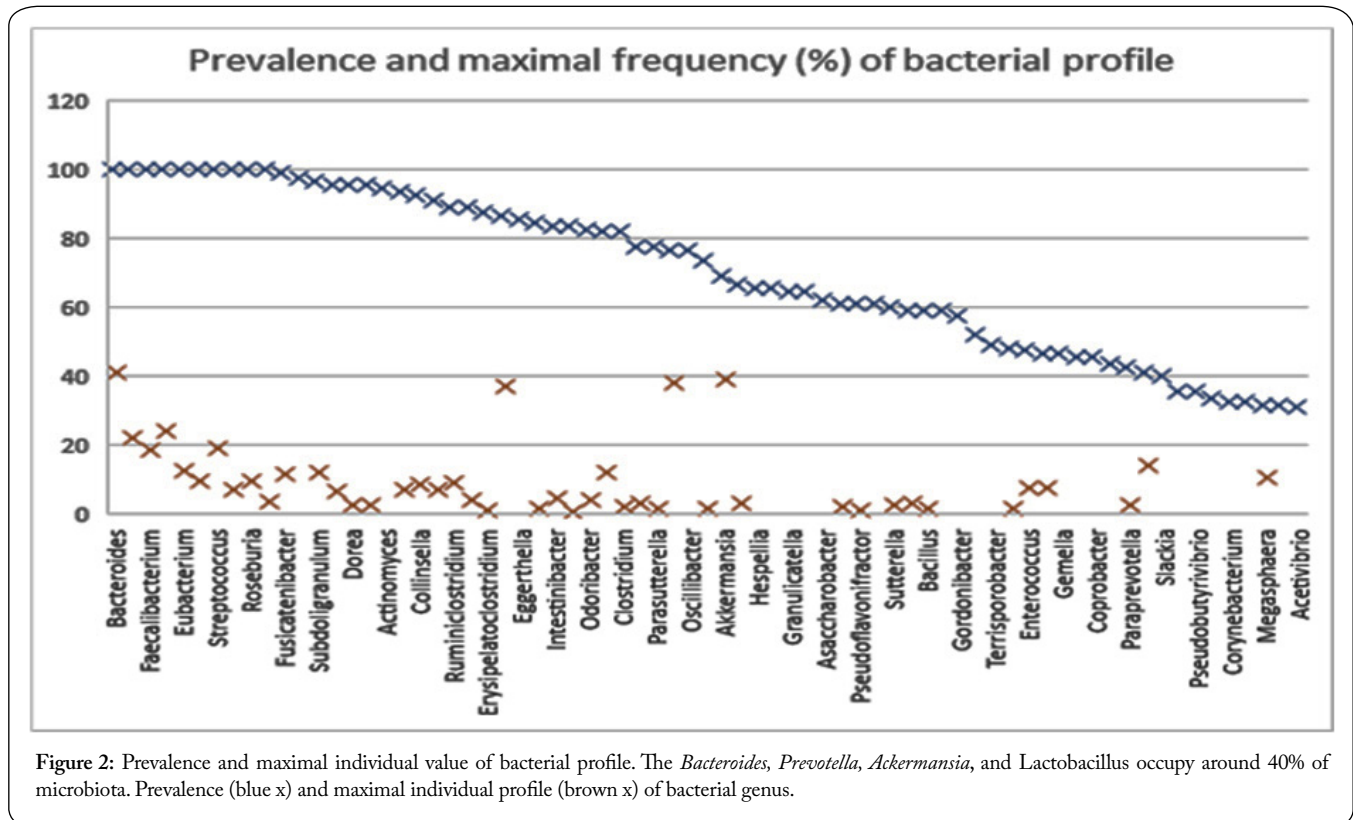
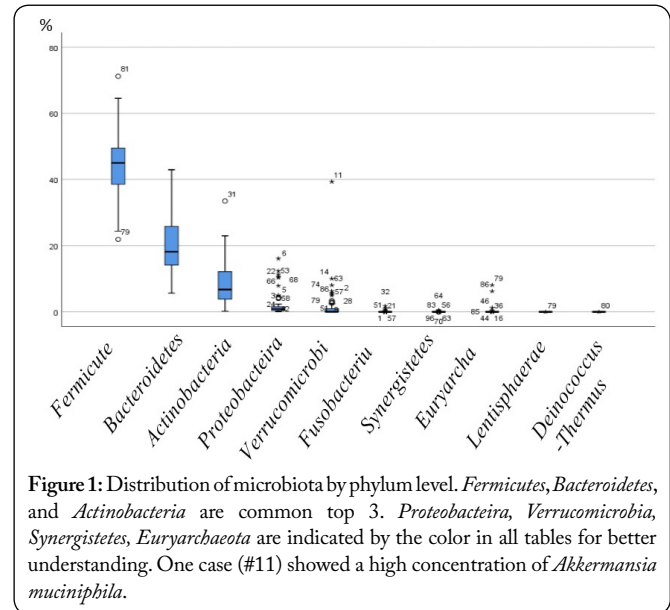
Sex	Rice_cat	Age_cat						Total
		20	30	40	50	60	70	
Male	White rice	0	1	1	1	0	0	3
	Mix rice	1	0	0	2	1	0	4
	Brown rice	1	1	3	2	3	1	11
	Total	2	2	4	5	4	1	18
Female	White rice	1	0	4	2	2	0	9
	Mix rice	1	4	8	4	6	4	27
	Brown rice	1	4	9	14	15	11	54
	Total	3	8	21	20	23	15	90

Brown rice was used in 11/18 (61%) in males and 54/90 (60%) in females. White rice was 3/18 (17%) in males and 9/90 (10%) in females. Other persons used mixture of partially polished rice, germ rice, and other rice.

Bowel movement

Defecate once a day was common; 8/18 in males and 23/90 in females. Five per week was 6/18 in males and 53/90 in females. Only 5 were less than 3/week.

Banana shape stools were half in both men (50%) and women (52%), then soft stools in male (39%) and female (44%). However, these differences were not statistically significant.



Microbiota profiles

Bacterial profiles (%) at phylum level were *Firmicutes* 44.3 ± 9.9, *Bacteroidetes* 20.7 ± 8.8, *Actinobacteria* 8.3 ± 6.3, *Proteobacteria* 1.7 ± 2.7, *Verrucomicrobia* 1.2 ± 4.2 (max 39.4), *Synergistetes* 0.02 ± 0.076 (max 0.54), *Euryarchaeota* 0.16 ± 1.0 (max 8.1), *Lentisphaerae* 0.0 ± 0.0003, *Deinococcus-Thermus* 0.0 ± 0.0003, and rejected 23.4 +/- 8.8 (Figure 1). No statistically significant difference was present among sex and age category at phylum level (Table 2). In each table, phylum of the bacteria is shown by the same color: *Firmicutes* (yellow), *Bacteroides* (green), *Actinobacteria* (pink), *Proteobacteria* (brown), and *Verrucomicrobia* (violet) to know the interrelationship of microbiota.

0.01% (Table 3 lower part). Rejected hits were 29.6% and undetermined rate was 9.4%.

Correlation among these bacteria showed that *Firmicutes* showed negative correlation with all other phylum.

Frequency of microbiota by genus level is shown in Table 3. In addition of the mean, median and s.d., max and prevalence rate are added to know the distribution of microbiota. Common bacteria more than 1% profile were *Bacteroides* (12.7%), *Blautia* (8.3%), *Faecalibacterium* (7.9%), *Bifidobacterium* (6.3%), *Prevotella* (5.3%), *Eubacterium* (4.9%), *Ruminococcus* (3.8%), *Fusicatenibacter* (2.6%), *Collinsella* (1.9%), *Streptococcus* (2.4%), *Subdoligranulum* (2.1%), *Anaerostipes*

Table 2: Microbiota profile at phylum level by sex-, age category. Color of the phylum is common in the following tables. There was no significant difference between age categories in both sex.

Age_cat	20 (n = 2)		30 (n = 2)		40(n = 4)		60 (n = 4)		70 (n =1)	
M	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Firmicutes</i>	49.281		49.372		39.442	8.102	40.926	6.806	59.130	
<i>Bacteroidetes</i>	17.429		25.739		28.711	10.008	29.735	9.030	16.098	
<i>Actinobacteria</i>	8.415		8.081		11.996	9.379	6.258	7.122	3.829	
<i>Proteobacteria</i>	0.857		0.688		0.960	0.755	1.046	0.563	1.053	
<i>Verrucomicrobia</i>	0.000		0.002		0.687	1.369	0.116	0.892	0.000	
<i>Fusobacteria</i>	0.000		0.000		0.001	0.003	0.035	0.001	0.000	
<i>Synergistetes</i>	0.000		0.000		0.000	0.000	0.001	0.239	0.000	
<i>Euryarchaeota</i>	0.000		0.002		0.001	0.001	0.000	0.000	0.000	
<i>Lentisphaerae</i>	0.000		0.000		0.001	0.002	0.000	0.001	0.000	
<i>DeinococcusThermus</i>	0.000		0.000		0.000	0.000	0.000	0.000	0.000	
reject_per	24.019		16.115		18.201	6.561	21.886	5.629	19.891	
	30 (n = 8)		40 (n = 21)		50 (n = 21)		60 (n = 23)		70 (n = 15)	
F	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Firmicutes</i>	46.050	8.678	38.845	10.638	49.558	10.768	43.581	10.480	45.828	8.115
<i>Bacteroidetes</i>	20.032	9.605	20.723	8.823	19.691	9.230	19.477	9.847	18.670	5.589
<i>Actinobacteria</i>	6.727	0.249	11.092	8.705	9.422	6.735	7.900	5.034	6.501	4.111
<i>Proteobacteria</i>	0.950	1.921	2.059	2.641	0.885	0.616	1.870	3.172	1.730	2.720
<i>Verrucomicrobia</i>	0.154	0.060	1.080	2.123	1.048	2.307	1.317	2.297	2.913	10.095
<i>Fusobacteria</i>	0.002	0.000	0.900	4.067	0.034	0.150	0.088	0.364	0.072	0.270
<i>Synergistetes</i>	0.003	0.000	0.012	0.050	0.017	0.065	0.023	0.066	0.004	0.011
<i>Euryarchaeota</i>	0.000	0.000	0.000	0.001	0.017	0.054	0.327	1.312	0.032	0.121
<i>Lentisphaerae</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>DeinococcusThermus</i>	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
reject_per	26.082	5.367	25.290	8.376	19.329	7.233	25.417	11.134	24.250	7.826

Our analyzing system of microbiota profile recognized 469 species from about more than one million taxa. Number of genus more than 1.0% was 15, and more than 0.01% and max more than 1.0% was 57 among 214 genera (Table 3). The number of more than 80% prevalence of microbiota among participants was 22, in which 5 (*Flavonifractor*, *Romboutsia*, *Actinomyces*, *Eggerthella*, and *Odoribacter*) were very low concentration less than 0.01% (Figure 2). Prevalence of 50-79% was 20 genus in which 11 were very low profile less than

(1.7%), *Akkermansia* (1.2%), and *Roseburia* (1.7%) (Table 3). In sex difference, females showed more *Fusicatenibacter* (2.0 vs. 2.8%), *Intestinibacter* (0.17 vs. 0.33%), *Paraprevotella* (0.04 vs. 0.24%) and less *Fusobacterium* (1.08 vs. 0.03%). *Akkermansia* was also high in females (0.6 vs. 1.3%) but it was due to high percentage of profile (39.2%) in one women.

The frequency of major microbiota, such as *Faecalibacterium* and *Bifidobacterium* were not different by age category (Figure 3).

Table 3: Frequency of microbiota of all participants at genus level. Mean, standard deviation, scatter, maximum value and prevalence are shown to characterize the profile distribution. Upper part is microbiota more than 0.01%, and lower part shows microbiota with more than 10% prevalence, even though the profile is less than 0.01%.

Genus	Phylum	mean	Median	SD	Scatter	Max	Prevalence
<i>Bacteroides</i>	B	12.745	11.552	7.902	62.442	40.893	100
<i>Blautia</i>	F	8.276	7.661	4.004	16.035	22.124	100
<i>Faecalibacterium</i>	F	7.920	7.720	3.982	15.857	18.730	100
<i>Bifidobacterium</i>	A	6.334	4.236	5.878	34.547	24.138	100
<i>Prevotella</i>	B	5.273	0.011	10.093	101.876	37.164	86.4
<i>Eubacterium</i>	F	4.935	4.580	2.749	7.559	12.359	100
<i>Ruminococcus</i>	F	3.780	3.617	2.440	5.953	9.824	100
<i>Fusicatenibacter</i>	F	2.688	2.305	2.081	4.332	11.448	99.1
<i>Streptococcus</i>	F	2.385	1.189	3.580	12.816	19.089	100
<i>Subdoligranulum</i>	F	2.089	1.574	1.977	3.910	12.305	96.4
<i>Collinsella</i>	A	1.916	1.970	1.613	2.601	8.690	92.7
<i>Anaerostipes</i>	F	1.714	1.352	1.450	2.104	7.212	100
<i>Roseburia</i>	F	1.774	1.422	1.596	2.547	9.402	100
<i>Coproccoccus</i>	F	1.123	0.773	1.197	1.434	6.978	90.9
<i>Akkermansia</i>	V	1.164	0.016	4.088	16.709	39.178	69.1
<i>Alistipes</i>	B	0.978	0.629	1.086	1.178	6.506	95.5
<i>Parabacteroides</i>	B	0.802	0.590	0.904	0.818	6.881	93.6
<i>Phascolarctobacterium</i>	B	0.738	0.340	0.928	0.862	3.300	77.3
<i>Megamonas</i>	F	0.773	0.000	2.364	5.587	14.265	40.9
<i>Dorea</i>	F	0.729	0.648	0.514	0.265	2.576	95.5
<i>Lachnospirillum</i>	F	0.715	0.503	0.648	0.420	3.837	100
<i>Lactobacillus</i>	F	0.597	0.010	3.730	13.913	38.274	76.4
<i>Megasphaera</i>	F	0.462	0.000	1.652	2.729	10.339	31.8
<i>Romboutsia</i>	F	0.415	0.220	0.489	0.239	2.682	95.5
<i>Sutterella</i>	P	0.407	0.160	0.547	0.299	2.692	60
<i>Escherichia</i>	P	0.356	0.013	1.666	2.775	12.050	81.8
<i>Ruminiclostridium</i>	F	0.361	0.046	0.998	0.997	9.116	89.1
<i>Dialister</i>	F	0.387	0.006	0.716	0.513	2.983	59.1
<i>Clostridium</i>	F	0.223	0.038	0.404	0.163	1.866	81.8
<i>Barnesiella</i>	B	0.252	0.028	0.441	0.194	2.043	60.9
<i>Intestinibacter</i>	F	0.304	0.077	0.577	0.333	4.377	83.6
<i>Enterococcus</i>	F	0.280	0.000	1.271	1.614	7.665	47.3
<i>Lachnospira</i>	F	0.235	0.016	0.511	0.261	3.197	66.4
<i>Fusobacterium</i>	Fuso	0.205	0.000	1.777	3.159	18.557	22.7
<i>Odoribacter</i>	B	0.204	0.129	0.438	0.192	4.020	82.7
<i>Paraprevotella</i>	B	0.203	0.000	0.468	0.219	2.542	42.7
<i>Mitsuokella</i>	F	0.191	0.000	0.962	0.925	7.667	16.4
<i>Veillonella</i>	F	0.175	0.030	0.488	0.238	3.858	89.1
<i>Bacillus</i>	F	0.161	0.006	0.296	0.088	1.763	59.1
<i>Methanosphaera</i>	*	0.147	0.000	0.970	0.942	8.047	10.9
<i>Enterobacter</i>	P	0.146	0.000	0.766	0.587	7.367	46.4
<i>Parasutterella</i>	P	0.141	0.027	0.266	0.071	1.467	77.3
<i>Turicibacter</i>	F	0.130	0.013	0.289	0.084	1.766	73.6
<i>Haemophilus</i>	P	0.114	0.027	0.213	0.046	1.734	84.5
<i>Catenibacterium</i>	F	0.093	0.000	0.440	0.193	3.157	13.6
<i>Bilophila</i>	P	0.090	0.075	0.080	0.006	0.356	83.6
<i>Erysipelatoclostridium</i>	F	0.074	0.024	0.150	0.022	1.060	87.3
<i>Tyzzerella</i>	F	0.072	0.000	0.223	0.050	1.607	48.2

<i>Hafnia</i>	P	0.062	0.000	0.500	0.250	4.961	4.5
<i>Klebsiella</i>	P	0.045	0.000	0.243	0.059	2.315	27.3
<i>Butyrivibrio</i>	F	0.059	0.000	0.396	0.156	3.890	13.6
<i>Acidaminococcus</i>	F	0.044	0.000	0.166	0.028	1.388	23.6
<i>Sarcina</i>	F	0.036	0.000	0.371	0.138	3.896	3.6
<i>Pseudoflavonifractor</i>	F	0.016	0.003	0.110	0.012	1.158	60.9
<i>Butyricoccus</i>	F	0.017	0.000	0.128	0.016	1.168	7.3
<i>Sharpea</i>		0.013	0.000	0.138	0.019	1.445	0.9
<i>Faecalicoccus</i>	F	0.012	0.000	0.117	0.014	1.226	5.5
Microbiota with less than 0.1% profile in average or more than 1% max, but more than 10% prevalence							
<i>Flavonifractor</i>	97.3	<i>Terrisporobacter</i>		49.1	<i>Abiotrophia</i>		19.1
<i>Romboutsia</i>	95.5	<i>Gemella</i>		46.4	<i>Atopobium</i>		19.1
<i>Actinomyces</i>	94.5	<i>Anaerotruncus</i>		45.5	<i>Adlercreutzia</i>		17.3
<i>Eggerthella</i>	85.5	<i>Coprobacter</i>		45.5	<i>Neisseria</i>		17.3
<i>Odoribacter</i>	82.7	<i>Butyricimonas</i>		43.6	<i>Weissella</i>		17.3
<i>Oscillibacter</i>	76.4	<i>Slackia</i>		40	<i>Cloacibacillus(Syn)</i>		16.4
<i>Hespellia</i>	65.5	<i>Oxalobacter</i>		35.5	<i>Peptostreptococcus</i>		16.4
<i>Rothia</i>	65.5	<i>Pseudobutyrvibrio</i>		35.5	<i>Solobacterium</i>		16.4
<i>Granulicatella</i>	64.5	<i>Marvinbryantia</i>		33.6	<i>sp.Eubacteriumsuliincertae_sedis</i>		15.5
<i>Lactonifractor</i>	64.5	<i>Corynebacterium</i>		32.7	<i>Citrobacter</i>		14.5
<i>Asaccharobacter</i>	61.8	<i>Desulfovibrio*</i>		32.7	<i>Anaerofilum</i>		13.6
<i>Holdemania</i>	60.9	<i>Lactococcus</i>		31.8	<i>Olsenella</i>		13.6
<i>Bacillus</i>	59.1	<i>Acetivibrio</i>		30.9	<i>Peptoniphilus</i>		13.6
<i>Eisenbergiella</i>	59.1	<i>Allisonella</i>		30.9	<i>Shigella</i>		13.6
<i>Gordonibacter</i>	57.3	<i>Christensenella</i>		30.9	<i>Kluyvera</i>		12.7
<i>Intestinimonas</i>	51.8	<i>Senegalimassilia</i>		29.1	<i>Oribacterium</i>		12.7
		<i>Campylobacter</i>		23.6	<i>Porphyromonas</i>		12.7
		<i>Robinsoniella</i>		22.7	<i>Aggregatibacter</i>		11.8
		<i>Coprobacillus</i>		21.8	<i>Leuconostoc</i>		10
		<i>Parvimonas</i>		21.8	<i>Raoultella</i>		10
		<i>Parasporobacterium</i>		20.9			

Frequency of microbiota at species level showed a high prevalence of *Faecalibacterium prausnitzii* (5.28%), and then *Blautia wexlerae* (3.67%), *Fusicatenibacter saccharivorans* (3.41%), *Megamonas funiformis* (3.35%), *Collinsella aerofaciens* (3.21%), and *Bacteroides vulgatus* (3.12%). *Bifidobacterium adolescentis* (2.35%) and *B. longum* (1.92%), *Bacteroides uniformis* (2.22%), *B. plebelius* (1.96%), and *B. dorei* (1.71%), and *Akkermansia muciphila* (2.16%) were followed (Table 4).

Microbiota profile by rice category

Brown rice eaters showed less *Actinobacteria* (12.1 vs. 8.5% $p = 0.078$) and *Fusobacterium* (1.6 vs. 0.018%, $p = 0.011$) compared to white rice eater. At the genus level *Anaerostipes* (2.6 vs. 1.7% $p = 0.071$) and *Fusobacterium* (1.5 vs. 0.02% $p = 0.02$) were low in brown rice and high in *Alistipes* (0.46 vs. 0.92% $p = 0.01$) and *Ruminiclostridium* (0.08 vs. 0.42% $p = 0.027$) (Table 5).

There were many other bacteria to show difference, so selected bacteria which showed different prevalence by white rice and brown rice eaters further analyzed the correlation among them (Table 6). Although *Lachnoclostridium*,

Lactobacillus, *Interstiniabacter*, *Enterococcus*, *Mitsuokella*, *Tyzzerella*, and *Erysipelatoclostrium* in *Fermicutes* phylum, and *Alistipes* and *Odoribacter* in *Bacteroides* phylum showed significant correlation, *Ruminococcus*, *Fusicatenibacter* and *Anaerostipes* showed negative correlation with above genera. *Bifidobacterium* only showed positive correlation with *Fusobacterium*.

Chief component analysis of the above genera showed that 3rd axis showed significant difference between white rice and brown rice eaters (Table 7). Decrease of *Intestinibacter* and *Turicibacter* in 3rd component axis seemed to be influenced by brown rice eating.

Effects of dietary habit on bacterial profile

The possibility that the above changes of microbiota had been influenced by other food stuffs was evaluated from the baseline FFF data, because the intake of foods for side dishes showed a different characteristic by the rice category [12]. The food intake indexes were standardized by multiplying the daily frequency of food intake x standard dose per meal. Brown rice

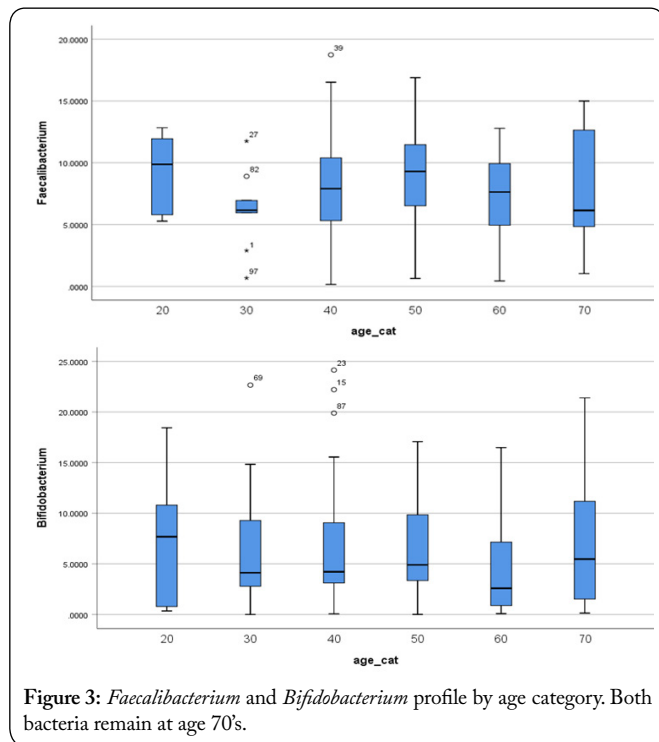


Table 4: Frequent microbiota above 0.8% profile in the 469 species level.

	Mean	Median	Max
<i>Faecalibacterium prausnitzii</i>	5.277	5.095	16.678
<i>Blautia wexlerae</i>	3.663	2.751	18.225
<i>Fusicatenibacter saccharivorans</i>	3.412	2.839	12.308
<i>Megamonas funiformis</i>	3.349	0.000	27.873
<i>Collinsella aerofaciens</i>	3.205	2.245	28.828
<i>Bacteroides vulgatus</i>	3.120	1.336	26.213
<i>Bifidobacterium adolescentis</i>	2.351	0.659	27.195
<i>Bacteroides uniformis</i>	2.220	1.550	9.82465
<i>Akkermansia muciniphila</i>	2.159	0.000	48.405
<i>Bacteroides plebeius</i>	1.955	0.000	24.374
<i>Bifidobacterium longum</i>	1.924	0.786	17.294
<i>Bacteroides dorei</i>	1.709	0.311	15.651
<i>Subdoligranulum variabile</i>	1.662	1.109	7.505
<i>Ruminococcus gnavus</i>	1.586	0.468	20.292
<i>Blautia luti</i>	1.536	0.870	12.353
<i>Ruminococcus faecis</i>	1.452	0.549	11.173
<i>Parabacteroides distasonis</i>	1.413	0.589	12.362
<i>Prevotella copri</i>	1.242	0.000	30.304
<i>Eubacterium hallii</i>	1.001	0.827	4.875
<i>Bacteroides stercoris</i>	0.994	0.008	11.780
<i>Bacteroides coprocola</i>	0.979	0.000	10.009
<i>Escherichia/Shigella</i>	0.911	0.045	29.707
<i>Ruminococcus obeum</i>	0.902	0.381	5.835
<i>Eubacterium rectale</i>	0.860	0.156	11.093

eaters consumed significantly more doses than white rice eaters in carrots, red beans, green yellow vegetables, radishes, ginger, burdocks, lotus roots, sweet potatoes, yam, sesame, peanuts, chestnuts, mushrooms, dried mushrooms, sea weed, kelp, and soy milk.

Correlation analysis between these food stuffs and microbiota profile did not coincide with brown rice, so the microbiota changed independently by rice eating habit (Table 8).

Discussion

Numerous animal models and human studies consistently demonstrated that gut microbiota can modulate host health [13-17]. We found that *genmai* (brown rice) eating was beneficial to keep healthy body weight, BMI and a good bowel movement [6, 18]. Brown rice eaters preferred to eat plant based Japanese foods, avoiding meat, and dairy products [12]. They disliked oily and spicy taste, and their selection was based upon fresh, organic, no additives, without genetically-modified foodstuff and domestic production.

A good bowel movement and the stool figure suggested a good intestinal environment which led to avoid obesity and kept one in health. A bowel movement of the brown rice eaters suggested a good intestinal environment.

We tried to clarify the differences in microbiota by brown rice and white rice, but the participants of white rice eaters in this nested study were quite healthy, and the number was small. They would not represent white rice eaters, so the comparison would be difficult. Although there would be a selection bias and many confounding factors, the results were obtained from more than 100 rice eaters, so the relationship between staple rice eating and intestinal microbiota was able to be evaluated.

Bacteroides (12.7%), *Blautia* (8.3%), *Faecalibacterium* (7.9%) were top 3 common genera, then *Bifidobacterium* (6.3%), *Prevotella* (5.3%), *Eubacterium* (4.9%), *Ruminococcus* (3.8%) were followed. *Faecalibacterium prausnitzii* (5.28%), and then *Blautia wexlerae* (3.67%) were most frequent at species level. The former was well known butyrate producer, and the latter is considered to control immunity.

Dietary fiber, rich in brown rice, was reported to have a good influence on bowel movements. It arranged intestinal environment by maintaining good bacterial flora [19-22]. *Bifidobacterium adolescentis* (2.35%) and *B. longum* (1.92%), and *Akkermansia muciniphila* (2.16%) were noteworthy because of their function for intestinal environment. Diets containing high levels of nondigestible dietary fiber was shown to pass the upper gut, and lead to higher levels of bacterial fermentation in the colon. It resulted in mildly acidic circumstance in the proximal colon that seem to fit butyrate production [23].

From the recent intestinal bacterial research short chain fatty acid, especially butyrate producing bacteria is a focus of studies. Butyrate becomes energy source for the colonic epithelial cells, maintain the gut barrier functions, and develop immune regulation and anti-inflammatory properties. The two most dominant bacterial species in the human colon

Table 5: Frequency of microbiota by rice consumption. Significant increase by ANOVA is shown in *Alistipes*, *Lachnospirillum*, *Lactobacillus*, *Enterococcus*, *Veillonella*, *Paraprevotella*, *Erysipelatoclostridium*, *Senegalimassilia*, *Citrobacter*, and decrease in *Anaerostipes*, *Lachnospirillum*, *Fusobacterium*, *Intestinibacter*, *Mitsuokella*, *Tyzzzerella*, *Odoribacter*, and *Turicibacter*.

		White rice				Mix				Brown rice				p
		Mean	SD	Median	Max	Mean	SD	Median	Max	Mean	SD	Median	Max	
<i>Bacteroides</i>	B	13.408	9.201	13.785	31.535	12.423	6.923	11.170	35.111	12.334	7.526	11.367	33.943	
<i>Blautia</i>	F	7.867	2.948	8.182	13.585	9.079	4.299	8.398	22.124	7.913	3.964	7.491	20.808	
<i>Faecalibacterium</i>	F	7.013	3.387	6.795	11.344	8.124	4.498	7.908	18.730	8.047	3.905	7.822	16.887	
<i>Bifidobacterium</i>	A	6.007	6.965	4.242	22.208	6.634	5.897	4.044	18.439	6.394	5.779	4.419	24.138	
<i>Prevotella</i>	B	7.380	12.812	0.012	37.164	4.055	8.605	0.009	29.089	5.626	10.394	0.011	35.147	
<i>Eubacterium</i>	F	4.772	3.312	4.384	12.359	4.452	2.436	4.318	10.810	5.264	2.756	4.926	11.605	
<i>Ruminococcus</i>	F	3.197	2.828	2.646	9.824	3.764	2.237	4.012	8.184	3.937	2.468	3.799	9.600	
<i>Fusicatenibacter</i>	F	3.018	2.559	2.642	7.812	2.604	1.679	2.309	7.337	2.719	2.188	2.300	11.448	
<i>Collinsella</i>	A	1.576	1.294	1.575	3.453	1.802	1.668	1.689	7.447	2.078	1.646	1.992	8.690	
<i>Streptococcus</i>	F	1.740	1.859	1.137	6.099	1.741	3.457	0.712	19.089	2.834	3.889	1.452	17.414	
<i>Subdoligranulum</i>	F	1.790	1.679	1.428	4.656	1.675	1.209	1.569	3.936	2.387	2.287	1.690	12.305	
<i>Anaerostipes</i>	F	2.571	1.533	2.332	5.077	1.514	1.088	1.370	4.043	1.658	1.560	1.260	7.212	0.066
<i>Akkermansia</i>	V	0.696	1.839	0.000	6.241	2.447	7.180	0.016	39.178	0.673	1.610	0.025	9.967	
<i>Roseburia</i>	F	2.008	1.802	1.406	6.987	1.681	1.548	1.388	7.798	1.771	1.626	1.366	9.402	
<i>Megamonas</i>	F	0.748	2.534	0.000	8.793	0.845	2.711	0.000	14.265	0.768	2.224	0.000	11.851	
<i>Parabacteroides</i>	B	0.646	0.472	0.395	1.457	0.789	0.551	0.632	1.999	0.839	1.090	0.454	6.881	
<i>Alistipes</i>	B	0.463	0.434	0.394	1.478	1.309	1.483	0.678	6.506	0.925	0.907	0.630	3.901	0.01
<i>Coprococcus</i>	F	1.628	2.111	0.794	6.978	1.017	1.025	0.715	3.544	1.116	1.045	0.789	4.994	
<i>Phascolarctobacterium</i>	B	0.669	0.832	0.283	2.274	0.798	1.066	0.207	3.224	0.736	0.898	0.343	3.300	
<i>Dorea</i>	F	0.929	0.651	0.674	2.249	0.730	0.558	0.695	2.029	0.706	0.464	0.637	2.576	
<i>Lachnospirillum</i>	F	1.048	1.165	0.643	3.837	0.648	0.473	0.528	2.215	0.658	0.568	0.450	3.211	0.076
<i>Escherichia</i>	P	0.056	0.097	0.020	0.339	0.779	2.748	0.012	12.050	0.214	1.029	0.013	7.636	
<i>Megasphaera</i>	F	0.213	0.724	0.000	2.511	0.288	1.449	0.000	8.065	0.605	1.876	0.000	10.339	
<i>Sutterella</i>	P	0.569	0.588	0.471	1.530	0.483	0.680	0.283	2.692	0.353	0.467	0.122	1.980	
<i>Romboutsia</i>	F	0.502	0.581	0.248	1.823	0.382	0.475	0.156	1.784	0.423	0.488	0.270	2.682	
<i>Ruminiclostridium</i>	F	0.082	0.127	0.035	0.397	0.312	0.711	0.034	3.475	0.427	1.192	0.052	9.116	
<i>Dialister</i>	F	0.345	0.860	0.003	2.983	0.444	0.798	0.007	2.843	0.380	0.664	0.011	2.634	
<i>Fusobacterium</i>	Fuso	1.548	5.357	0.000	18.557	0.034	0.187	0.000	1.042	0.018	0.092	0.000	0.684	0.02
<i>Lactobacillus</i>	F	0.012	0.027	0.003	0.096	0.460	1.416	0.008	6.422	0.200	0.579	0.018	3.242	0.011
<i>Clostridium</i>	F	0.202	0.318	0.030	0.980	0.215	0.372	0.028	1.715	0.232	0.441	0.044	1.866	
<i>Barnesiella</i>	B	0.316	0.622	0.024	2.043	0.347	0.537	0.197	2.038	0.203	0.346	0.024	1.940	
<i>Intestinibacter</i>	F	0.595	1.273	0.015	4.377	0.276	0.505	0.086	2.659	0.259	0.387	0.075	2.310	0.081
<i>Enterococcus</i>	F	0.039	0.131	0.000	0.454	0.011	0.050	0.000	0.279	0.459	1.632	0.003	7.665	0.045
<i>Veillonella</i>	F	0.046	0.046	0.042	0.147	0.100	0.201	0.011	0.924	0.227	0.613	0.033	3.858	0.022
<i>Paraprevotella</i>	B	0.013	0.043	0.000	0.148	0.265	0.523	0.000	2.066	0.215	0.485	0.000	2.542	0.002
<i>Enterobacter</i>	P	0.071	0.174	0.000	0.596	0.117	0.492	0.000	2.737	0.178	0.937	0.000	7.367	
<i>Mitsuokella</i>	F	0.508	1.761	0.000	6.099	0.344	1.418	0.000	7.667	0.066	0.230	0.000	1.240	0.05
<i>Tyzzzerella</i>	F	0.174	0.353	0.007	1.110	0.039	0.099	0.000	0.462	0.039	0.128	0.000	0.694	0.019
<i>Lachnospira</i>	F	0.192	0.441	0.000	1.394	0.222	0.496	0.013	1.972	0.206	0.396	0.024	1.724	
<i>Odoribacter</i>	B	0.391	1.145	0.037	4.020	0.255	0.397	0.174	2.095	0.148	0.126	0.123	0.521	0.093
<i>Notdetermined</i>		0.082	0.088	0.049	0.295	0.102	0.204	0.044	1.131	0.049	0.040	0.040	0.164	
<i>Erysipelatoclostridium</i>	F	0.020	0.021	0.011	0.051	0.082	0.138	0.030	0.660	0.065	0.116	0.020	0.486	0.005
<i>Bacillus</i>	F	0.212	0.511	0.001	1.763	0.181	0.290	0.064	1.332	0.143	0.253	0.002	0.948	
<i>Klebsiella</i>	P	0.000	0.000	0.000	0.000	0.056	0.187	0.000	0.825	0.050	0.289	0.000	2.315	

<i>Parasutterella</i>	P	0.121	0.264	0.023	0.939	0.191	0.342	0.030	1.467	0.124	0.227	0.027	0.973	
<i>Methanosphaera</i>	*	0.000	0.002	0.000	0.006	0.000	0.000	0.000	0.003	0.249	1.256	0.000	8.047	
<i>Turicibacter</i>	F	0.385	0.517	0.020	1.155	0.181	0.355	0.029	1.766	0.062	0.137	0.013	0.890	0.054
<i>Haemophilus</i>	P	0.145	0.160	0.083	0.466	0.091	0.142	0.021	0.648	0.120	0.251	0.032	1.734	
<i>Bilophila</i>	P	0.087	0.052	0.091	0.214	0.116	0.105	0.076	0.356	0.081	0.068	0.073	0.297	
<i>Flavonifractor</i>	F	0.041	0.046	0.028	0.135	0.078	0.113	0.036	0.410	0.064	0.112	0.023	0.758	
<i>Eggerthella</i>	A	0.077	0.105	0.039	0.375	0.095	0.146	0.033	0.557	0.051	0.071	0.018	0.281	
<i>Slackia</i>	A	0.029	0.070	0.000	0.228	0.078	0.129	0.000	0.447	0.066	0.114	0.000	0.472	
<i>Acidaminococcus</i>	F	0.143	0.397	0.000	1.388	0.028	0.122	0.000	0.678	0.034	0.104	0.000	0.729	
<i>Desulfovibrio</i>	*	0.080	0.187	0.000	0.550	0.013	0.040	0.000	0.216	0.064	0.186	0.000	0.939	
<i>Coprobacter</i>	F	0.063	0.148	0.006	0.517	0.065	0.151	0.000	0.686	0.061	0.127	0.000	0.517	
<i>Senegalimassilia</i>		0.020	0.041	0.000	0.106	0.076	0.122	0.000	0.399	0.064	0.140	0.000	0.827	0.036
<i>Catenibacterium</i>	F	0.037	0.084	0.000	0.217	0.141	0.474	0.000	2.092	0.083	0.470	0.000	3.157	
<i>Hafnia</i>	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.104	0.649	0.000	4.961	
<i>Butyrivibrio</i>	F	0.000	0.001	0.000	0.004	0.003	0.010	0.000	0.053	0.098	0.513	0.000	3.890	
<i>Terrisporobacter</i>	F	0.058	0.137	0.000	0.457	0.029	0.068	0.000	0.252	0.058	0.131	0.003	0.644	
<i>Citrobacter</i>	P	0.000	0.000	0.000	0.000	0.008	0.041	0.000	0.229	0.006	0.023	0.000	0.160	0.044
<i>Butyricimonas</i>	P	0.027	0.043	0.003	0.123	0.030	0.099	0.000	0.539	0.040	0.075	0.000	0.404	
<i>Hespellia</i>	F	0.033	0.041	0.020	0.138	0.034	0.042	0.033	0.172	0.031	0.033	0.024	0.137	
<i>Actinomyces</i>	A	0.036	0.053	0.016	0.151	0.023	0.035	0.014	0.197	0.031	0.031	0.019	0.150	
<i>Raoultella</i>	P	0.046	0.146	0.000	0.509	0.000	0.002	0.000	0.012	0.001	0.004	0.000	0.026	
<i>Asaccharobacter</i>	A	0.036	0.056	0.014	0.185	0.030	0.057	0.003	0.227	0.032	0.064	0.005	0.343	
<i>Lactococcus</i>	F	0.002	0.005	0.000	0.017	0.005	0.016	0.000	0.081	0.040	0.164	0.000	0.999	
<i>Sarcina</i>	F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.060	0.483	0.000	3.896	
<i>Eisenbergiella</i>	F	0.008	0.012	0.003	0.037	0.020	0.037	0.006	0.186	0.018	0.078	0.002	0.610	
<i>Acetivibrio</i>	F	0.018	0.061	0.000	0.213	0.012	0.032	0.000	0.135	0.029	0.062	0.000	0.303	
<i>Pseudoflavonifractor</i>	F	0.006	0.006	0.005	0.018	0.005	0.008	0.002	0.034	0.023	0.143	0.002	1.158	
<i>Lactonifactor</i>	F	0.008	0.017	0.001	0.061	0.010	0.012	0.006	0.057	0.011	0.031	0.003	0.198	
<i>Gordonibacter</i>	A	0.007	0.010	0.004	0.036	0.011	0.018	0.003	0.085	0.014	0.017	0.007	0.086	
<i>Allisonella</i>	F	0.007	0.015	0.000	0.039	0.012	0.022	0.000	0.076	0.011	0.025	0.000	0.146	
<i>Oscillibacter</i>	F	0.008	0.010	0.005	0.027	0.023	0.029	0.008	0.127	0.012	0.012	0.009	0.050	
<i>Butyricoccus</i>	F	0.000	0.000	0.000	0.000	0.038	0.210	0.000	1.168	0.001	0.006	0.000	0.043	
<i>Intestinimonas</i>	F	0.009	0.020	0.000	0.064	0.007	0.010	0.003	0.039	0.007	0.023	0.002	0.179	
<i>Holdemania</i>	F	0.007	0.012	0.003	0.037	0.006	0.009	0.002	0.034	0.009	0.012	0.005	0.067	
<i>Cloacibacillus</i>	Syner	0.020	0.066	0.000	0.229	0.001	0.003	0.000	0.014	0.018	0.080	0.000	0.532	
<i>Anaerotruncus</i>	F	0.009	0.017	0.000	0.048	0.008	0.021	0.000	0.113	0.007	0.015	0.002	0.073	
<i>Rothia</i>	A	0.008	0.011	0.003	0.032	0.010	0.024	0.004	0.135	0.012	0.019	0.004	0.071	
<i>Marvinbryantia</i>	F	0.001	0.004	0.000	0.014	0.007	0.015	0.000	0.065	0.012	0.030	0.000	0.200	0.007
<i>Granulicatella</i>	F	0.006	0.007	0.005	0.026	0.005	0.009	0.002	0.040	0.007	0.010	0.004	0.051	
<i>Weissella</i>	F	0.002	0.005	0.000	0.018	0.000	0.002	0.000	0.009	0.008	0.032	0.000	0.224	
<i>Oxalobacter</i>	P	0.006	0.012	0.000	0.039	0.005	0.008	0.000	0.036	0.006	0.010	0.000	0.043	
<i>Pseudobutyrvibrio</i>	F	0.005	0.008	0.001	0.021	0.002	0.006	0.000	0.031	0.005	0.015	0.000	0.110	
<i>Shigella</i>	P	0.000	0.000	0.000	0.000	0.003	0.010	0.000	0.043	0.002	0.012	0.000	0.094	
<i>Pyramidobacter</i>	Syner	0.000	0.000	0.000	0.000	0.002	0.006	0.000	0.035	0.008	0.045	0.000	0.299	
<i>Gemella</i>	F	0.002	0.003	0.000	0.009	0.003	0.007	0.000	0.038	0.003	0.004	0.000	0.022	
<i>Paraeggerthella</i>	A	0.000	0.001	0.000	0.004	0.000	0.000	0.000	0.000	0.001	0.007	0.000	0.055	
<i>Corynebacterium</i>	A	0.002	0.003	0.000	0.009	0.003	0.007	0.000	0.039	0.002	0.003	0.000	0.014	
<i>Christensenella</i>	F	0.001	0.002	0.000	0.008	0.003	0.006	0.000	0.024	0.002	0.005	0.000	0.024	
<i>Adlercreutzia</i>	A	0.003	0.010	0.000	0.036	0.001	0.003	0.000	0.017	0.003	0.015	0.000	0.117	

Table 7: Chief component analysis. Chief component 3, 10, 11 showed significant correlation with brown rice category.

	Component										
	1	2	3	4	5	6	7	8	9	10	11
<i>Butyricoccus</i>	0.84	-0.051	0.026	-0.048	-0.145	-0.095	-0.051	0.023	-0.009	0.21	0.013
<i>Erysipelatoclostridium</i>	0.803	0.042	-0.032	0.156	0.164	0.028	0.023	-0.056	-0.228	0.244	-0.083
<i>Tyzzerella</i>	0.766	-0.063	0.053	0.085	0.304	0.059	-0.109	-0.022	0.03	-0.188	-0.092
<i>Ruminococcus</i>	-0.462	0.185	0.093	0.264	0.163	-0.361	-0.184	-0.109	-0.169	0.312	-0.114
<i>Odoribacter</i>	-0.072	0.704	-0.115	-0.068	-0.133	-0.16	-0.155	0.036	-0.066	-0.126	-0.231
<i>Alistipes</i>	-0.062	0.688	0.062	0.01	-0.07	-0.059	-0.026	-0.179	0.226	-0.178	0.3
<i>Akkermansia</i>	0.052	0.560	0.059	0	0.248	0.143	0.082	0.013	0.048	0.39	0.032
<i>Intestinibacter</i>	0.059	-0.019	0.851	0.004	0.024	-0.129	-0.054	0.067	-0.179	0.022	0.109
<i>Turicibacter</i>	-0.052	-0.011	0.833	-0.046	-0.097	0.33	-0.02	-0.11	0.139	-0.08	-0.077
<i>Citrobacter</i>	0.051	-0.018	-0.003	0.854	-0.01	-0.013	0.015	0.056	-0.156	0.071	-0.151
<i>Lactobacillus</i>	0.032	-0.049	-0.039	0.664	0.137	0.015	-0.095	0.04	0.084	-0.209	0.123
<i>Anaerostipes</i>	-0.04	-0.132	-0.021	-0.465	0.3	0.121	-0.235	-0.001	-0.247	-0.013	-0.402
<i>Lachnoclostridium</i>	0.177	-0.062	-0.049	0.052	0.795	-0.064	-0.049	-0.022	0.12	0.062	-0.017
<i>Faecalibacterium</i>	0.134	-0.481	0.055	-0.099	-0.545	-0.351	-0.265	-0.02	0.131	-0.028	-0.096
<i>Fusobacterium</i>	0.02	-0.028	0.126	-0.022	0.038	0.854	-0.073	0.004	-0.033	-0.001	-0.067
<i>Mitsuokella</i>	0.015	-0.032	-0.024	-0.002	0.084	-0.063	0.806	-0.177	-0.136	-0.109	-0.049
<i>Senegalimassilia</i>	-0.108	-0.033	-0.047	-0.043	-0.101	-0.007	0.684	0.312	0.104	0.066	0.016
<i>Veillonella</i>	-0.009	-0.062	0.072	0.021	0.201	-0.111	-0.083	0.768	-0.097	-0.061	0.159
<i>Enterococcus</i>	-0.002	-0.014	-0.103	0.095	-0.255	0.108	0.16	0.723	0.16	0	-0.129
<i>Marvinbryantia</i>	-0.116	0.05	-0.114	-0.011	0.017	0.029	-0.068	-0.016	0.77	0.112	-0.026
<i>Bifidobacterium</i>	-0.067	-0.081	-0.249	0.042	-0.327	0.45	-0.099	-0.228	-0.566	-0.014	0.019
<i>Robinsoniella</i>	0.176	-0.119	-0.061	-0.128	0.026	-0.036	-0.061	-0.047	0.14	0.842	0.043
<i>Paraprevotella</i>	-0.087	0.006	0.03	-0.014	0.029	-0.031	-0.055	0.045	-0.063	0.035	0.866
			p = 0.009							p = 0.059	p = 0.047
ANOVA analysis between rice category											

Table 8: Correlation between microbiota and food stuffs by FFQ. Brown rice significantly correlated with *Firmicutes* and *Synergistetes*. Other foodstuffs do not show similar correlation.

	<i>Firmicutes</i>	<i>Bacteroidetes</i>	<i>Actinobacteria</i>	<i>Verrucomicrobia</i>	<i>Fusobacteria</i>	<i>Synergistetes</i>	<i>Euryarchaeota</i>	<i>Lentisphaerae</i>	<i>Brown rice</i>	<i>Croissant</i>	<i>Dsnish</i>	<i>Tofu</i>	<i>G-Y vegetable</i>	<i>Soy protein</i>	<i>Taro</i>
<i>Firmicutes</i>	1	-.286**	-0.198	-.339**	-.290**	-.245*	-.329**	-.225*	.207*	-0.173	0.012	.211*	0.085	-0.103	.261**
<i>Bacteroidetes</i>	-.286**	1	-.282**	0.024	-0.165	-0.094	0.029	0.077	-0.103	0.170	.202*	-0.021	-0.126	0.055	-0.137
<i>Actinobacteria</i>	-0.198	-.282**	1	-0.156	.258*	0.110	-0.113	-0.086	-0.050	0.094	0.154	-.219*	-0.096	-0.062	0.034
<i>Proteobacteria</i>	-0.086	-0.049	0.030	-0.021	0.055	-0.035	-0.009	-0.024	-0.053	-0.135	-0.041	.212*	.219*	0.164	-0.094
<i>Verrucomicrobia</i>	-.339**	0.024	-0.156	1	0.021	0.069	0.132	0.090	-0.094	.349**	-0.041	0.003	-0.063	0.040	-0.045
<i>Fusobacteria</i>	-.290**	-0.165	.258*	0.021	1	-0.033	-0.020	-0.018	-0.104	-0.027	0.014	-0.095	-0.007	-0.058	-0.088
<i>Synergistetes</i>	-.245*	-0.094	0.110	0.069	-0.033	1	.457**	.247*	.271**	-0.111	-0.062	-0.149	-0.167	0.044	-0.064
<i>Euryarchaeota</i>	-.329**	0.029	-0.113	0.132	-0.020	.457**	1	.564**	0.072	-0.069	-0.035	-0.118	-0.091	.212*	-0.058
<i>Lentisphaerae</i>	-.225*	0.077	-0.086	0.090	-0.018	.247*	.564**	1	0.036	-0.062	-0.072	-0.086	0.027	.418**	-0.086
<i>Deinococcus Thermus</i>	0.076	-0.046	-0.042	-0.028	-0.022	-0.029	-0.018	-0.011	0.150	-0.047	-0.054	-0.093	0.013	-0.053	-0.008
reject_per	-.358**	-.455**	-.203*	-0.042	0.054	0.199	.228*	0.126	-0.014	-0.171	-0.294**	-0.068	0.091	0.026	-0.096

with a significant contribution to butyrate production are *rectale* in cluster XIVa [24-27]. Cross-feeding interactions were also present between *Bifidobacteria* and other butyrate-

producing bacteria [28-30].

In the case of *F. prausnitzii* increased to reach a peak toward adolescence, decreased during adulthood and in the elderly [31]. In our study, the high composition of *F. prausnitzii* was maintained even in the old age by rice eating.

The distribution of butyrate producing bacteria among *Firmicutes* phylum seemed to be uneven [32]. Butyrate was not only produced from dietary fiber but from lactate, encompassing *A. caccae*, *A. butyraticus*, *A. hadrus*, and *E. hallii*. Furthermore, bacteria related to *Eubacterium hallii* and *Anaerostipes caccae* were shown to be able to convert acetate and lactate into butyrate [26, 33]. *Roseburia/E. rectale* species and *F. prausnitzii*, grew well at pH 5.5, whereas they declined dramatically at pH 6.5 when *Bacteroides* spp., became dominant. It has been shown that butyrate-producing colon bacteria, such as *Eubacterium rectale*, *Faecalibacterium prausnitzii*, and *Roseburia* spp., can consume prebiotics such as inulin type fructans (ITF).

Even though the number of white rice eater was small, some difference was found in the frequency of microbiota. This may be caused by some functional ingredients in the brown rice, such as γ -oryzanol, GABA and other components [34]. Feruloylated oligosaccharides was shown to have beneficial effects on metabolism and function [35].

Thus, the influence of brown rice on health is extremely large and could be called "medical rice" [34].

Comparison of our cases with other Japanese microbiota profile

Nishijima et al. [36], found that the composition of the Japanese gut microbiome showed more abundant in *Bifidobacterium* than other 11 nations by analyzing gut microbiota of 106 Japanese. The *Bifidobacterium/bacteroides* ratio predominance was also reported in Japanese school-age children [37]. *Bifidobacterium catenulatum* was present in 38% of the adults in other study [38]. Hisada et al. [10], reported predominance of *Firmicutes* as well as ours.

Recent community-wide analyses of the gut microbiota in Ireland showed that the intestinal microbiota varied by their dietary habits and living condition. [39, 40]

De Filippo et al. [41], compared the gut microbiota between Rural African and Italian children, and found that high dietary fiber consuming African in 2-6 years old (14.2 g/d) had low *Firmicutes* (27.3%) and high *Bacteroides* (57.7%), while low DF (8.4 g/d) consuming Italian children showed high *Firmicutes* (63.7%) and low *Bacteroides* (22.4%). Main staple food of Africans was millet grain, sorghum, black eyed peas, so the different composition of dietary fibers could lead different composition of microbiota. The dietary habits should cause such difference, so further analysis is necessary to clarify the relationship between coexistence or network of microbiota and *genmai* eating.

The weakness of the study is the selection bias of healthy people's participation. Diet can easily change the microbiota, but an ordinary diet could show stable microbiota composition.

Stable presence of the dominant butyrate producing bacteria, and the relationship to other bacteria at genus level was clarified. Furthermore, suppressed *Fusobacterium* may contribute to health. These could be clarified more in a follow up study in the future. *Genmai* eaters in the macrobiotic groups are usually calm and peaceful. In addition to the health benefit, recent study on the microbiota-gut-brain relationship is also a target of future study [42, 43].

Conclusion

Habitual rice eaters showed *Faecalibacterium* and *Blautia* dominant profile of intestinal microbiota. *Faecalibacterium prausnitzii*, and then *Blautia wexlerae* were most frequent at species level. Brown rice eaters showed additional benefits by high prevalence of *Firmicutes* and low prevalence of *Fusobacterium*. These combinations of profiles could lead to integrated healthy life.

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Conflict of Interest

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