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Relationships between barley consumption and gut microbiome characteristics in a healthy Japanese population: a cross-sectional study

（健康な日本人集団における大麦摂取と腸内細菌叢の関係：横断調査）

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Relationships between barley consumption and gut microbiome characteristics in a healthy Japanese population: a cross-sectional study

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目次

Abstract 1
Abbreviations 2
Background 3
Methods 4
Study design 4
Measurements 5
DNA extraction and 16S rRNA gene amplicon sequencing 5
Bioinformatics analysis 6
Statistical analyses 7
Results 8
Participants 8
Enterotypes 12
Microbiome 13
Multiple regression analyses of all the participants 14
Network analysis of microbiome 15
Discussion 15
Declarations 18
Ethics approval and consent to participate 18
Consent for publication 18
Availability of data and materials 19
Competing interests 19
Funding 19
Authors' contributions 19
Acknowledgements 19
References 20
Supplementary data

Abstract

Background: Barley contains abundant soluble β-glucan fibers, which have established health benefits. In addition, the health benefits conferred by the gut microbiota have attracted considerable interest. However, few studies have focused on the barley intake and microbiota of the Japanese population. In this study, we aimed to identify the relationship between the barley consumption and gut microbiota composition of the Japanese population.

Methods: A total of 236 participants were recruited in Japan, and 94 participants with no complications of diabetes, hypertension, or dyslipidemia were selected for the study. We analyzed fecal samples from the participants, their medical check-up results, and responses to questionnaires about dietary habits. The participants were grouped according to their median barley intake. Then, we assessed the relative abundance of 50 microbial genera. Characteristic bacteria were evaluated for their relationship with barley by multiple regression analysis, adjusted for disease and dietary habits in all participants. We also analyzed the networks and clustering of the 20 selected genera.

Results: According to the comparison between the barley groups, *Bifidobacterium*, *Butyricicoccus*, *Collinsella*, *Ruminococcus 2*, and *Dialister* were characteristic candidate microbiota of the group that consumed large amounts of barley ($P < 0.05$). The relationship between barley and *Bifidobacterium* remained after adjusting for disease and dietary habits, and *Butyricicoccus* remained after adjusting for disease. Furthermore, network and cluster analyses revealed that barley consumption was directly correlated with *Bifidobacterium* and *Butyricicoccus*.

Conclusions: Barley consumption generates changes in the intestinal microbiota of the Japanese population. We found that *Bifidobacterium* and *Butyricicoccus* abundance was positively associated with barley consumption.

Keywords: barley; beta-glucan; microbiome; *Bifidobacterium*; *Butyricicoccus*

Abbreviations

VIF: Variance inflation factor

PCoA: Principal coordinate analysis

FDA: False discovery rate

SCFA: Short-chain fatty acid

Background

The gut microbiota is important for health, and thus, several investigations have focused on the human gut microbiome. It has been reported that diet can alter the gut microbiome [1]. Microbiota-accessible carbohydrates, such as dietary fiber [2], are resistant to digestion in the small intestine and enter the large intestine undigested; therefore, they are likely to improve host metabolism [2].

Barley is an important cereal that contains the soluble fiber β-glucan [3]. Barley improves metabolic dysfunction, increases the diversity of the gut microbiota, and increases bacteria such as *Blautia* [4]. Additionally, barley lowers postprandial blood glucose in healthy [3] and diabetic patients [5] and lowers cholesterol concentrations in Japanese people with mild metabolic syndrome after 12 weeks of consumption [6]. Therefore, barley consumption has many potential benefits to global health.

Barley consumption affects the gut microbiota and host health [7, 8]. For example, a crossover study in Sweden found that barley intake increased blood concentrations of butyric acid (produced by intestinal bacteria) and decreased postprandial hyperglycemia [7]. Research in the USA found that barley intake increased the gut microbiota diversity and *Blautia* abundance and improved host cholesterol levels [4]. Another Swedish study found that barley intake promoted a high ratio of *Prevotella*/*Bacteroide*s and improved host blood glucose metabolism [4]. These results suggest that barley might beneficially modulate the composition of the gut microbiota and improve host metabolic health.

Indeed, the effects of barley on the gut microbiota have been established in several countries [4, 7, 8]. Furthermore, as the intestinal microbiota is affected by dietary habits and genetic factors, it is important to evaluate its effectiveness in different populations [9]. The Japanese gut microbiota is characterized by abundant *Blautia* and *Bifidobacterium*, which utilize the dietary fiber from seaweed, a common item in the Japanese diet [1]. Consuming barley with white rice is also a specific dietary habit

in Japan, but little is known about its effects on the microbiota. Most studies on barley and the microbiota have regarded exhaled hydrogen concentrations as an alternative measurement of intestinal bacteria [7, 10, 11]; however, few studies have analyzed the relationship between the microbiota and barley consumption. Therefore, we aimed to define the relationship between barley and gut microbiota composition in healthy Japanese adults using next-generation sequencing.

Methods

Study design

The study was approved by the Yamanashi University Ethics Committee (approval No. 1824), the National Institutes of Biomedical Innovation, Health and Nutrition Ethics Committee (approval No. 169-04), and the Chiyoda Paramedical Care Clinic Ethics Committee (approval No. 15000088). This study was conducted in accordance with the Declaration of Helsinki (2013) and was based on a registered study (UMIN000033479). Cross-sectional data were evaluated from the first year of the study to undertake an exploratory overview of the gut microbiome of a population that consumes barley. Sampling was conducted from August 2018 to March 2019.

We enrolled 272 participants, which were employees of the barley processing company Hakubaku Co., Ltd. Our target sample included at least 100 participants. We excluded those with disorders (Risk 2) and pre-disorders (Risk 1) of diabetes, hypertension, and dyslipidemia from the main analysis. Details of the exclusion criteria for disorders are shown in **Table S1** (see Additional file 1). We classified the participants into two groups based on the median barley consumption rate (high, 32.3–253 and low, 0–31.8 g/1000 kcal·d.

Measurements

The primary outcome was the association between barley consumption and the alpha-diversity of the microbiome, and the secondary outcome was the abundance of the 50 dominant microbiota sorted by mean relative abundance (**Tables S4 and S5**, see Additional file 1). We collected a copy of the participants' medical check-up results. Dietary habits other than barley consumption were assessed using a brief self-administered diet history questionnaire (BDHQ) (Gender Medical Research, Inc., Tokyo, Japan). Barley consumption (g/1000 kcal·d) was calculated using a questionnaire and the daily energy value from the BDHQ. Rice bowl size (200, 160, 140, and 100 g), proportion of barley mixed with white rice (0%, 5%, 10%, 15%, 30%, and 50%), barley-mixed rice consumed per month (0, 0.5, 1, 4, 8, and 16 days/month), and barley consumption rate (g/d) were determined. Medical history, including medication (especially during the month of sampling), and consumption of fermented foods and supplements were determined using questionnaires.

DNA extraction and 16S rRNA gene amplicon sequencing

Fecal samples were collected at home with guanidine thiocyanate (GuSCN) solution, and DNA was extracted and stored at 10–30 °C for up to 30 d [12]. Briefly, 0.2 mL of fecal samples, 0.3 mL of No. 10 lysis buffer (Kurabo Industries Ltd., Osaka, Japan), and 0.5 g of 0.1-mm glass beads (WakenBtech Co., Ltd., Tokyo, Japan) were homogenized using a PS1000 Cell Destroyer (Bio Medical Science, Tokyo, Japan) at 4260 rpm for 50 s at 25 °C. The homogenate was centrifuged at 13 000 × g for 5 min at 25 °C, and the DNA was extracted from the supernatant using a Gene Prep Star PI-80X automated DNA isolation system (Kurabo Industries Ltd). DNA concentration was determined with the ND-1000 NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The samples were

stored at −30 °C. The 16S rRNA gene was amplified from fecal DNA and sequenced [12]. The V3–V4 region of the 16S rRNA gene was amplified using the following primers (5′→3′): TCGTCGGCAGCGTCAGATGTGTATAAGCGACAGCCTACGGGNGGCWGCAG and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. The DNA library for Illumina MiSeq was prepared using Nextera XT Index Kit v2 Set A (Illumina Inc., San Diego, CA, USA), and its concentration was determined with the QuantiFluor dsDNA System (Promega Corp., Madison, WI, USA). The 16S rRNA gene was sequenced using Illumina MiSeq (Illumina) as described by the manufacturer.

Bioinformatics analysis

The sequence reads from Illumina MiSeq were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (v1.9.1) [13]. We used QIIME Analysis Automating Script (Auto-q) [14] to proceed from trimming paired-end reads to operational taxonomic unit (OTU) selection. We used open-reference OTU picked with the UCLUST software against the SILVA v128 reference sequence to select OTUs based on sequence similarity (>97%). The taxonomy (phylum, class, order, family, and genus) and relative abundance were calculated using the SILVA v128 database [13, 14]. The intestinal microbiota was compared in 10 000 randomly selected reads per sample.

Statistical analyses

Calculation of alpha-diversity

Data were exported as BIOM files and imported into R (version 3.6.0). Diversity was analyzed using the phyloseq R-package. Alpha-diversity indices of observed OTU, Chao 1, Shannon, and Simpson indices were calculated using the estimate_richness function.

Comparison of barley groups

To compare the results of the medical check-ups and dietary habits between the high and low barley groups, we used Student's *t*-tests. The alpha-diversity and relative abundance of each genus were analyzed using Mann–Whitney *U*-tests. *P* values were adjusted using false discovery rate (FDR) methods. To confirm the reliability of the analyses, we explored the relationship between barley consumption groups (0 = low and 1 = high) and each microbiome using multiple regression analyses with all participants. We expressly set the amounts of *Bifidobacterium*, *Butyricicoccus*, *Collinsella*, *Ruminococcus 2*, and *Dialister* as outcomes. We adjusted the model for age, sex, risk of diabetes, dyslipidemia, and hypertension for model 1. In addition to model 1, we adjusted the model for consumption rate (g/1000 kcal) of cereals, sugar and sweetener, legumes, and beverages for model 2 and for cereals, sugar and sweetener, legumes, beverages, green vegetables, other vegetables, fish, and confectionery for model 3. We used the vif function of the car R-package to evaluate variance inflation factors (VIFs). All VIFs were < 5 and considered acceptable for these analyses [15].

Principal coordinate analysis of microbiomes

We classified the participants into enterotypes A, B, and C using the pam function of the cluster R-package. We then summarized the composition of intestinal microbiota by principal coordinate analysis (PCoA) using the vegdist function of the vegan R-package and the quasieuclid and dudi.pco

functions of the ade4 R-package. Data were calculated using the Bray–Curtis method. Subsequently, the environmental factor arrows were fit to the PCoA figure using the envfit function of the vegan R-package. Significant genera were assessed using permutations of environmental variables.

Network analysis of significant microbiomes and barley

To visualize the associations between barley and 20 microbiome genera selected by $P < 0.1$, we implemented a network analysis (Table S4 and S5, see Additional file 1). The network is shown with lines of correlation with $|r| > 0.15$ (Kendall rank-sum tests). Different colors on the plots indicate different community groups. We fit the correlation data frame to the cc.df function of the igraph R-package using the reshape2 R-package and calculated microbiota community groups using the leading.eigenvector.community function.

All analyses were carried out in R (version 3.6.0), and tests were two-sided; $P < 0.05$ was considered significant. All graphs except for those from the network analysis were created with the R-package ggplot2 [16].

Results

Participants

We obtained informed consent from 272 individuals, of which 33 participants resigned, and six were excluded because of non-compliance and data loss. Ninety-four participants had no disorders and were included in the main analyses, and 236 were included in the multiple regression analyses (**Figure 1**). Additionally, the numbers of participants who consumed antibiotic drugs, laxative drugs, and drugs

or supplements for controlling intestinal function (prebiotics and probiotics) were 4, 6, and 10, respectively, out of 236 participants. We included these participants in the analyses.

The characteristics of the barley groups did not significantly differ (**Table 1**). However, fasting glucose concentrations tended to be higher in the high barley group ($P = 0.054$). Disorder risk did not significantly differ between the groups (Table S1, see Additional file 1). **Table 2** and **Table S2** (see Additional file 1) show the differences in dietary habits between the barley groups. The barley intake was 13.9 ± 11.3 g/1000 kcal and 70.9 ± 43.6 g/1000 kcal in the low and high barley groups, respectively. The consumption rates of dietary fiber ($P < 0.001$), cereals ($P = 0.048$), sugar and sweetener, and beverages were significantly different between the barley groups, and those of legumes ($P = 0.056$) tended to be higher in the high barley group. Therefore, we selected these four dietary categories for adjustment in the multiple regression analyses (model 2). Additionally, green leafy vegetables, carrot, and pumpkin in the green vegetable category, boiled fish in the fish category, and Japanese confectionery and ice cream in the confectionery category had a significant or slight difference between the barley groups. Also, seaweed and mushroom, which are known to contain soluble fiber, were in the "other vegetable" category. Therefore, we added these four variables to model 2 and conducted multiple regression analyses (model 3).

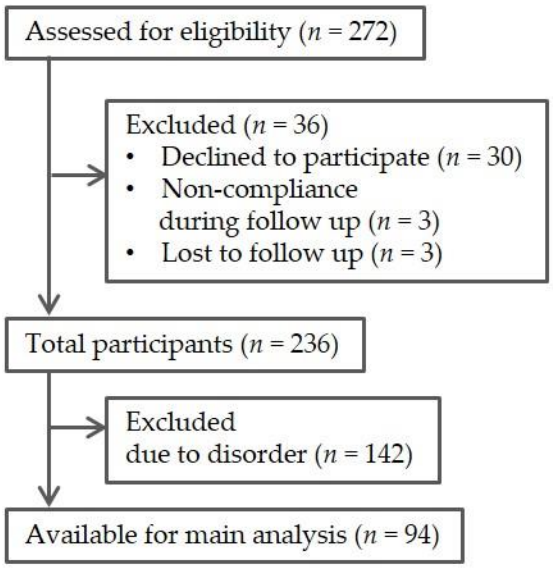

Figure 1 Flow chart of participants. Disorder means risk of diabetes, hypertension, and dyslipidemia.

Table 1 Characteristics of the participants in the barley groups

Variable	Total (*n* = 94) 0–253 g/1000 kcal d	Low barley[1] (*n* = 47) 0–31.8 g/1000 kcal·d	High barley[1] (*n* = 47) 32.3–253 g/1000 kcal·d	*P* [2]
Male, *n* (%)	54 (57%)	32 (68%)	22 (47%)	
Age (years)	36 ± 10	36 ± 10	36 ± 11	0.82
Body mass index (kg/m^2)	21.5 ± 3.4	21.3 ± 4.1	21.6 ± 2.5	0.67
Systolic pressure (mmHg)	111 ± 11	110 ± 11	112 ± 11	0.25
Diastolic pressure (mmHg)	68 ± 9	67 ± 9	69 ± 8	0.39
Fasting glucose (mg/dL)	86 ± 7	84 ± 7	87 ± 7	0.054
HbA1c (%)	5.3 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	0.26
TG (mg/dL)	69 ± 28	67 ± 30	71 ± 26	0.42
HDL-cholesterol (mg/dL)	65 ± 13	66 ± 13	63 ± 12	0.26
LDL-cholesterol (mg/dL)	95 ± 15	93 ± 14	97 ± 16	0.15

Data are shown as means ± SD or n (%). [1]Range of barley consumption.

[2]Student's *t*-test

Table 2 Comparison of diet between the barley groups

Variable	Low barley (*n* = 47) 0–31.8 g/1000 kcal·d[1]	High barley (*n* = 47) 32.3–253 g/1000 kcal·d[1]	*P* [2]
Daily Nutrition			
Energy (kcal)	1693 ± 456	1765 ± 562	0.50
Protein (g/1000 kcal)	35 ± 6	34 ± 6	0.58
Fat (g/1000 kcal)	30 ± 6	30 ± 6	0.85
Carbohydrate (g/1000 kcal)	130 ± 20	129 ± 18	0.75
Dietary fiber (g/1000 kcal)	4.9 ±1.3	6.1 ±1.7	<0.001
Na (mg/1000 kcal)	3608 ± 948	3871 ± 1094	0.22
Dietary habits (g/1000 kcal ·d)			
Barley	13.9 ± 11.3	70.9 ± 43.6	<0.001
Cereal	213 ± 55	236 ± 58	0.048
Legumes	20 ± 15	27 ± 20	0.06
Green vegetable	37 ± 23	44 ± 32	0.2537
Other vegetable	62 ± 29	72 ± 45	0.2001
Fruit	36 ± 32	35 ± 30	0.87
Fish	32 ±15	31 ±16	0.74
Sugar and sweetener	3.9 ± 3.7	2.4 ± 2.4	0.03
Confectionery	26 ± 20	25 ± 17	0.81
Beverage	439 ± 271	335 ± 226	0.047

Data are shown as means ± SD.

[1]Range of barley consumption.

[2] Student's *t*-test

Enterotypes

Figure 2a describes the number of participants with each enterotype. Enterotype B was dominant and enterotypes A and C were less abundant in each group. The numbers of each enterotype did not significantly differ between the groups (P = 0.20). Figure 2b shows an overview of the PCoA of the microbiome. The distribution of the plots was laid out in a "∧" shape and separated into three groups. Clusters A and B were distributed in the negative direction of PCoA1 and cluster C in the positive direction. Clusters A and B were divided by PCoA2, and cluster B was in an intermediate position between A and C. The driven genera in each enterotype were *Bacteroides* (A), *Blautia* (B), and *Prevotella 9* (C) (correlation coefficients; all P < 0.001). The most common enterotypes of the 94 participants were in the following order: B (60%), A (28%), and C (13%). The distribution of enterotypes was similar between the barley groups (B > A > C) and tended to be in the positive direction of PCoA2 in the high barley group, but the values were not significant (correlation coefficient, P = 0.08, **Figure S1**, see Additional file 1).

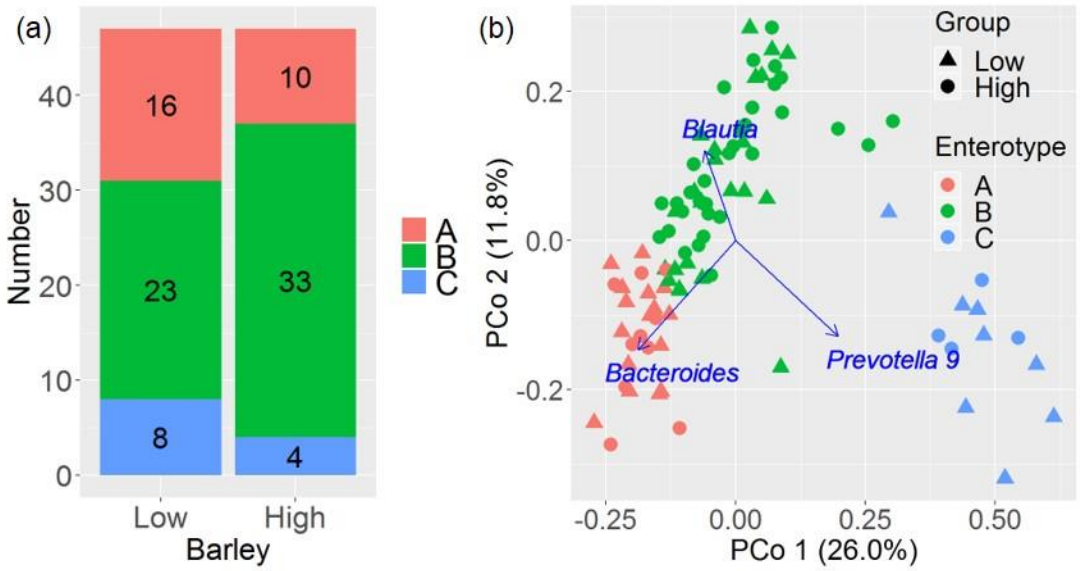

Figure 2 Microbiome enterotypes in high and low barley groups (*n* = 94) aged 19–65 years in 2018. (a) Comparison of numbers of enterotypes A, B, or C in each group. (b) Plot of PCoA. Color indicates enterotype; symbols indicate high or low barley consumption. Arrows indicate the top three environmental factors.

Microbiome

Alpha-diversity did not significantly differ between the barley groups (**Table S3**, see Additional file 1). The high barley group had a higher abundance of *Bifidobacterium* (*P* = 0.01), *Collinsella* (*P* = 0.03), *Butyricicoccus* (*P* = 0.002), *Dialister* (*P* = 0.04), and *Ruminococcus 2* (*P* = 0.04) without FDR adjustment (**Table 3**, Table S4, see Additional file 1). *Subdoligranulum* (*P* = 0.08), *Anaerostipes* (*P* = 0.0502), *Acidaminococcus* (*P* = 0.07), and Ruminococcaceae UCG-013 (*P* = 0.06) tended to be high in the high barley group. Therefore, we selected *Bifidobacterium*, *Collinsella*, *Butyricicoccus*, *Dialister*, and *Ruminococcus 2* as candidate characteristic genera associated with barley consumption.

Table 3 Relative abundance (%) of microbiome bacterial genera in low and high barley groups

	Low barley (*n* = 47) 0-31.8 g/1000 kcal·d [1]	High barley (*n* = 47) 32.3-253 g/1000 kcal·d [1]		
Genus (relative abundance %)	Median (interquartile range)	Median (interquartile range)	*P* [2] (crude)	*P* [3] (FDR)
Bifidobacterium	2.73 (0.00, 5.10)	5.61 (0.00, 8.12)	0.01	0.37
Collinsella	0.92 (0.00, 1.82)	1.95 (0.00, 2.20)	0.03	0.42
Subdoligranulum	1.12 (0.00, 1.80)	1.83 (0.00, 2.32)	0.08	0.44
Anaerostipes	0.89 (0.02, 1.28)	1.06 (0.07, 1.92)	0.0502	0.42

Butyricicoccus	0.43 (0.00, 0.46)	0.65 (0.12, 0.65)	0.002	0.09
Ruminococcaceae *UCG-013*	0.09 (0.00, 0.27)	0.22 (0.00, 0.34)	0.06	0.44
Dialister	0.00 (0.00, 0.31)	0.07 (0.00, 0.43)	0.04	0.42
Acidaminococcus	0.00 (0.00, 0.30)	0.03 (0.00, 0.66)	0.07	0.44
Ruminococcus 2	0.00 (0.00, 0.37)	0.01 (0.00, 0.86)	0.04	0.42

[1]Range of barley consumption rate

[2] Mann–Whitney *U*-test (crude *P* value)

[3] Mann–Whitney *U*-test adjusted with FDR (false discovery rate) method

Multiple regression analyses of all the participants

We assessed associations between barley and *Bifidobacterium*, *Butyricicoccus*, *Collinsella*, *Dialister*, and *Ruminococcus 2* using multiple regression analyses (**Table 4**, **Table S6**, see Additional file 1). The results of model 1 and 2 are shown in Table 4, and the results of model 1–3 are shown in Table S6 (see Additional file 1) with detailed data. *Bifidobacterium* had a consistent relationship with barley, and *Butyricicoccus* had a relation in model 1. Other bacteria had no significant relationship with barley.

Table 4 Association between the intestinal bacteria and barley intake[1] by multivariate linear regression analyses.

	Crude[2]		Model 1[3]		Model 2[4]	
Genus	r (SE)	*P*	R (SE)	*P*	R (SE)	*P*
Bifidobacterium	2.52 (0.70)	0.012	2.52 (1.00)	0.012	2.61 (1.03)	0.012
Butyricicoccus	0.11 (0.05)	0.03	0.11 (0.05)	0.03	0.08 (0.05)	0.102
Collinsella	0.23 (0.30)	0.45	0.27 (0.30)	0.38	0.26 (0.31)	0.41
Dialister	0.08 (0.09)	0.37	0.10 (0.09)	0.24	0.08 (0.09)	0.41
Ruminococcus 2	0.10 (0.15)	0.47	0.10 (0.15)	0.53	0.11 (0.16)	0.48

[1]The barley intake group 0: low barley group (0–32.3 g/1000 kcal), 1: high barley group (32.9–253 g/1000 kcal)

[2]Crude [r (SE)]: Coefficients of a single linear regression model.

[3]Model 1 [R (SE)]: Adjusted with sex, age, risk of diabetes, dyslipidemia, and hypertension.

[4]Model 2 [R (SE)]: In addition to model 1, adjusted with an intake of cereal, sugar and sweetener, legume, and beverage.

Network analysis of microbiome

Figure S2 (see Additional file 1) shows the results of the network analysis. *Bifidobacterium*, *Butyricicoccus*, *Ruminococcus 2*, Ruminococcaceae *UCG-013*, *Lachnospira*, and *Tyzzerella 3* were directly associated with barley intake, and except for *Ruminococcus 2*, they were classified in the same community group. *Anaerostipes* was not directly associated with barley intake but was associated via *Lachnospira*, Ruminococcaceae *UCG-13*, or *Tyzzerella 3* to barley intake. In addition, *Anaerostipes* belonged to the same community as barley, *Butyricicoccus*, and *Bifidobacterium*. *Ruminococcus 2* was directly associated with barley but did not belong to the same community group as barley, *Butyricicoccus*, *Bifidobacterium*, and *Anaerostipes*. Finally, *Dialister* and *Collinsella* seemed to have no relation with barley because they were positioned far from barley and classified in different groups.

Discussion

We identified the characteristics of the gut microbiota in a Japanese population that consumes barley. Previous studies reported that the soluble fiber in barley increases the prevalence of intestinal bacteria, such as *Prevotella 9* and *Blautia*, and alpha-diversity [4, 8]. In our study, *Bifidobacterium* and *Butyricicoccus* abundance tended to increase (Table 4), and we consider these bacteria as specific

candidates that relate to barley consumption. However, barley intake might have a limited effect because there were no changes in alpha-diversity.

The consumption of barley tended to be positively related to PCoA2, but this result was not significant (p = 0.08, Figure S1, see Additional file 1). The numbers of each enterotype did not differ between the barley groups. The absolute numbers of enterotype B were 33 (high) and 23 (low). These results suggested that barley consumption slightly shifted the enterotype to B; however, further studies are necessary to confirm this result. Our results of PCoA (Figure 2b) were similar to those of Arumugam et al. [9], in which the distribution is in a "Λ" shape comprising enterotypes A (driven by *Bacteroides*), B (driven by *Blautia*), and C (driven by *Prevotella 9*). However, the influential genus of enterotype B, *Blautia*, was different [9]. Arumugam et al. reported that the driving genera of enterotype A (*Bacteroides*) and C (*Prevotella 9*) are robust between cohorts, but for B it depends on the cohort. Furthermore, *Blautia* was reported as one of the major driving genera [9].

In this study, *Bifidobacterium* and *Butyricicoccus* were identified as characteristic bacteria in the high barley group. *Bifidobacterium* was associated with rye-containing beta-glucan consumption in a randomized control trial [17] and increased with dietary fiber in a systematic review [18]. *Butyricicoccus* was associated with barley in an animal study [19]. Most bacteria relatively close to barley in the network analyses (Figure S2, see Additional file 1) are known for their characteristics of producing short-chain fatty acids (SCFAs). For example, *Butyricicoccus*, *Subdoligranulum*, *Ruminococcus 2*, and Ruminococcaceae *UCG-013* belong to the Ruminococcaceae family, which is well-known for producing SCFAs. *Anaerostipes* belongs to the Lachnospiraceae family, which is also known to produce SCFAs. Many bacteria were more highly correlated with other bacteria than with barley. This suggests that in addition to the effects of barley intake, there are many bacteria that are indirectly altered by interactions

between bacteria. *Bifidobacterium* has been reported to produce SCFAs, such as acetic acid from carbohydrates [20], and are known as characteristic bacteria of Japanese people [1]. *Butyricicoccus* is a prevalent butyric acid producer associated with inflammatory bowel disease prevention [21]. Additionally, SCFA production from dietary fiber can acidify the gut environment, which alters the composition of the microbiota [22]. Therefore, these barley-related bacteria might have a positive influence on the hosts.

Although an association with barley was suggested, the results for *Bifidobacterium* may not be unique to barley, as *Bifidobacterium* abundance has been reported to increase with the consumption of rye [17] and other dietary fiber [18]. *Bifidobacterium* itself is a characteristic bacteria found in abundance in the Japanese population [1]. Therefore, it may increase in abundance with a typical Japanese diet. Most barley intake is from barley mixed with rice, which is a typical Japanese food pattern, so other dietary factors may be confounding. However, in this study, the association between *Bifidobacterium* and barley was observed even after adjusting for various dietary factors (Table 4, Table S6, see Additional file 1), so we consider the results robust. Although there are few reports on *Butyricicoccus* in humans, randomized controlled trials have shown that its abundance increased after consuming a Mediterranean diet [23]. Because *Butyricicoccus* is highly capable of fermenting dietary fiber, it is possible that the increase was caused by the whole grains, legumes, and vegetables in the Mediterranean diet, but the details are not known. This is the first study to show an association between barley and *Butyricicoccus* in humans, and it will be interesting to see whether the effect was specific to barley or this cohort. However, because the association between barley and *Butyricicoccus* was found only in model 2, confounding by other dietary factors cannot be ruled out, limiting the interpretation of the present results.

This study has several limitations. The participants were employees of a company that manufactures barley products and would thus most likely consume more barley in general. The possibility of other confounding factors such as dietary habits cannot be completely excluded; thus, the effects of barley could be overestimated. We did not measure SCFAs or microbiome functions, which restricts the usefulness of our results. A follow-up intervention study with another cohort is warranted to overcome these limitations.

In conclusion, barley might change the intestinal microbiota of the Japanese population. We selected *Bifidobacterium* and *Butyricicoccus* as candidate characteristic genera indicating barley consumption.

Declarations

Ethics approval and consent to participate

The study was approved by the Yamanashi University Ethics Committee (approval No. 1824), the National Institutes of Biomedical Innovation Health and Nutrition Ethics Committee (approval No. 169-04), and the Chiyoda Paramedical Care Clinic Ethics Committee (approval No. 15000088). The study was registered with the University Hospital Medical Information Network (ID: UMIN000033479). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files). Other data described in the manuscript, code book, and analytic code will be made available from corresponding author upon reasonable request.

Competing interests

The department where Z.Y. works has received research grants for other studies from Hakubaku Co., Ltd. T.M., Y.G., M.N., S.N., and T.K. are employees of Hakubaku Co., Ltd. All other authors have no Competing Interest to declare.

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Authors' contributions

Project administration: T.M., K.H., Y.G., H.M., T.K., M.N., S.N., and K.K. Resources: H.M., K.K., M.M., K.H., J.P., H.K., K.M., and J.K. Investigation and Writing, original draft: T.M., K.H., and J.P. Writing, revising and editing: T.M., J.K., and Z.Y. All authors have read and approved the final manuscript.

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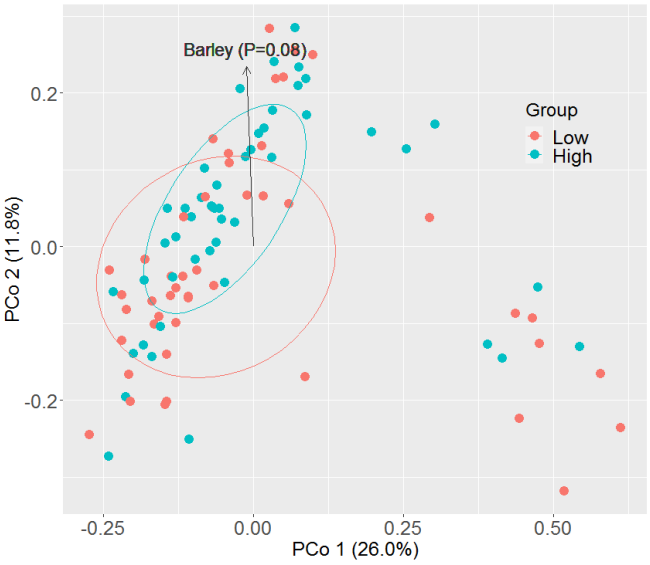

Figure S1 PCoA result. Color means high or low consumption of barley. The arrow means high barley group (*n* 94, aged 19-65 years, Japan, 2018).

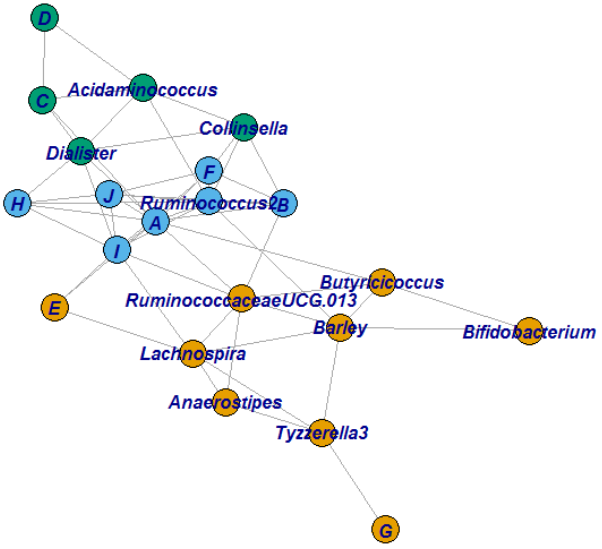

Figure S2 The result of network analysis. Line means correlation $|r|>0.15$ (Kendall rank-sum tests). Colors mean community groups. Described names are those in which the difference between low barley and high barley is $P<0.1$ (Mann-Whitney *U*-test) (*n* 94, aged 19-65 years, Japan, 2018).

The bacteria described as characters are as follow; *A*: *Subdoligranulum*, *B*: *Eubacterium Hallii group*, *C*: *Prevotella2*, *D*: *Megasphaera*, *E*: *Roseburia*, *F*: *Veillonella*, *G*: *Lachnospiraceae family uncultured*, *H*: *Dorea*, *I*: *Ruminococcus1*, *J*: *Eubacterium coprostanoligenes group*.

Table S1 Criterion of risk group of diabetes, hypertension, and dislipidemia.

Variable	*n* (Male)	Low barley (*n* 119)	High barley (*n* 117)	Criterion
Diabetes risk				
Risk 2	8 (8)	3	5	HbA1c>=6.5% or Fasting blood glucose>=126 mg/dL or Under medication
Risk 1	5 (5)	4	1	Who were not Risk 2, and Fasting blood glucose>=110 mg/dL
Risk 0	223 (161)	110	113	Who were neither Risk 1 nor Risk 2
Hypertension risk				
Risk 2	46 (43)	20	26	SBP>=140 mmHg or DBP>=90 mmHg or Under medication
Risk 1	20 (16)	11	9	Who were not Risk 2, and either SBP>=130 mgHg or DBP>=85 mgHg
Risk 0	170 (115)	86	84	Who were neither Risk 1 nor Risk 2
Dislipidemia risk				
Risk 2	97 (84)	46	51	TG>=150 mg/dL or HDL-cholesterol<40 mg/dL or LDL-cholesterol>=140 mg/dL or Under medication
Risk 1	31 (25)	17	14	Who were not Risk 2, and LDL-cholesterol>=120 mg/dL
Risk 0	108 (65)	54	54	Who were neither Risk 1 nor Risk 2

Risk 2 means disease, Risk 1 means border line, and Risk 0 means healthy participants.

Range of barley consumption; Low barley: 0-31.8 g/1000 kcal･day, High barley: 32.3-253 g/1000 kcal･day

Table S2 Comparison of diet between low barley group and high barley group on cross-sectional study (*n* 94).

Variable (g/1,000 kcal·d)	Low barley (*n* 47) 0-31.8 g/1,000 kcal·d[1]	High barley (*n* 47) 32.3-253 g/1,000 kcal·d[1]	*P* [2]	Model 2[3]	Model 3[3]
Category					
Cereal	213 ± 55	236 ± 58	0.048	O	O
Potato	19 ± 16	21 ± 14	0.40		
Sugar and sweetener	3.9 ± 3.7	2.4 ± 2.4	0.03	O	O
Legume	20 ± 15	27 ± 20	0.056	O	O
Green vegetable	37 ± 24	44 ± 31	0.25		O
Other vegetable [4]	62 ± 29	72 ± 45	0.2001		O
Fruit	36 ± 32	35 ± 30	0.87		
Fish	32 ± 15	31 ± 16	0.74		O
Meat	48 ± 26	45 ± 15	0.47		
Egg	18 ± 10	20 ± 13	0.45		
Milk	54 ± 43	48 ± 44	0.49		
Oil	6.9 ± 2.2	7.2 ± 2.2	0.53		
Confectionery	26 ± 20	25 ± 17	0.81		O
Beverage [4]	439 ± 271	335 ± 226	0.047	O	O
Seasoning and spice [4]	128 ± 45	135 ± 70	0.57		
In detail					
Rice	141 ± 54	173 ± 61	0.007		
Natto	3.6 ± 4.2	10.4 ± 12.0	<0.001		
Green leafy vegetables	11 ± 10	17 ± 17	0.04		
Carrot and Pumpkin	6.7 ± 5.9	10.1 ± 8.4	0.03		
Seaweed	3.5 ± 2.8	4.0 ± 3.9	0.51		

Mushroom	4.4 ± 2.5	5.0 ± 4.1	0.37
Boiled fish	25 ± 28	15 ± 16	0.052
Japanese confectionery	3.8 ± 3.4	2.8 ± 2.5	0.08
Ice cream	18 ± 19	12 ± 12	0.08
Coffee	136 ± 113	84 ± 91	0.015
Cola	67 ± 65	37 ± 50	0.013

Data are shown as Means ± SD
1) Range of barley consumption.
2) Student's *t* -test
3) Model variables were used as the independent variables on tha multiple regression analyses.
4) Other vegetable include mushroom and seaweed.
Beverage include fruit or vegetable drink.
Seasoning include liquid seasoning.

Table S3 Alpha-diversity, compared low barley with high barley group on cross-sectional study (aged 19-65 years in 2018, Japan).

	Low barley (*n* 47) range: 0-31.8 g/1000 kcal・d	High barley (*n* 47) range: 32.3-253 g/1000 kcal・d		
Variable	Median [Interquartile range]	Median [Interquartile range]	*P* value[1)]	P_{FDR} value[2)]
Observed	561 [482, 649]	549 [500, 628]	0.86	0.87
Chao1	1146 [919, 1290]	1170 [971, 1419]	0.41	0.68
Shannon	3.77 [3.46, 3.95]	3.77 [3.57, 4.14]	0.38	0.68
Simpson	0.94 [0.90, 0.95]	0.94 [0.92, 0.96]	0.21	0.68
Fisher	128 [106, 156]	125 [111, 149]	0.87	0.87

[1)] Compared low and high barley groups using Mann–Whitney *U*-test (crude *P* value)

[2)] Compared low and high barley groups using Mann–Whitney *U*-test adjusted with *FDR* method

Table S4 Relative abundance(%) of the frequent 50 microbiomes (genus levels), compared low barley with high barley group on cross-sectional study (aged 19-65 years in 2018, Japan).

Genus	Low barley (*n* 47) range: 0-31.8 g/1000 kcal・d Median [Interquartile range]	High barley (*n* 47) range: 32.3-253 g/1000 kcal・d Median [Interquartile range]	*P* value[1)]	P_{FDR} value[2)]
Bacteroides	31.27 [0.01, 28.98]	29.28 [1.71, 27.52]	0.50	0.79
Blautia	6.15 [0.78, 7.48]	5.81 [1.63, 6.67]	0.64	0.85
Bifidobacterium	2.73 [0.00, 5.10]	5.61 [0.00, 8.12]	0.01	0.37
Faecalibacterium	5.76 [0.00, 6.02]	6.08 [0.00, 6.34]	0.54	0.79
Prevotella 9	0.01 [0.00, 6.96]	0.00 [0.00, 3.90]	0.20	0.59
Eubacterium rectale group	1.10 [0.00, 2.28]	1.56 [0.00, 2.62]	0.73	0.87
Parabacteroides	2.28 [0.00, 2.62]	1.64 [0.00, 1.96]	0.18	0.59
Subdoligranulum	1.12 [0.00, 1.80]	1.83 [0.00, 2.32]	0.08	0.44
Collinsella	0.92 [0.00, 1.82]	1.95 [0.00, 2.20]	0.03	0.42
Sutterella	1.41 [0.00, 1.82]	1.43 [0.00, 2.02]	0.99	0.99
Megamonas	0.00 [0.00, 3.03]	0.00 [0.00, 0.76]	0.20	0.59
Ruminococcus torques group	1.27 [0.03, 1.67]	1.09 [0.01, 1.73]	0.36	0.72
Anaerostipes	0.89 [0.02, 1.28]	1.06 [0.07, 1.92]	0.0502	0.42
Lachnoclostridium	1.22 [0.19, 1.69]	1.23 [0.04, 1.48]	0.53	0.79
Fusicatenibacter	1.41 [0.00, 1.49]	1.00 [0.00, 1.40]	0.83	0.90
Fusobacterium	0.00 [0.00, 1.14]	0.00 [0.00, 1.65]	0.91	0.95
Eubacterium hallii group	0.97 [0.00, 1.28]	0.78 [0.00, 0.97]	0.60	0.83
Alistipes	0.21 [0.00, 0.82]	0.40 [0.00, 1.30]	0.34	0.72
Lachnospira	0.41 [0.00, 0.78]	0.62 [0.00, 1.10]	0.22	0.59

Prevotella 2	0.00 [0.00, 0.34]	0.00 [0.00, 1.46]	0.99	0.99
Megasphaera	0.00 [0.00, 0.65]	0.00 [0.00, 1.15]	0.28	0.66
Roseburia	0.66 [0.00, 1.04]	0.40 [0.00, 0.72]	0.18	0.59
Veillonella	0.03 [0.00, 1.06]	0.03 [0.00, 0.70]	0.47	0.79
Phascolarctobacterium	0.01 [0.00, 0.79]	0.00 [0.00, 0.70]	0.58	0.83
Escherichia Shigella	0.07 [0.00, 0.72]	0.05 [0.00, 0.75]	0.73	0.87
Lachnospiraceae family uncultured	0.49 [0.01, 0.72]	0.59 [0.00, 0.70]	0.91	0.95
Alloprevotella	0.00 [0.00, 0.56]	0.00 [0.00, 0.74]	0.77	0.89
Dorea	0.40 [0.00, 0.81]	0.35 [0.00, 0.43]	0.32	0.72
Ruminococcus 2	0.00 [0.00, 0.37]	0.01 [0.00, 0.86]	0.04	0.42
Butyricicoccus	0.43 [0.00, 0.46]	0.65 [0.12, 0.65]	0.00	0.09
Lachnospiraceae UCG-008	0.52 [0.00, 0.57]	0.32 [0.00, 0.48]	0.81	0.90
Ruminococcus 1	0.01 [0.00, 0.28]	0.01 [0.00, 0.69]	0.34	0.72
Acidaminococcus	0.00 [0.00, 0.30]	0.03 [0.00, 0.66]	0.07	0.44
Ruminococcaceae family uncultured	0.23 [0.00, 0.42]	0.24 [0.00, 0.54]	0.64	0.85
Parasutterella	0.02 [0.00, 0.38]	0.02 [0.00, 0.57]	0.50	0.79
Streptococcus	0.12 [0.00, 0.52]	0.20 [0.00, 0.43]	0.50	0.79
Mitsuokella	0.00 [0.00, 0.59]	0.00 [0.00, 0.35]	0.38	0.73
Prevotellaceae NK3B31 group	0.00 [0.00, 0.48]	0.00 [0.00, 0.28]	0.71	0.87
Dialister	0.00 [0.00, 0.31]	0.07 [0.00, 0.43]	0.04	0.42
Ruminiclostridium 5	0.12 [0.00, 0.36]	0.15 [0.01, 0.32]	0.66	0.85
Ruminococcaceae UCG-013	0.09 [0.00, 0.27]	0.22 [0.00, 0.34]	0.06	0.44
Barnesiella	0.00 [0.00, 0.23]	0.05 [0.00, 0.36]	0.22	0.59
Ruminococcaceae UCG-002	0.00 [0.00, 0.17]	0.01 [0.00, 0.39]	0.19	0.59

Rhodospirillaceae family uncultured	0.00 [0.00, 0.26]	0.00 [0.00, 0.27]	0.43	0.79
Ruminococcus gauvreauii group	0.00 [0.00, 0.23]	0.00 [0.00, 0.25]	0.79	0.90
Paraprevotella	0.00 [0.00, 0.31]	0.00 [0.00, 0.17]	0.20	0.59
Odoribacter	0.04 [0.00, 0.21]	0.10 [0.00, 0.24]	0.48	0.79
Eubacterium coprostanoligenes group	0.00 [0.00, 0.14]	0.01 [0.00, 0.30]	0.21	0.59
Bilophila	0.10 [0.00, 0.18]	0.14 [0.00, 0.25]	0.24	0.60
Tyzzerella 3	0.00 [0.00, 0.10]	0.00 [0.00, 0.29]	0.22	0.59

[1)] Compared low and high barley groups using Mann–Whitney *U*-test (crude *P* value)

[2)] Compared low and high barley groups using Mann–Whitney *U*-test adjusted with *FDR* method

Table S5 Results of Multiple regression analysis (dichotomy and continuous) and Kendall rank correlation between barley consumption rate (g/1000 kcal・day) and intestinal microbiota on cross-sectional study (aged 19-65 years in 2018, Japan).

	Multiple regression (Barley:dichotomy)[1)]			Multiple regression (Barley:continuous)[2)]			Kendall rank correlation[3)]	
Genus	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	*P* value
Bacteroides	-1.51	3.15	0.63	-0.06	0.04	0.57	-0.05	0.48
Blautia	-0.85	0.85	0.32	-0.02	0.01	0.50	-0.05	0.47
Bifidobacterium	2.78	1.63	0.09	0.11	0.02	0.04	0.17	0.01
Faecalibacterium	-0.21	1.09	0.85	-0.04	0.01	0.23	0.07	0.32
Prevotella 9	-2.60	2.91	0.38	-0.07	0.03	0.44	-0.09	0.27
Eubacterium rectale group	0.55	0.58	0.34	0.02	0.01	0.37	0.02	0.80
Parabacteroides	-0.65	0.39	0.10	-0.01	0.00	0.35	-0.11	0.12
Subdoligranulum	0.43	0.46	0.36	-0.01	0.01	0.57	0.06	0.38
Collinsella	0.50	0.52	0.34	0.02	0.01	0.15	0.12	0.10
Sutterella	0.24	0.44	0.59	-0.01	0.01	0.42	-0.03	0.67
Megamonas	-1.87	1.15	0.11	-0.06	0.01	0.13	-0.11	0.19
Ruminococcus torques group	0.16	0.41	0.70	0.00	0.00	0.94	-0.05	0.45
Anaerostipes	0.71	0.36	0.05	0.01	0.00	0.36	0.08	0.25
Lachnoclostridium	-0.17	0.27	0.54	0.00	0.00	0.96	-0.04	0.54
Fusicatenibacter	-0.06	0.27	0.82	-0.01	0.00	0.46	-0.02	0.80
Fusobacterium	0.74	0.85	0.39	0.01	0.01	0.61	-0.03	0.72
Eubacterium hallii group	-0.42	0.24	0.08	-0.01	0.00	0.50	0.00	0.98
Alistipes	0.29	0.37	0.44	0.00	0.00	1.00	0.05	0.44
Lachnospira	0.23	0.25	0.36	0.01	0.00	0.26	0.16	0.03

Prevotella 2	1.41	0.64	0.03	0.01	0.01	0.68	-0.01	0.90
Megasphaera	0.74	0.60	0.22	0.08	0.01	0.00	0.06	0.43
Roseburia	-0.40	0.22	0.08	-0.01	0.00	0.16	-0.07	0.32
Veillonella	-0.37	0.44	0.40	0.00	0.01	0.86	0.14	0.07
Phascolarctobacterium	-0.19	0.22	0.39	0.00	0.00	0.66	0.00	0.96
Escherichia Shigella	0.06	0.47	0.90	0.02	0.01	0.26	0.03	0.69
Lachnospiraceae family uncultured	0.02	0.14	0.90	0.01	0.00	0.01	0.07	0.31
Alloprevotella	0.18	0.56	0.75	-0.01	0.01	0.56	-0.06	0.49
Dorea	-0.32	0.19	0.10	-0.01	0.00	0.39	-0.06	0.36
Ruminococcus 2	0.45	0.23	0.06	0.00	0.00	0.51	0.15	0.05
Butyricicoccus	0.17	0.07	0.02	0.00	0.00	0.05	0.21	0.00
Lachnospiraceae UCG-008	-0.11	0.11	0.32	0.00	0.00	0.60	0.01	0.93
Ruminococcus 1	0.36	0.21	0.08	0.01	0.00	0.18	0.10	0.20
Acidaminococcus	0.37	0.20	0.07	0.00	0.00	0.82	0.08	0.29
Ruminococcaceae family uncultured	0.03	0.13	0.81	0.01	0.00	0.15	0.01	0.85
Parasutterella	0.18	0.20	0.38	0.02	0.00	0.01	0.08	0.27
Streptococcus	-0.11	0.17	0.53	0.00	0.00	0.79	0.07	0.32
Mitsuokella	-0.21	0.45	0.65	0.00	0.01	0.93	0.02	0.84
Prevotellaceae NK3B31 group	-0.34	0.41	0.41	-0.01	0.00	0.53	0.00	0.99
Dialister	0.17	0.13	0.19	0.00	0.00	0.92	0.08	0.27
Ruminiclostridium 5	-0.04	0.11	0.72	0.00	0.00	0.95	0.01	0.89
Ruminococcaceae UCG-013	0.06	0.08	0.49	0.00	0.00	0.27	0.17	0.02
Barnesiella	0.09	0.12	0.46	0.00	0.00	0.63	0.08	0.29

Ruminococcaceae UCG-002	0.14	0.13	0.25	0.00	0.00	0.98	0.07	0.38
Rhodospirillaceae family uncultured	0.02	0.21	0.92	0.00	0.00	0.64	-0.09	0.25
Ruminococcus gauvreauii group	0.01	0.11	0.93	0.00	0.00	0.38	-0.01	0.93
Paraprevotella	-0.15	0.13	0.23	0.00	0.00	0.86	-0.13	0.11
Odoribacter	0.01	0.08	0.87	0.00	0.00	0.88	0.02	0.76
Eubacterium coprostanoligenes group	0.13	0.10	0.21	0.01	0.00	0.03	0.07	0.38
Bilophila	0.05	0.05	0.29	0.00	0.00	0.46	0.06	0.40
Tyzzerella 3	0.19	0.13	0.16	0.02	0.00	0.00	0.16	0.04

[1)] Results of multiple regression analysis. Genus levels of microbiome relative abundance (%) were used as the dependent variable, barley consumption rate (0="Low barley group", 1="High barley group"), age (years), and sex (0="female", 1="male") were used as the independent variable. Estimate: Linear regression coefficient, SE: standard error.

[2)] Results of multiple regression analysis. Genus levels of microbiome relative abundance (%) were used as the dependent variable, barley consumption rate (g/1000 kcal・day), age (years), and sex (0="female", 1="male") were used as the independent variable. Estimate: Linear regression coefficient, SE: standard error.

[3)] Results of correlation test between barley consumption rate (g/1000 kcal・day) and genus levels of intestinal microbiota relative abundance (%) using Kendall method. Estimate: Kendall's correlation coefficient.

Table S6 Association between the relative abundance of microbiome bacteria and barley intake group (0 = low, 1 = high)[1] by multivariate linear regression analyses (n = 236).

	Model 1			Model 2			Model 3		
	R	SE	*P* value	R	SE	*P* value	R	SE	*P* value
Bifidobacterium									
Barley (0: low, 1: high)[1]	2.52	1.00	0.012 *	2.61	1.03	0.012 *	2.59	1.03	0.013 *
Sex (0: female, 1: male)	-1.31	1.20	0.28	-0.79	1.24	0.52	-1.22	1.33	0.36
Age (10 years)	0.36	0.49	0.46	0.25	0.51	0.63	0.35	0.51	0.49
Risk of diabetes (0–2)	0.45	1.32	0.73	0.79	1.34	0.55	0.96	1.34	0.48
Risk of dyslipidemia (0–2)	-0.17	0.60	0.77	-0.14	0.60	0.81	-0.09	0.60	0.87
Risk of hypertension (0–2)	-1.31	0.71	0.07 #	-1.03	0.73	0.16	-0.98	0.73	0.18
Cereal (g/1000kcal·d)				-0.01	0.01	0.44	-0.02	0.01	0.101
Sugar and sweetner (g/1000kcal·d)				0.26	0.13	0.04 *	0.27	0.13	0.03 *
Legume (g/1000kcal·d)				0.00	0.03	0.95	0.01	0.03	0.62
Beverage (g/1000kcal·d)				0.00	0.00	0.06 #	-0.01	0.00	0.009 **
Green vegetable (g/1000kcal·d)							-0.03	0.02	0.23
Other vegetable (g/1000kcal·d)							-0.01	0.02	0.68
Fish (g/1000kcal·d)							-0.06	0.03	0.04 *
Confectionery (g/1000kcal·d)							-0.02	0.04	0.60
Butyricicoccus									
Barley (0: low, 1: high)[1]	0.11	0.05	0.03 *	0.08	0.05	0.102	0.08	0.05	0.13
Sex (0: female, 1: male)	0.02	0.06	0.73	0.03	0.06	0.64	0.07	0.07	0.32
Age (10 years)	0.01	0.02	0.75	0.01	0.03	0.60	0.01	0.03	0.73
Risk of diabetes (0–2)	0.02	0.07	0.70	0.03	0.07	0.64	0.04	0.07	0.54
Risk of dyslipidemia (0–2)	-0.02	0.03	0.44	-0.02	0.03	0.41	-0.03	0.03	0.39
Risk of hypertension (0–2)	-0.01	0.04	0.81	-0.01	0.04	0.72	0.00	0.04	0.95
Cereal (g/1000kcal·d)				0.00	0.00	0.43	0.00	0.00	0.11
Sugar and sweetner (g/1000kcal·d)				0.00	0.01	0.78	0.00	0.01	0.76
Legume (g/1000kcal·d)				0.00	0.00	0.16	0.00	0.00	0.17
Beverage (g/1000kcal·d)				0.00	0.00	0.34	0.00	0.00	0.92

Green vegetable (g/1000kcal・d)							0.00	0.00	0.22
Other vegetable (g/1000kcal・d)							0.00	0.00	0.44
Fish (g/1000kcal・d)							0.00	0.00	0.99
Confectionery (g/1000kcal・d)							0.00	0.00	0.15
Collinsella									
Barley (0: low, 1: high)[1]	0.27	0.30	0.37	0.26	0.31	0.41	0.15	0.31	0.63
Sex (0: female, 1: male)	0.44	0.36	0.23	0.49	0.38	0.20	0.41	0.41	0.31
Age (10 years)	-0.09	0.15	0.52	-0.11	0.15	0.49	-0.09	0.16	0.56
Risk of diabetes (0–2)	-0.26	0.40	0.51	-0.24	0.41	0.56	-0.17	0.41	0.68
Risk of dyslipidemia (0–2)	0.03	0.18	0.86	0.04	0.18	0.84	0.04	0.18	0.83
Risk of hypertension (0–2)	-0.06	0.21	0.77	-0.05	0.22	0.81	0.04	0.22	0.87
Cereal (g/1000kcal・d)				0.00	0.00	0.70	0.00	0.00	0.75
Sugar and sweetner (g/1000kcal・d)				0.00	0.04	0.93	0.00	0.04	0.97
Legume (g/1000kcal・d)				0.00	0.01	0.86	0.00	0.01	0.79
Beverage (g/1000kcal・d)				0.00	0.00	0.69	0.00	0.00	0.61
Green vegetable (g/1000kcal・d)							0.00	0.01	0.82
Other vegetable (g/1000kcal・d)							0.01	0.01	0.08 #
Fish (g/1000kcal・d)							-0.02	0.01	0.02 *
Confectionery (g/1000kcal・d)							0.00	0.01	0.85
Ruminococcus 2									
Barley (0: low, 1: high)[1]	0.10	0.15	0.53	0.11	0.16	0.48	0.09	0.16	0.58
Sex (0: female, 1: male)	-0.26	0.19	0.16	-0.18	0.19	0.35	-0.12	0.21	0.58
Age (10 years)	0.09	0.07	0.26	0.08	0.08	0.29	0.07	0.08	0.35
Risk of diabetes (0–2)	0.11	0.20	0.60	0.09	0.21	0.65	0.14	0.21	0.50
Risk of dyslipidemia (0–2)	0.00	0.09	1.00	0.02	0.09	0.81	0.03	0.09	0.73
Risk of hypertension (0–2)	-0.11	0.11	0.34	-0.11	0.11	0.32	-0.09	0.11	0.45
Cereal (g/1000kcal・d)				0.00	0.00	0.29	0.00	0.00	0.42
Sugar and sweetner (g/1000kcal・d)				-0.01	0.02	0.55	-0.01	0.02	0.53
Legume (g/1000kcal・d)				-0.01	0.00	0.20	0.00	0.00	0.41
Beverage (g/1000kcal・d)				0.00	0.00	0.20	0.00	0.00	0.23
Green vegetable (g/1000kcal・d)							0.00	0.00	0.34

Other vegetable (g/1000kcal・d)							0.00	0.00	0.37
Fish (g/1000kcal・d)							0.00	0.00	0.34
Confectionery (g/1000kcal・d)							0.01	0.01	0.33
Dialister									
Barley (0: low, 1: high)[1]	0.10	0.09	0.24	0.08	0.09	0.41	0.06	0.09	0.50
Sex (0: female, 1: male)	0.20	0.11	0.06 #	0.14	0.11	0.19	0.20	0.12	0.098 #
Age (10 years)	-0.03	0.04	0.42	-0.02	0.04	0.64	-0.03	0.05	0.53
Risk of diabetes (0–2)	-0.08	0.12	0.48	-0.10	0.12	0.41	-0.07	0.12	0.54
Risk of dyslipidemia (0–2)	-0.06	0.05	0.27	-0.07	0.05	0.19	-0.07	0.05	0.22
Risk of hypertension (0–2)	-0.04	0.06	0.54	-0.06	0.06	0.37	-0.04	0.07	0.53
Cereal (g/1000kcal・d)				0.00	0.00	0.12	0.00	0.00	0.06 #
Sugar and sweetner (g/1000kcal・d)				-0.01	0.01	0.25	-0.01	0.01	0.23
Legume (g/1000kcal・d)				0.00	0.00	0.29	0.00	0.00	0.20
Beverage (g/1000kcal・d)				0.00	0.00	0.09 #	0.00	0.00	0.05 #
Green vegetable (g/1000kcal・d)							0.00	0.00	0.85
Other vegetable (g/1000kcal・d)							0.00	0.00	0.35
Fish (g/1000kcal・d)							0.00	0.00	0.70
Confectionery (g/1000kcal・d)							0.00	0.00	0.19

[1]The range of barley intake is 0-32.3 g/1,000kcal・d in low, 32.9-253 g/1,000kcal・d in high.

Model 1: Adjusted with sex, age, risk of diabetes, dyslipidemia, and hypertension.

Model 2: In addition to model 1, adjusted with an intake of cereal, sugar and sweetener, legume, and beverage.

Model 3: In addition to model 2, adjusted with green vegetable, other vegetable and confectionery