Original Article

A randomized, double-blinded study evaluating effect of matcha green tea on human fecal microbiota

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Matcha green tea is made from powdered green tea leaves. Unlike regular green tea, Matcha green tea is believed to exert beneficial effects on the gut microbiota, as it is richer in nutrients such as tea catechins and insoluble dietary fiber. In the present study, we aimed to investigate the effects of consumption of Matcha green tea on the gut microbiota. Human participants were randomly assigned to a placebo (n = 16) or a Matcha green tea (n = 17) drink group and asked to drink the treatments for two weeks. Feces were collected from the participants pre- and post-treatment and fecal microbiota composition was analyzed by 16S rRNA metagenomic sequencing. The beta-diversity of microbial composition significantly (p<0.05) changed in MGT group but not in placebo group. In addition, the number of unique bacterial genera significantly (p<0.05) changed in the Matcha green tea group was 30, while it was only 3 in the Placebo group. Increase and decrease in abundances of *Coprococcus* and *Fusobacterium*, respectively, in the gut microbiota of Matcha green tea group, conferred potential health benefits to the host. The present study was registered in the UMIN Clinical Trial Registry (UMIN000043857).

Key Words*: Matcha green tea, gut microbiota, *Fusobacterium*, *Coprococcus

The gut microbiota is a complex microbial community that exists in the gastrointestinal tracts of humans and animals and regulates their health. Dysbiotic gut microbiotas are usually associated with gastrointestinal diseases such as the irritable bowel syndrome and the inflammatory bowel disease (IBD),[1] as well as with various systemic disorders such as diabetes and autism.[2,3] Therefore, recent research has focused on finding strategies to prevent diseases by maintaining the homeostasis of the gut microbiota and ultimately to achieve a higher quality of life for humans. Studies have found that, while factors such as genetics, age, levels of exercise and stress and antibiotic administration can affect it in various degrees, nutrition in particular can have a high impact on the wellness of the gut microbiota.[4–6]

Unlike regular green tea, which is a leaf infusion product, matcha green tea (MGT) is made from ground-up Japanese green tea (*Camellia sinensis*) leaves. MGT contains higher amounts of dietary fibers, carotenoids and fat-soluble vitamins such as vitamin K than does regular green tea.[7,8] MGT leaves are cultivated in a sun-shaded condition for several weeks before harvest, which contributes to increase amino acids, including theanine, which enables MGT to consume as a thicker suspension, providing twice higher amount of catechins and caffeine than green tea.[7] MGT is thought to be a potential functional food for the gut microbiota.[9–11] In this context, it is believed that catechins found in green tea possess anti-pathogenic properties, which result in their well-known improving effect on the gut microbiota.[12] In addition, in contrast with regular green tea, MGT contains dietary fiber, which is used as dietary substrate by many enteric bacteria.[13] The recommended dietary fiber intake of Japanese adult males is at least 21 g/day and that of females is around 18 g/day.[14] By contrast, the average dietary fiber intake of those in their twenties is only 16 g/day, which is considered deficient.[15] Therefore, it is theorized that MGT could have a greater, positive modulative effect on the gut microbiota than do regular green tea and catechin supplements alone.

Caffeine- and theanine-rich composition of MGT has attracted attention for its effects on brain function.[16] Clinical trials have shown that MGT improves cognitive function not only in the elderly but also in young adults.[16,17] Vitamin K contained in MGT has also been reported to improve cognitive function and prevent aging of the cortical capillaries, suggesting that MGT may be useful in improving brain function.[7,18,19] In addition, theanine in MGT has been suggested stress reducing function in mice and humans.[20,21] Recent growing evidences are suggesting the existence of bidirectional gut-brain axis and stress is considered as a factor of gut microbiota modulation.[22,23] These findings suggest that the effect of MGT on brain function like relaxant effect may contribute to changes in gut microbiota through brain-gut axis.

While the effects of green tea and its biologically-active compounds (e.g., catechins) on the gut microbiota have previously been investigated, that of MGT intake has not been thoroughly studied.[11,24–26] Moreover, to the best of our knowledge, the association of MGT intake and the gut microbiota alteration is yet to be reported. Therefore, in the present study, we aimed to examine the effect of continuous MGT consumption on the gut microbiota and elucidate the possible use of MGT as a functional food.

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Materials and Methods

Ethics statements and research subjects. The present study was registered in the UMIN Clinical Trial Registry (UMIN000043857). The experimental protocol was approved by the Ethical Committee of the Kyoto Prefectural University and conducted as per their guidelines (approval number: 149 and 2018/Mar/30) and conducted according to the guidelines of the Declaration of Helsinki. Healthy male and female at university-age and postgraduate-age (18–25 years old) were enrolled in this study. Written informed consents were obtained from all participants. Thirty-three people living in Kyoto, Japan (10 males, 23 females, age 18–25) were initially recruited for the experiment and randomly allocated to MGT ($n = 17$) and placebo ($n = 16$) groups.

Experimental design. Prior to the start of the experiment, we supplied either 28 MGT (containing 1.5 g of MGT) or 28 placebo (containing fragrance and Gardenia pigment) capsules to each participant in the MGT and the Placebo groups, respectively. The participants prepared drinks with the supplied capsules and 80 ml of hot water, using a Dolce gust machine (Nestlé Japan Ltd., Tokyo, Japan). The experiment participants drank the treatments in the morning and the evening for 2 weeks, following their daily routine. The capsules were collected after the use, and participants who had not used all capsules were excluded from further analysis. Feces were collected from the participants pre- (Week 0) and post-consumption (Week 2) of the experimental drinks. On the samples collection days, the participants filled up forms with questions about the defecation frequency per week and fecal formation, as per the Bristol Stool Form Scale (BSFS).[27] A flow-chart describing this experimental procedure is provided in Fig. 1.

Sample collection and DNA extraction. Fecal samples of the size of grains of rice were collected using stool collection brush and storage tube sets (Wako Pure Chemicals, Osaka, Japan). The storage tubes contained DMSO/EDTA/saturated sodium chloride (DESS).[28] After mixing well, the samples were stored at room temperature for up to 14 days until DNA extraction.[28]

To extract the DNA from the samples, they were first centrifuged (10,000 × g for 3 min at room temperature) and the supernatants removed. Next, the fecal pellets were washed with phosphate buffered saline. Then, whole bacterial DNA was extracted using a Quick Gene DNA Tissue Kit S (KURABO, Okayama, Japan). DNA extraction was conducted exactly as instructed in the manufacturer's manual (KURABO).

16S rRNA metagenomic analysis. Library preparation, DNA sequencing and sequence data analysis were carried out as previously described.[3] For the heat-denaturing process, 10 pM of the library combined with phiX Control (ver. 3, Illumina; expected 20%) was used.

During the sequence data analysis step, the obtained Fastq data was quality filtered, and USEARCH,[29] VSEARCH,[30] and Quantitative Insights Into Microbial Ecology (QIIME)[31] were used to remove dereplication, singletons, to define OTU (operational taxonomic units), and to assign taxonomy to each out, as previously reported.[32] Calculation and visualization of the alpha and beta diversity indices were conducted using the R package "phyloseq".[33]

Statistical analysis. Physical characteristics (age, BMI) between the Placebo and the MGT groups were compared with the Wilcoxon rank sum test. The BSFS data, the defecation frequency data, the alpha diversity of the fecal microbiotas and the relative abundances (%) of bacterial genera obtained pre- and post-consumption of the experimental drinks were compared with the Wilcoxon signed rank sum test. Comparison of the beta diversity in the feces was carried out using a permutational multivariate analysis of variance (PERMANOVA) in QIIME ver. 1.9.1. Values are expressed as the means ± SD. Differences between the means were considered to be significant if $p<0.05$. All statistical analyses were carried out using R (ver. 4.0.2), unless otherwise specified.

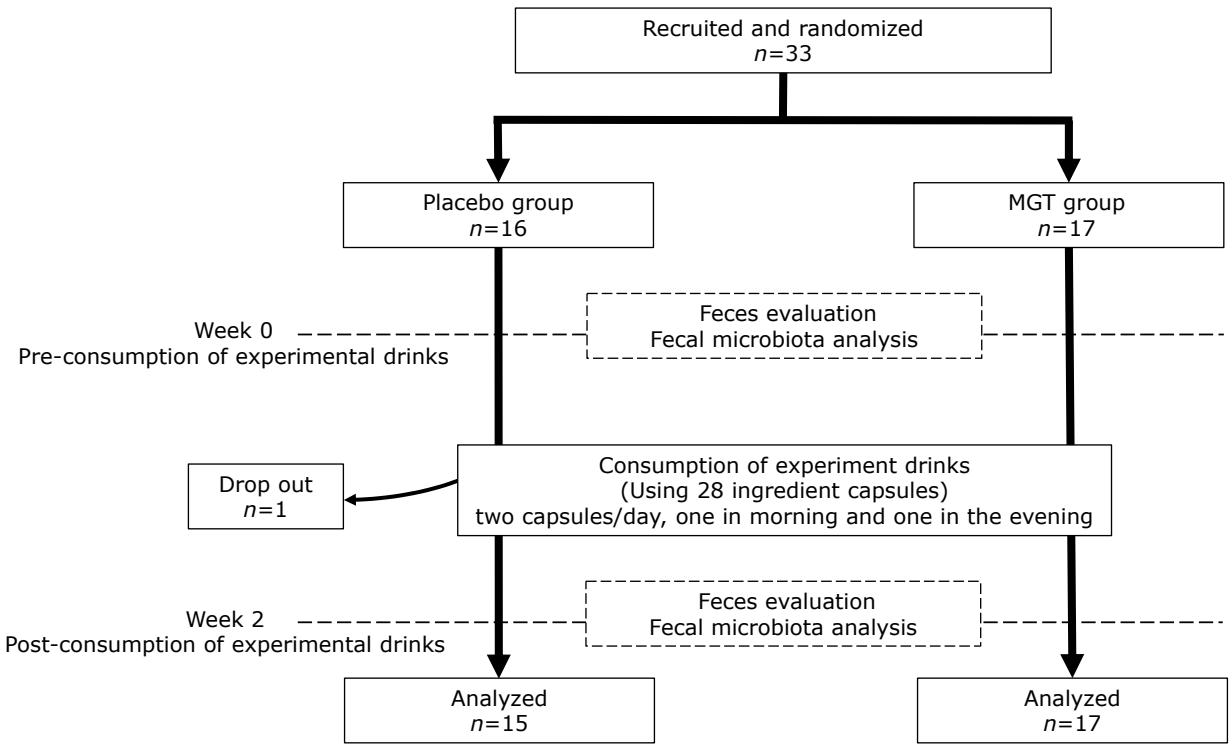

Fig. 1. Flow-chart of the experimental design.

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Results

Experiment participants and evaluation of feces. During the experimental period, one participant from the Placebo group was removed after one week, because they accidentally failed to take the treatment. Therefore, the number of participants in the Placebo group totaled to 15 (Fig. 1). The number of subjects analyzed was 5 males and 10 females in the Placebo group and 5 males and 12 females in the MGT group, with no significant gender differences between the two groups by chi-square test.

Prior to the start of the experiment, it was confirmed that there were no significant differences in ages (20.9 ± 2.0, vs 21.5 ± 1.8 years old; $p = 0.36$) and the body mass indices (BMI) (20.9 ± 3.3 vs 21.3 ± 3.0; $p = 0.53$) between the participants in the Placebo and MGT groups. The BSFS and Defecation frequency are described below (Table 1). There were no significant pre- and post-experiment changes in these two fecal parameters.

Analysis of the fecal microbiota. The alpha diversity of the fecal microbiotas is shown below (Table 2). Both experimental groups showed significantly lower Chao1 (bacterial genus richness) and Shannon (bacterial genus abundant equality) indices post-consumption of the experimental drinks.

As for the beta diversity, a principal coordinate analysis (PCoA) showed that the fecal microbiota composition two weeks post-MGT consumption significantly changed when compared with that pre-MGT consumption (Fig. 2). In contrast, the microbiota composition of the Placebo group showed no changes between the pre- and the post-placebo drink consumption periods (Fig. 2). A comparison of the bacterial composition in the feces during the pre- and post-experimental drink consumption periods showed that the abundances of 52 bacterial genera changed in the MGT group. In Placebo group, the number of bacterial genera of which abundance significantly changed during pre- and post-experimental period was 25, of which 22 bacterial genera were common with MGT group (Supplemental Table 1*). When excluding these commonly changed bacterial genera, only three bacterial genera, including two unclassified *Erysipelotrichaceae* and *Streptcoccus*, significantly decreased in the Placebo group (Table 3) during two weeks experimental drink consumption period. In contrast, the abundances of 30 bacterial genera,

Table 1. Defecation frequency and Bristol Stool Form Scale pre- and post-consumption of experimental drinks

	Placebo group			MGT group		
	Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value	Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value
Defecation frequency (times per week)	9.73 ± 13.32	11.20 ± 16.75	0.44	6.65 ± 4.14	6.65 ± 4.05	>0.99
Bristol Stool Form Scale	3.29 ± 1.68	3.14 ± 1.56	>0.99	3.71 ± 1.10	3.53 ± 1.23	>0.99

Table 2. Alpha diversity index of fecal microbiotas pre- and post-consumption of experimental drinks

	Placebo group			MGT group		
	Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value	Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value
Chao1	508.39 ± 82.69	428.58 ± 42.68	<0.01**	560.60 ± 122.75	447.55 ± 68.48	<0.01**
Shannon	5.48 ± 0.66	4.85 ± 0.33	<0.01**	5.66 ± 0.87	4.87 ± 0.65	<0.01**

**p<0.01.

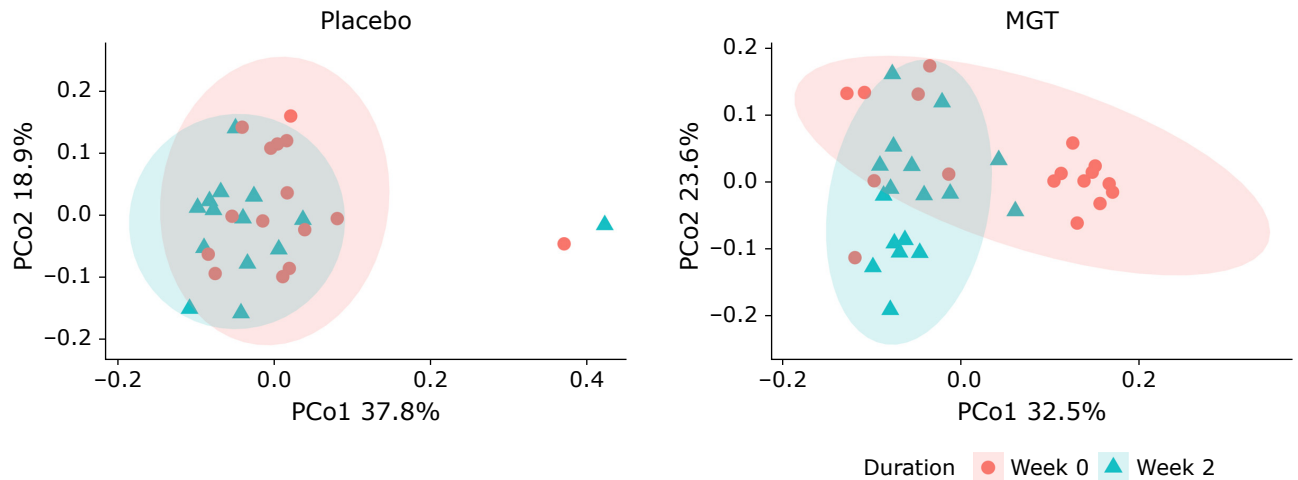

Fig. 2. Beta-diversity index of fecal microbiotas in Placebo and MGT groups pre- and post-consumption of experimental drinks. A principal coordinate analysis (PCoA) was conducted based on weighted UniFrac distances. There was no significant change in beta-diversity in the placebo group ($p = 0.42$), while there was a significant change in the MGT group over the study period by PERMANOVA (p<0.001). The ellipses enclosing the clusters indicate a 95% confidence interval.

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Table 3. Bacterial genera whose relative abundances changed pre- and post-consumption of the placebo drink

Taxonomy			Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value
Order	Family	Genus			
Erysipelotrichales	*Erysipelotrichaceae*	Unclassified	1.138 ± 1.251	0.775 ± 1.014	<0.01**
Erysipelotrichales	*Erysipelotrichaceae*	Other	0.005 ± 0.010	<0.001	0.04*
Lactobacillales	*Streptococcaceae*	*Streptococcus*	1.933 ± 1.170	1.145 ± 0.958	0.02*

*p<0.05, **p<0.01.

Table 4. Bacterial genera whose relative abundances changed pre- and post-consumption of the MGT drink

Taxonomy			Pre-consumption (Week 0)	Post-consumption (Week 2)	*p* value
Order	Family	Genus			
Bacillales	*Staphylococcaceae*	*Staphylococcus*	0.002 ± 0.003	0.011 ± 0.018	0.03*
Bacteroidales	[*Barnesiellaceae*]	Unclassified	1.175 ± 1.022	0.057 ± 0.109	<0.01**
Bacteroidales	[*Odoribacteraceae*]	*Butyricimonas*	0.283 ± 0.251	0.025 ± 0.089	<0.01**
Bacteroidales	*Rikenellaceae*	Unclassified	1.627 ± 1.153	0.464 ± 0.663	<0.01**
Bacteroidales	*Rikenellaceae*	*Alistipes*	0.243 ± 0.219	0.003 ± 0.005	<0.01**
Bifidobacteriales	*Bifidobacteriaceae*	*Alloscardovia*	0.002 ± 0.003	<0.001	0.03*
Clostridiales	[*Mogibacteriaceae*]	Unclassified	0.129 ± 0.093	0.045 ± 0.072	<0.01**
Clostridiales	*Christensenellaceae*	*Christensenella*	0.007 ± 0.008	<0.001	<0.01**
Clostridiales	*Clostridiaceae*	*Sarcina*	0.041 ± 0.039	<0.001	<0.01**
Clostridiales	*EtOH8*	Unclassified	0.011 ± 0.011	<0.001	<0.01**
Clostridiales	*Lachnospiraceae*	*Coprococcus*	1.481 ± 1.320	2.610 ± 1.913	0.01*
Clostridiales	*Lachnospiraceae*	*Epulopiscium*	0.003 ± 0.004	<0.001	0.01*
Clostridiales	*Peptococcaceae*	*rc4-4*	0.008 ± 0.009	<0.001	<0.01**
Clostridiales	*Ruminococcaceae*	*Butyricicoccus*	0.433 ± 0.411	0.902 ± 0.771	0.02*
Clostridiales	*Ruminococcaceae*	*Ruminococcus*	5.816 ± 3.775	2.058 ± 2.487	<0.01**
Clostridiales	*Ruminococcaceae*	Other	0.373 ± 0.283	0.097 ± 0.140	0.02*
Clostridiales	*Veillonellaceae*	*Megasphaera*	0.057 ± 0.050	0.017 ± 0.026	<0.01**
Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	0.081 ± 0.061	0.153 ± 0.139	<0.01**
E2	[*Methanomassiliicoccaceae*]	*Methanomassiliicoccus*	0.007 ± 0.009	<0.001	0.01*
Erysipelotrichales	*Erysipelotrichaceae*	*Catenibacterium*	0.012 ± 0.013	<0.001	<0.01**
Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	0.163 ± 0.245	0.061 ± 0.095	0.04*
Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.082 ± 0.208	0.115 ± 0.184	0.03*
Pseudomonadales	*Moraxellaceae*	*Acinetobacter*	0.041 ± 0.028	0.098 ± 0.095	0.02*
RF32	Unclassified	Unclassified	0.159 ± 0.155	0.014 ± 0.060	<0.01**
SHA-98	Unclassified	Unclassified	0.005 ± 0.006	0.001 ± 0.004	0.02*
Synergistales	*Dethiosulfovibrionaceae*	*Pyramidobacter*	0.013 ± 0.015	<0.001	<0.01**
Synergistales	*Synergistaceae*	*Synergistes*	0.010 ± 0.013	<0.001	<0.01**
Turicibacterales	*Turicibacteraceae*	*Turicibacter*	0.135 ± 0.311	0.038 ± 0.071	0.04*
YS2	Unclassified	Unclassified	0.033 ± 0.033	<0.001	<0.01**
	Unclassified c__Clostridia		0.037 ± 0.035	<0.001	<0.01**

*p<0.05, **p<0.01.

including *Fusobacterium* and *Coprococcus*, changed in the MGT group (Table 4).

Discussion

In the present study, we investigated the effect of two weeks MGT consumption on the fecal microbiota. MGT consumption for two weeks did not affect the fecal formation or defecation frequency. However, the beta-diversity resulting from a PCoA indicated that MGT continuously consumed for two weeks possibly affected the gut microbiota composition. Among the bacterial genera whose abundance significantly changed pre- and post-consumption of MGT, *Fusobacterium* and *Coprococcus* were noteworthy. For example, *Fusobacterium* is considered to be a harmful bacterium, as a high proportion of it was reported to be present in the gut microbiota when IBD and colorectal cancer were diagnosed.[4,34,35] In contrast, *Coprococcus* has been reported in lower abundance in IBD patients[36,37] Moreover, *Coprococcus* produces butyric acid, which is a short-chain fatty acid that is beneficial to the host's health.[38] Therefore, it is believed that the consumption of MGT may have had a beneficial modulatory effect on the human gut microbiota.

Liu *et al.* (2020)[39] reported that epigallocatechin-3-gallate inhibited the growth of *Fusobacterium in vitro.* Therefore, we theorized that in the present study, catechins contained in MGT and perhaps its bacterial metabolites such as 4-phenylbutyric

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acid may have played a role in the decrease of *Fusobacterium*. With respect to *Coporococcus*, it was reported that the ingestion of insoluble dietary fiber increased its abundance in the gut microbiotas of piglets.[40] Moreover, using an *in vitro* digestion and fermentation model, Pérez-Burillo *et al.* (2020)[41] found increases in the concentration of butyrate and the activity of *Coprococcus* when they applied a green tea extract to feces suspended in fermentation medium. Therefore, we believed that components of MGT such as insoluble fiber may have helped increase the abundance of *Coprococcus*. MGT components have been observed to have antioxidant, anti-inflammatory and carbohydrate absorption inhibitory effects,[11] which may have also indirectly affected the gut microbiota composition studied in the present work.

In the present study, some changes in the fecal microbiotas pre- and post- consumption of the experimental drinks followed the same trend, regardless of the treatment. For example, the alpha diversity index significantly decreased and the abundance of *Bacteroides* and *Faecalibacterium* significantly increased pre- and post-consumption of both experimental drinks, although the reason for these changes remained unclear. Nonetheless, one plausible reason may be the stress caused by an abnormally high temperature observed during the experimental period. To be precise, the present work was conducted in Kyoto, Japan in July 2018, which had an average temperature of 29.8°C, which was about 2.5°C higher than the average temperature previously recorded between 1991 and 2020 (27.3°C).[42,43]

In conclusion, the present study demonstrated that MGT consumption for two weeks affected the fecal microbiota. An increase of beneficial *Coprococcus* and a decrease of potential pathogenic *Fusobacterium* in the gut microbiota due to MGT consumption would likely be beneficial long-term to the host's health.

Authors Contributions

Conceptualization, RI and YF; methodology, RI and YF; validation, SM and RI; formal analysis, SM; investigation, SM and YK; resources, YF and RI; data curation, RI and SM; writing – original draft preparation, SM; writing – review and editing, RI, TT, and YN; visualization, SM; supervision, RI, TT, and YN; project administration, RI; funding acquisition, RI. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

All authors have no conflict of interest. The funders had no role in the design of the study, the sample collection, analyses or interpretation of data, the writing of the manuscript or in the decision to publish the results.

References

1 Lee BJ, Bak YT. Irritable bowel syndrome, gut microbiota and probiotics. *J Neurogastroenterol Motil* 2011; **17**: 252–266.
2 Allin KH, Nielsen T, Pedersen O. Mechanisms in endocrinology: gut microbiota in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 2015; **172**: R167–R177.
3 Inoue R, Sakaue Y, Sawai C, *et al*. A preliminary investigation on the relationship between gut microbiota and gene expressions in peripheral mononuclear cells of infants with autism spectrum disorders. *Biosci Biotechnol Biochem* 2016; **80**: 2450–2458.
4 Takagi T, Naito Y, Inoue R, *et al*. Differences in gut microbiota associated with age, sex, and stool consistency in healthy Japanese subjects. *J Gastroenterol* 2019; **54**: 53–63.
5 Smits SA, Leach J, Sonnenburg ED, *et al*. Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* 2017; **357**: 802–806.
6 Clarke SF, Murphy EF, O'Sullivan O, *et al*. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 2014; **63**: 1913–1920.
7 Sakurai K, Shen C, Ezaki Y, *et al*. Effects of Matcha green tea powder on cognitive functions of community-dwelling elderly individuals. *Nutrients* 2020; **12**: 3639.
8 Fukushima Y, Taguchi C, Kishimoto Y, Kondo K. Japanese carotenoid database with α- and β-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene, and fucoxanthin and intake in adult women. *Int J Vitam Nutr Res* 2021. DOI: 10.1024/0300-9831/a000707
9 Phongnarisorn B, Orfila C, Holmes M, Marshall LJ. Enrichment of biscuits with Matcha green tea powder: its impact on consumer acceptability and acute metabolic response. *Foods* 2018; **7**: 17.
10 Dietz C, Dekker M, Piqueras-Fiszman B. An intervention study on the effect of matcha tea, in drink and snack bar formats, on mood and cognitive performance. *Food Res Int* 2017; **99 (Pt 1)**: 72–83.
11 Kochman J, Jakubczyk K, Antoniewicz J, Mruk H, Janda K. Health benefits and chemical composition of Matcha green tea: a review. *Molecules* 2020; **26**: 85.
12 Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. *Biochim Biophys Acta* 1993; **1147**: 132–136.
13 Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes* 2017; **8**: 172–184.
14 Overview of the Dietary Reference Intakes for Japanese (2020). Ministry of Health, Labor and Welfare. https://www.mhlw.go.jp/content/10900000/000862500.pdf. Accessed 6 Jun 2022.
15 2019 National Health and Nutrition Survey Report. Ministry of Health, Labor and Welfare. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/kenkou/eiyou/r1-houkoku_00002.html. Accessed 6 Jun 2022.
16 Baba Y, Kaneko T, Takihara T. Matcha consumption maintains attentional function following a mild acute psychological stress without affecting a feeling of fatigue: a randomized placebo-controlled study in young adults. *Nutr Res* 2021; **88**: 44–52.
17 Baba Y, Inagaki S, Nakagawa S, Kobayashi M, Kaneko T, Takihara T. Effects of daily Matcha and caffeine intake on mild acute psychological stress-related cognitive function in middle-aged and older adults: a randomized placebo-controlled study. *Nutrients* 2021; **13**: 1700.
18 Iwai R, Ishii T, Fukushima Y, *et al*. Matcha and its components control angiogenic potential. *J Nutr Sci Vitaminol* 2021; **67**: 118–125.
19 Alisi L, Cao R, De Angelis C, *et al*. The relationships between vitamin K and cognition: a review of current evidence. *Front Neurol* 2019; **10**: 239.
20 Unno K, Furushima D, Hamamoto S, *et al*. Stress-reducing function of Matcha green tea in animal experiments and clinical trials. *Nutrients* 2018; **10**: 1468.
21 Monobe M, Nomura S, Ema K, Horie H. Influence of continued ingestion of matcha on emotional behaviors after social stress in mice. *Biosci Biotechnol Biochem* 2019; **83**: 2121–2127.
22 Molina-Torres G, Rodriguez-Arrastia M, Roman P, Sanchez-Labraca N, Cardona D. Stress and the gut microbiota-brain axis. *Behav Pharmacol* 2019; **30 (2 and 3-Spec Issue)**: 187–200.
23 Cryan JF, O'Riordan KJ, Cowan CSM, *et al*. The microbiota-gut-brain axis. *Physiol Rev* 2019; **99**: 1877–2013.
24 Hursel R, Westerterp-Plantenga MS. Catechin- and caffeine-rich teas for control of body weight in humans. *Am J Clin Nutr* 2013; **98 (6 Suppl)**: 1682S–1693S.
25 Jin JS, Touyama M, Hisada T, Benno Y. Effects of green tea consumption on human fecal microbiota with special reference to *Bifidobacterium* species. *Microbiol Immunol* 2012; **56**: 729–739.
26 Janssens PLHR, Penders J, Hursel R, Budding AE, Savelkoul PHM, Westerterp-Plantenga MS. Long-term green tea supplementation does not

change the human gut microbiota. *PLoS One* 2016; **11**: e0153134.
27 Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 2016; **44**: 693–703.
28 Kawada Y, Naito Y, Andoh A, Ozeki M, Inoue R. Effect of storage and DNA extraction method on 16S rRNA-profiled fecal microbiota in Japanese adults. *J Clin Biochem Nutr* 2019; **64**: 106–111.
29 Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 2015; **31**: 3476–3482.
30 Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016; **4**: e2584.
31 Caporaso JG, Kuczynski J, Stombaugh J, *et al*. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; **7**: 335–336.
32 Andoh A, Inoue R, Kawada Y, *et al*. Elemental diet induces alterations of the gut microbial community in mice. *J Clin Biochem Nutr* 2019; **65**: 118–124.
33 McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013; **8**: e61217.
34 Lee Y, Eun CS, Lee AR, Park CH, Han DS. Fusobacterium isolates recovered from colonic biopsies of inflammatory bowel disease patients in Korea. *Ann Lab Med* 2016; **36**: 387–389.
35 Repass J; Reproduction Project: Cancer Biology. Replication study: *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Elife* 2018; **7**: e25801.
36 Nishino K, Nishida A, Inoue R, *et al*. Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. *J Gastroenterol* 2018; **53**: 95–106.
37 Scher JU, Ubeda C, Artacho A, *et al*. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. *Arthritis Rheumatol* 2015; **67**: 128–139.
38 Aoe S, Nakamura F, Fujiwara S. Effect of wheat bran on fecal butyrate-producing bacteria and wheat bran combined with barley on *Bacteroides* abundance in Japanese healthy adults. *Nutrients* 2018; **10**: 1980.
39 Liu Z, de Bruijn WJC, Bruins ME, Vincken JP. Reciprocal interactions between epigallocatechin-3-gallate (EGCG) and human gut microbiota *in vitro*. *J Agric Food Chem* 2020; **68**: 9804–9815.
40 Chen T, Chen D, Tian G, *et al*. Soluble fiber and insoluble fiber regulate colonic microbiota and barrier function in a piglet model. *Biomed Res Int* 2019; **2019**: 7809171.
41 Pérez-Burillo S, Hinojosa-Nogueira D, Pastoriza S, Rufián-Henares JA. Plant extracts as natural modulators of gut microbiota community structure and functionality. *Heliyon* 2020; **6**: e05474.
42 Monthly mean air temperature. Japan Meteorological Agency. https://www.data.jma.go.jp/obd/stats/etrn/view/monthly_s3_en.php?block_no=47759&view=1. Accessed 6 Jun 2022.
43 Climatological normals. Japan Meteorological Agency. https://www.data.jma.go.jp/obd/stats/etrn/view/nml_sfc_ym.php?prec_no=61&block_no=47759&year=&month=&day=&view=. Accessed 6 Jun 2022.

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