JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

# Intake of sucrose affects gut dysbiosis in patients with type 2 diabetes

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**Keywords**
Dietary habit, Gut microbiota, Type 2 diabetes

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*J Diabetes Investig* 2020; 11: 1623–1634

doi: 10.1111/jdi.13293

## ABSTRACT

**Aims/Introduction:** Gut dysbiosis is generally associated with type 2 diabetes mellitus. However, the effect of habitual dietary intake on gut dysbiosis in Japanese patients with type 2 diabetes mellitus has not yet been explicated. This study investigated whether alteration of the gut microbiota was influenced by dietary intake of sucrose in Japanese patients with type 2 diabetes mellitus.

**Materials and Methods:** In this cross-sectional study, 97 patients with type 2 diabetes mellitus and 97 healthy individuals were matched by age and sex, and then, fecal samples were obtained. Next-generation sequencing of the 16S ribosomal ribonucleic acid gene was carried out, and functional profiles for the gut microbiota were analyzed. We selected the top 30 gut microbial genera and top 20 functional profiles for the gut microbiota specified by the weighted average difference method. The association between gut microbial genera or functional profiles and habitual dietary intake was investigated by Spearman's rank correlation coefficient, and then, clustering analysis was carried out to clarify the impact of habitual dietary intake.

**Results:** The Actinobacteria phylum was highly abundant in patients with type 2 diabetes mellitus, whereas the Bacteroidetes phylum was less abundant. Diabetic-type gut microbes, specifically *Bacteroides* and *Bifidobacterium*, were altered by sucrose intake at the genus level. Furthermore, sucrose intake was associated with glycolysis/gluconeogenesis in the diabetic-type functional profiles of the gut microbiota.

**Conclusions:** The gut microbiota and functional profiles for the gut microbiota in patients with type 2 diabetes mellitus were significantly different from those in healthy individuals. Furthermore, we showed that sucrose intake was closely associated with these differences.

## INTRODUCTION

Patients with type 2 diabetes mellitus are increasing worldwide. Accumulating evidence suggests that the gut microbiota has a close relationship with the development of diseases, including type 2 diabetes mellitus[1–5]. In fact, previous studies showed a difference between the gut microbiota of patients with type 2 diabetes mellitus and that of healthy individuals, such as the genera *Roseburia*, *Clostridium*, *Prevotella*, *Bacteroides* and *Faecalibacterium*[4,5,6,7].

The microbiota characteristics of patients with type 2 diabetes mellitus varied between countries[6,7]. In addition, the gut microbiota characteristics of healthy individuals also showed different composition among the country and race[8]. To investigate differences between the gut microbiota of type 2 diabetes mellitus and that of healthy individuals, one should consider not only the race or country, but also sex and age, because the gut microbiota is affected by the host's age and sex[9]. It has also been reported that the functional profile of gut microbiota is an important factor in disease development[10]. A recent study showed that glycolysis/gluconeogenesis, which is a components of metabolism, is altered in patients with type 2 diabetes

Received 13 January 2020; revised 7 May 2020; accepted 8 May 2020

mellitus[11]. However, little is known about the differences between gut microbiota of Japanese patients with type 2 diabetes mellitus and that of healthy individuals[12].

Furthermore, the functional profile, especially metabolism, including carbohydrate metabolism and energy metabolism, differs from individuals of other countries[6]. Previous studies showed that the diet of Japanese people, including patients with type 2 diabetes, differed from the diet of other countries[13,14]. Thus, there is a possibility that the gut microbiota response to diet of Japanese people differs from that of people from other countries. In contrast, in recent years, continuous Westernization of diet has occurred in Japan[13,15]. However, no studies to date have investigated whether a habitual dietary intake affects the gut microbiota or the functional profiles of the gut microbiota in Japanese patients with type 2 diabetes mellitus.

In the current study, we compared the gut microbiota of Japanese patients with type 2 diabetes mellitus with that of age- and sex-matched healthy individuals, and investigated differences in the functional profiles of gut microbiota within patients with type 2 diabetes mellitus. Furthermore, we investigated the impact of dietary habits on these gut dysbiosis, and change in functional profiles for gut microbiota of type 2 diabetes mellitus patients.

## METHODS

### Study population and data collection

The ethics committee of the Kyoto Prefectural University of Medicine (no. ERB-C-534 and no. RBMR-E-466-5) approved this study, and we carried it out in accordance with the Declaration of Helsinki. Written informed consent was obtained from the participants before enrollment. A total of 554 individuals (109 individuals without diabetes and 445 patients with diabetes) were enrolled from November 2016 to December 2017. For the current study, 109 healthy individuals and 109 patients with type 2 diabetes mellitus were selected, and matched by age and sex. A lack of data on gut microbiota due to the absence of a fecal sample was an exclusion criterion.

Bodyweight, height and body mass index (BMI) data were collected for all participants. The participants were then surveyed with respect to type of medication for diabetes, hypertension, dyslipidemia, proton pump inhibitor usage and antibiotic usage within the preceding 3 months. Individuals who took antibiotics within 3 months before the study were excluded. Diagnosis of type 2 diabetes mellitus was based on the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[16]. Information on the duration of diabetes, family history of diabetes, bodyweight at 20 years-of-age and maximum bodyweight were obtained from the patients with type 2 diabetes mellitus. Based on a questionnaire, patients were divided into non-, past- or current smokers. Patients who carried out any kind of sport at least once a week were defined as regular exercisers[17].

Blood samples for analyses, including fasting plasma glucose, hemoglobin A1c, C-peptide and creatine levels, were obtained from the patients with type 2 diabetes mellitus. Glomerular filtration rate was calculated using the Japanese Society of Nephrology equation: estimated glomerular filtration rate = $194 \times \text{creatine}^{-1.094} \times \text{age}^{-0.287}$ (mL/min/1.73 $m^2$) ($\times 0.739$, if patient is female)[18]. Insulin resistance was calculated as 20 / (fasting C-peptide [ng/mL] × fasting plasma glucose [mg/dL])[19]. Insulin secretion capacity was evaluated based on the C-peptide immunoreactivity index and secretory units of islets in transplantation index[20]. Early morning spot urine samples were used for urinary albumin and creatinine levels. A mean value for urinary albumin excretion was determined from three urine collections. Diagnosis of neuropathy was defined by the diagnostic criteria of the Diagnostic Neuropathy Study Group[21]. Retinopathy was graded as follows: no diabetic retinopathy, simple diabetic retinopathy, pre-proliferative diabetic retinopathy or proliferative diabetic retinopathy[22]. Missing clinical data were dealt with using multiple imputation in SPSS ver 25.0 (SPSS Inc., Chicago, IL, USA) repeated 10 times to account for the variability associated with unknown values.

The data regarding habitual dietary intake were obtained from patients with type 2 diabetes mellitus using a brief-type self-administered diet history questionnaire[23]. The brief-type self-administered diet history questionnaire has been described in detail previously[24].

### Sampling, DNA extraction, sequencing and data analysis

Fecal sample collection and the analyses of gut bacterial composition were carried out as previously described[11,25,26]. Briefly, fecal samples were collected into a guanidine thiocyanate solution (feces collection kit; Techno Suruga Lab, Shizuoka, Japan). Genomic deoxyribonucleic acid (DNA) was isolated using the Nucleospin Microbial DNA kit (Macherey-Nagel, Düren, Germany), as per the manufacturer's instructions. The extracted DNA was then distilled by the Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA).

DNA was analyzed by 16S ribosomal ribonucleic acid (rRNA) metagenomic sequencing using the MiSeq platform (Illumina, San Diego, CA, USA) at the Biomedical Center at Takara Bio (Shiga, Japan). Two-step polymerase chain reaction was used for the purified DNA samples to obtain sequence libraries. The first polymerase chain reaction was carried out to amplify, and used a 16S (V3–V4) metagenomic library construction kit for next-generation sequencing (Takara Bio Inc., Kusatsu, Japan) with primer pairs 341F (5′-TCGTCGGCAGC GTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGC AG-3′) and 806R (5′-GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGGACTACHVGGGTWTCTAAT-3′) corresponding to the V3–V4 region of the 16S rRNA gene. The second polymerase chain reaction was carried out to add the index sequences for the Illumina sequencer with a barcode sequence using the Nextera XT index kit (Illumina). The prepared libraries were subjected to sequencing of 250 paired-end bases using the MiSeq Reagent v3 kit and the MiSeq (Illumina) at the Biomedical Center at Takara Bio.

Generation of the amplicon sequence variant (ASV) table, including quality filtering and chimeric variant filtering was carried out using the DADA2 plugin of Quantitative Insights Into Microbial Ecology 2 (QIIME2) version 2019.4[27]. The taxonomy of each ASV was assigned by the Sklearn classifier algorithm against Greengenes database version 13_8 (99% OTU dataset). Overall, 6,902 ASVs were obtained. Prediction of the functional profiles from the 16S rRNA dataset was carried out using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) version 2.1.4 software[28]. The 16S rRNA gene amplicons were functionally annotated and mapped onto networks in the Kyoto Encyclopedia of Genes and Genomes database (release 70.0), and functional profiles of the gut microbiota were predicted. We calculated a Nearest Sequenced Taxon Index and excluded five ASV, because of Nearest Sequenced Taxon Index >2.

### Statistical analysis

The differences of diversity of gut microbiota by α-diversity indices, including the observed species, the phylogenetic diversity whole tree, the Chao 1 index (ASV richness estimation) and the Shannon index (ASV evenness estimation), between the groups were evaluated by paired *t*-test. The β-diversity was determined by the UniFrac metric to evaluate distances between the samples and visualized by the PCoA plots, and diversity was evaluated by permutational multivariate analysis of variance (PERMANOVA).

The relative abundance of the phyla in the groups was evaluated by paired *t*-tests using JMP version 13.2 software (SAS Institute Inc., Cary, NC, USA). Furthermore, the relative abundance of the bacterial genera between the groups was evaluated by the weighted average differences (WAD) method using Tinn-Rgui version 1.19.4.7 and R version 1.36[29], and by paired *t*-tests using JMP. In this WAD method, genera were ranked by comprehensively assessing higher expression, higher weight and fold change. WAD was found to be an effective type of transcriptome analysis. The relative abundance of functional profiles for the gut microbiota in the groups were evaluated by the WAD method and by paired *t*-tests.

The top 30 gut microbial genera and top 20 functional profiles for the gut microbiota in the Japanese patients with type 2 diabetes mellitus were determined using the WAD algorithm using R. Because dysbiosis of gut microbiota involved in metabolic syndrome was reported to differ by sex[30], we carried out sensitivity analysis according to sex by the genera levels. Correlations between the abundance of bacterial genera or functional profiles for the gut microbiota and habitual dietary intake or anthropometric and metabolic parameters, were analyzed using Spearman's correlation coefficient. Hierarchical clustering[31] was carried out to investigate the association between habitual dietary essential nutrient intake, including carbohydrate, animal protein, vegetable protein, fat, animal fat, vegetable fat, polyunsaturated fat, monounsaturated fat, saturated fat, soluble dietary fiber, insoluble dietary fiber, salt, sucrose and fatty acid intake, and the diabetic type of gut microbiota or functional profiles for the gut microbiota. Furthermore, multiple regression analyses were carried out to check the effects of sucrose intake on genus *Bacteroides* and genus *Bifidobacterium*, which were the top two genera that change in type 2 diabetes mellitus, adjusting for sex, age, duration of diabetes, BMI, hemoglobin A1c, exercise, smoking and energy intake.

## RESULTS

### Study design and study participants

Overall, 218 patients (109 healthy individuals and 109 patients with type 2 diabetes mellitus) were enrolled in the study. Among them, six healthy individuals and six patients with type 2 diabetes mellitus did not submit fecal samples, and thus, we excluded the 12 matched pairs. Hence, 194 participants (97 individuals without diabetes and 97 patients with type 2 diabetes mellitus) were included (Figure S1). Both groups included 41 men and 56 women, with an average age of 66 ± 13 years (all data are expressed as the mean ± standard deviation). The average BMI of individuals with type 2 diabetes mellitus was higher than that of healthy individuals (24.8 ± 4.4 vs 21.5 ± 3.4 kg/m$^2$, $P < 0.001$, by paired *t*-test). The clinical characteristics of patients with type 2 diabetes mellitus are summarized in Table S1.

### Gut dysbiosis in Japanese patients with type 2 diabetes mellitus

No α-diversity indices were statistically significantly different between healthy individuals and patients with type 2 diabetes mellitus (the number of observed species, 206.14 ± 73.80 vs 215.10 ± 85.55, respectively, $P = 0.421$; the Chao 1 index, 206.55 ± 73.92 vs 215.42 ± 85.63, respectively, $P = 0.427$; phylogenetic diversity whole tree, 16.41 ± 5.1 vs 16.96 ± 5.5, respectively, $P = 0.446$; and the Shannon index, 5.76 ± 0.71 vs 5.67 ± 0.82, respectively, $P = 0.404$, all by paired *t*-test). The differences of overall structure of the gut microbiota in healthy individuals and those in patients with type 2 diabetes mellitus were then evaluated using β-diversity indices. According to the principal coordinate analysis plots, no difference was detected in the microbial structure between patients with type 2 diabetes mellitus and healthy individuals using unweighted metrics (PERMANOVA, $P = 0.218$). In contrast, a microbial structural difference between patients with type 2 diabetes mellitus and healthy individuals was shown using weighted metrics (PERMANOVA, $P < 0.0001$; Figure 1).

At the phylum level, the abundance of *Actinobacteria* in patients with type 2 diabetes mellitus was higher than in healthy individuals, whereas the abundance of *Bacteroidetes* in patients with type 2 diabetes mellitus was lower than those of healthy individuals (Figure S2). The top 30 gut microbial genera in the Japanese patients with type 2 diabetes mellitus were determined by the WAD algorithm, and the genera were ranked from top to bottom, as shown in Figure 2. The abundance of the genus *Bacteroides* in patients with type 2 diabetes

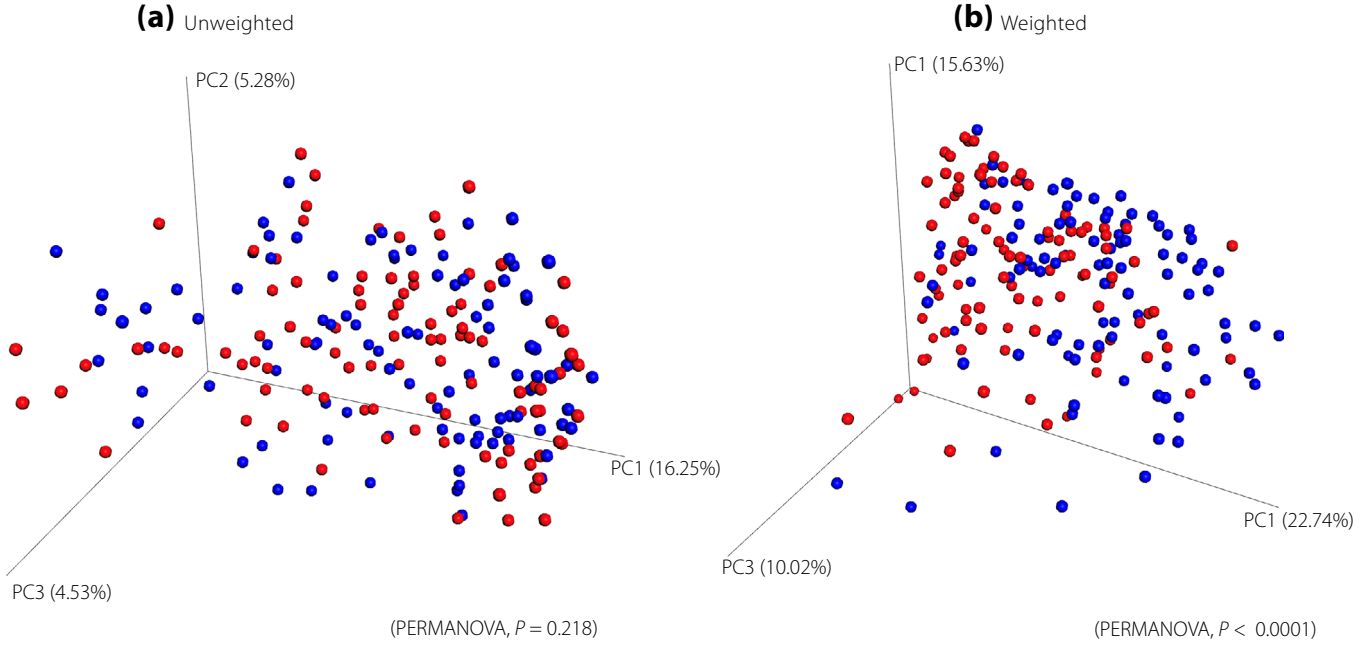


**Figure 1** | Gut microbiota structure for patients with type 2 diabetes mellitus differed significantly from healthy individuals. Differences in gut microbiota structure were evaluated by principal coordinate analysis plots using the data from the operational taxonomic unit. In the principal coordinate analysis plots, red circles represent patients with type 2 diabetes mellitus, and blue circles represent healthy individuals. (a) The distances of gut microbiota structure were calculated using unweighted UniFrac. No differences in the microbial structure between patients with type 2 diabetes mellitus and healthy individuals (PERMANOVA, $P = 0.218$) was apparent. (b) The distances of gut microbiota structure were calculated using weighted UniFrac. The calculated distance between patients with type 2 diabetes mellitus and healthy individuals was significantly different (PERMANOVA, $P < 0.0001$), suggesting that the balance of the gut microbiota structure was different in patients with type 2 diabetes mellitus. PC1, principal component 1; PC2, principal component 2; PC3, principal component 3.

mellitus was lower than that in healthy individuals, whereas the abundance of the genus *Bifidobacterium* in patients with type 2 diabetes mellitus was higher than that in healthy individuals. In addition, we also carried out subanalysis by sex, and the results are shown in Figure S3. The top two genera were genus *Bacteroides* and genus *Bifidobacterium*, which were the same as the whole analysis.

The top 20 functional profiles for gut microbiota in Japanese patients with type 2 diabetes mellitus were determined by the WAD algorithm, and functional profiles for gut microbiota were ranked from top to bottom, as shown in Figure 3. The citrate cycle (tricarboxylic acid cycle) was less prevalent in patients with type 2 diabetes mellitus than in healthy individuals. In contrast, glycolysis/gluconeogenesis was more prevalent in patients with type 2 diabetes mellitus than in healthy individuals.

### Association between habitual dietary intake, including sucrose intake, and gut dysbiosis in Japanese patients with type 2 diabetes mellitus

Correlations between habitual dietary essential nutrient intake and the genera abundance or the functional profiles for the gut microbiota are shown in Figure 4, Table S2, Figure 5 and Table S3. Interestingly, sucrose intake was negatively associated with *Bacteroides* ($r = -0.23$, $P = 0.031$) and *Parabacteroides* ($r = -0.24$, $P = 0.027$), and positively associated with *Bifidobacterium* ($r = 0.33$, $P = 0.002$) (Figure 4; Table S2; Figure S4). In addition, sucrose intake was positively associated with glycolysis/gluconeogenesis ($r = 0.38$, $P < 0.0001$) and lysine biosynthesis ($r = 0.25$, $P = 0.019$; Figure 5; Table S3).

The results of the multiple regression analyses are shown in Table 1. Sucrose intake was associated with decreasing genus *Bacteroides* ($\beta = -0.30$, $P = 0.015$) and increasing genus *Bifidobacterium* ($\beta = 0.44$, $P < 0.001$).

The association between fatty acids and genera abundance or functional profiles for the gut microbiota is shown in Figure S5, Table S4, Figure S6 and Table S5. Medium-chain fatty acids, such as caprylic acid, capric acid and lauric acid, were positively associated with an abundance of *Streptococcus*, and negatively associated with an abundance of *Ruminococcus* and *Parabacteroides*. In addition, medium-chain fatty acids were negatively associated with citrate cycle (tricarboxylic acid cycle), and were positively associated with glycolysis/gluconeogenesis.

The interrelationships between the other clinical characteristics and the gut microbiota or the functional profiles for the gut microbiota are shown in Figures S7 and S8. Age, duration of diabetes, and use of α-glucosidase inhibitors were associated with the abundance of a specific genera and functional profiles.

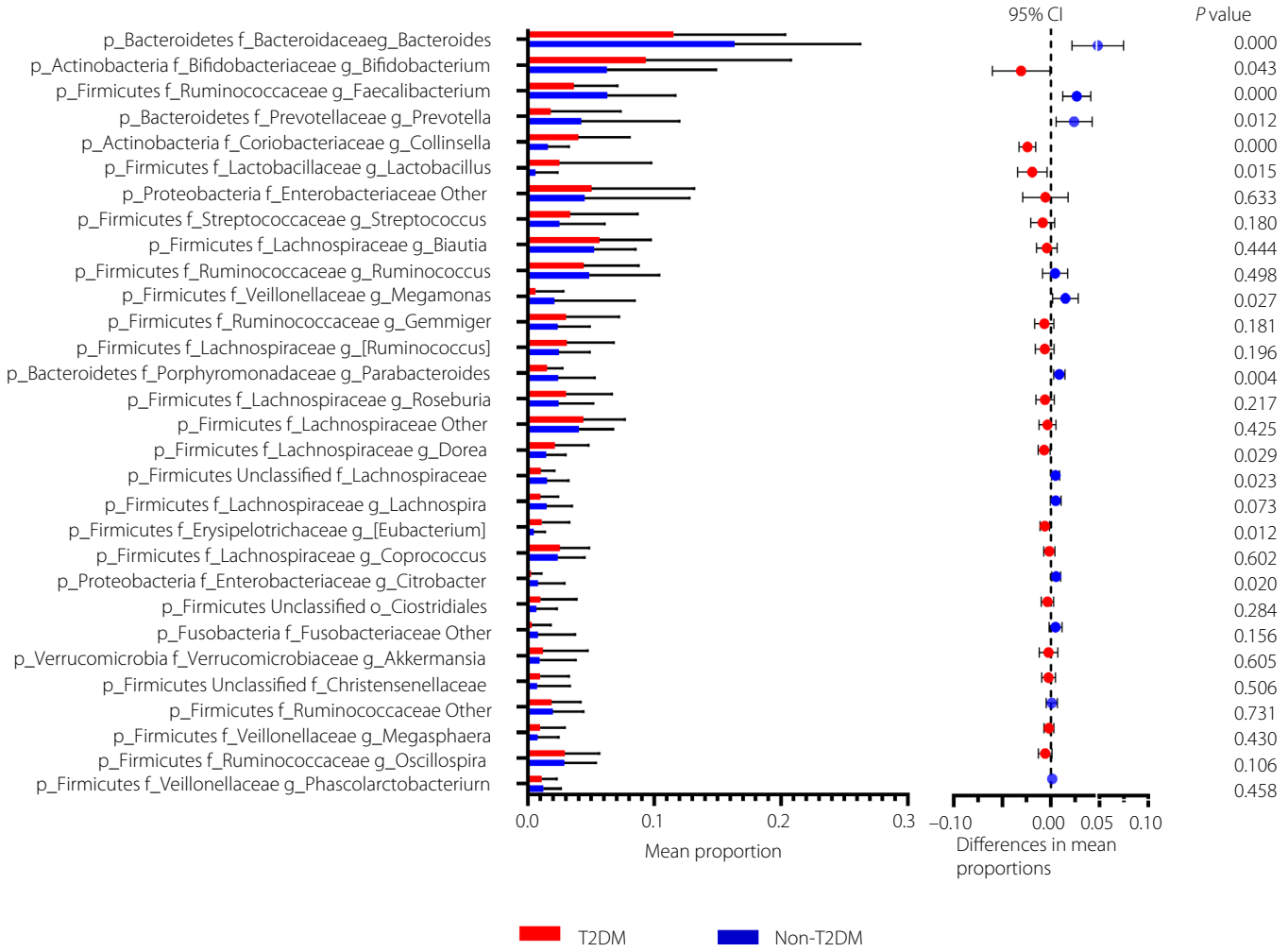


**Figure 2** | Dominant gut microbial genera in Japanese patients with type 2 diabetes mellitus (T2DM). The influence of genera for unique gut microbiota in Japanese patients with type 2 diabetes mellitus was assessed by the weighted average differences method, and the assessed influence of the genera was ranked from top to bottom. The top 30 gut microbial genera are shown, and differences between these gut microbial genera were evaluated by paired *t*-tests. Left, histograms show the mean proportion of the relative abundance of genera (mean + standard deviation); right, 95% confidence interval (CI) of the differences in the mean proportion and *P*-value by paired *t*-test is shown.

## DISCUSSION

In the current study, we investigated specific gut microbiota and their functional profiles for Japanese patients with type 2 diabetes mellitus. Importantly, we showed that the gut dysbiosis or change of gut functional microbial profiles associated with diabetes were closely related to dietary intake, especially habitual sucrose intake.

It is well known that the gut microbiota plays an important role in type 2 diabetes mellitus[6,7]. A whole-genome-based shotgun metagenomics study previously reported that certain functional profiles of the gut microbiota are associated with inflammatory diseases and autoimmune diseases[10]. However, few studies have shown that the functional profiles of the gut microbiota in Japanese patients with type 2 diabetes mellitus are different from those of healthy Japanese individuals[11].

In the current study, unweighted UniFrac metrics in patients with type 2 diabetes mellitus compared with those of healthy individuals were not statistically different. Conversely, weighted UniFrac metrics in the patients with type 2 diabetes mellitus compared with those of healthy individuals were statistically different. Various studies have reported that microbiota quantity and composition might be influenced by environmental factors. For example, it has been reported that the microbiota composition of vaginally delivered infants compared with those born by cesarean section differed[32]. Also, the quantity of microbiota could be changed by habitual dietary intake. In fact, high salt consumption has been shown to reduce intestinal survival of *Lactobacillus* spp[33]. These observations suggested that differences found in Japanese patients with type 2 diabetes mellitus compared with healthy Japanese individuals might be due to

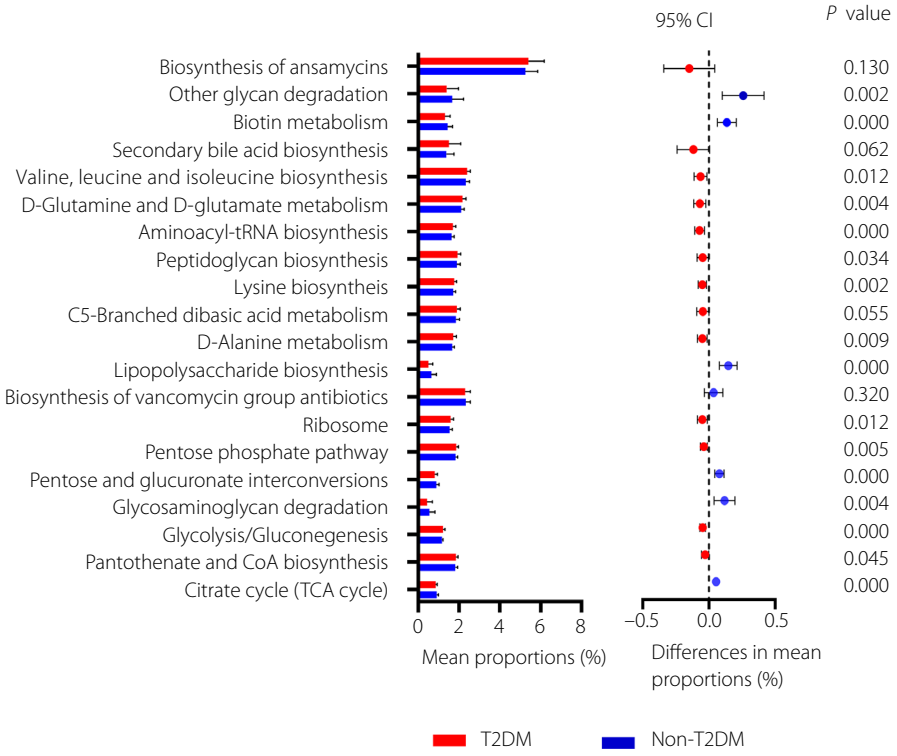


**Figure 3** | Change in metabolism profiles for the gut microbiota in Japanese patients with type 2 diabetes mellitus (T2DM). The degree of impairment in the gut microbial metabolism profile for Japanese patients with type 2 diabetes mellitus was assessed by the weighted average differences method, and the degree was ranked from top to bottom. The top 20 metabolism profiles for gut microbiota in the Japanese patients with type 2 diabetes mellitus are shown, and the difference of these gut microbiota profiles was evaluated by paired *t*-tests. Left, histograms show the mean proportion of the relative abundance of metabolism profiles of gut microbiota (mean + standard deviation); right, 95% confidence interval (CI) of the differences in the mean proportion and *P*-value by paired *t*-test is shown.

environmental factors, including habitual dietary intake. In fact, the diet of Japanese people, including patients with type 2 diabetes, differed from the diet of the people from other countries[13,14]. However, in recent years, continuous Westernization of diet has occurred in Japan[13,15].

In the present study, we found that reduction of genus *Bacteroides*[8,34–37] and increases of genus *Bifidobacterium*[37–39] were the predominant changes found in Japanese patients with type 2 diabetes mellitus, and sucrose intake, which is a one the characteristics of the Western diet[40], was correlated with these changes. In this the present, sucrose intake was associated with *Bacteroides* or *Bifidobacterium* after adjusting for several risk factors, including BMI[2]. It was reported that not all sucrose is absorbed in the small intestine, and some reach the colon[41]. In addition, the sucrose intake tended to be increased in Japanese patients with type 2 diabetes mellitus; thus, there is a possibility that not all sucrose is digested and absorbed in the small intestine, and some reaches the colon. A recent study showed that sucrose intake reduced *Bacteroides* levels by inhibiting the expression of the *BT3172* gene, which is essential for *Bacteroides*[42]. It was reported that decreasing genus *Bacteroides* is associated with insulin resistance[2,5], due to the reduction of branched chain amino acids[4] and short-chain fatty acids[4]. It is controversial that the relative abundance of *Bifidobacterium* in Japanese patients with type 2 diabetes mellitus is increasing or decreasing[12,37,43]. The study, which showed an increase of *Bifidobacterium* in Japanese patients with type 2 diabetes mellitus, showed that there is an association between the use of α-glucosidase inhibitors and an increase of *Bifidobacterium*[37]. In fact, the use of α-glucosidase inhibitors was associated with an increase of *Bifidobacterium* in the present study. Previous studies showed that some *Bifidobacterium* species possessed a fructose transporter associated with a highly active fructose metabolism in the intestinal tract and the production of acetic acid[44]. Furthermore, *Bifidobacterium* possesses a bifid shunt, which could efficiently produce adenosine triphosphate from glucose[45]. Sucrose is composed of glucose and fructose molecules; therefore, it is possible that *Bifidobacterium* was proliferated, by digesting sucrose. *Bifidobacterium* mainly exists in the colon. However, some *Bifidobacterium* exists in the duodenum[46]. Thus, there is a possibility that these *Bifidobacterium* that exist in the duodenum use sucrose. It has been reported that increased levels of *Bifidobacterium* leads to enhanced insulin signaling and reduced inflammation in

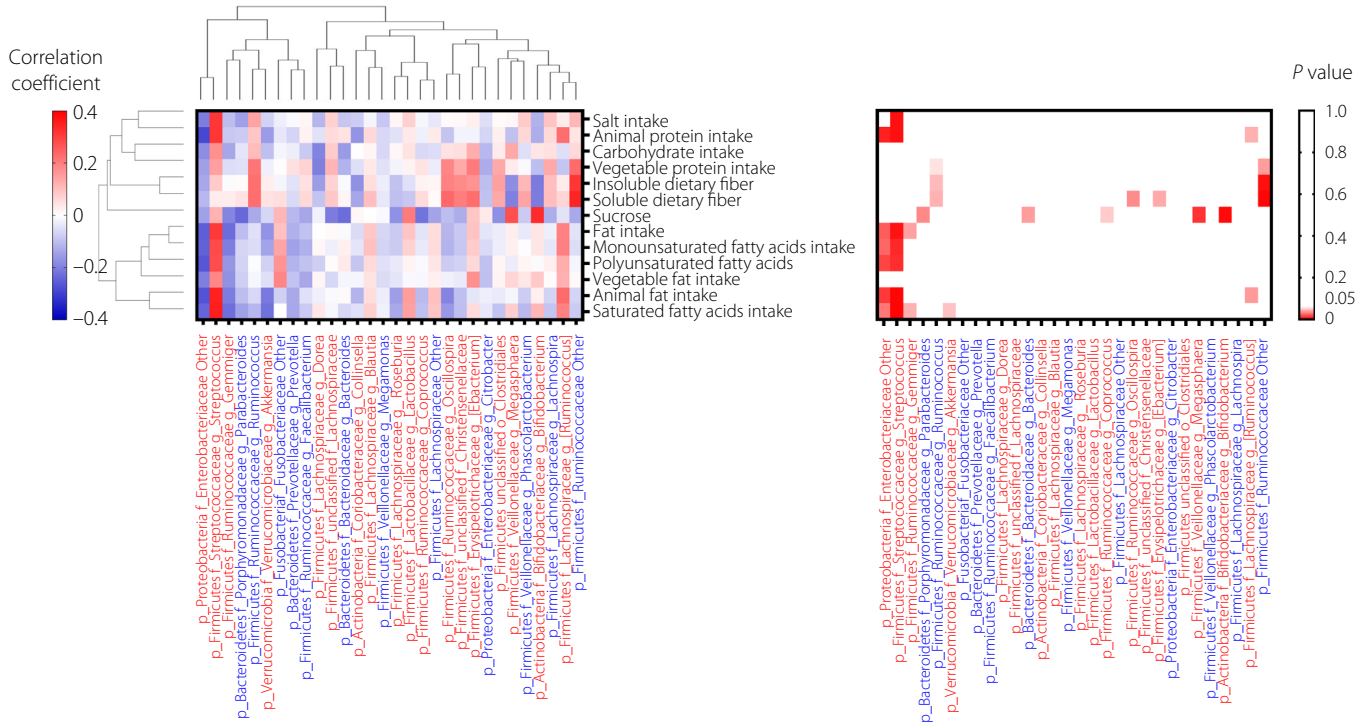


**Figure 4** | Cluster analysis for the association between habitual dietary intake and the relative quantity of the gut microbial genera for Japanese patients with type 2 diabetes mellitus. The effect of essential habitual dietary intake, including carbohydrates, animal protein, vegetable protein, fat, animal fat, vegetable fat, saturated fat, polyunsaturated fat, monounsaturated fat, soluble dietary fiber, insoluble dietary fiber, salt and sucrose or fatty acid intake, on diabetes-specific gut microbial genera was investigated by Spearman's correlation coefficient. Furthermore, hierarchical clustering was carried out to investigate the impact of habitual dietary essential nutrient intake on the diabetic type of gut microbiota. The habitual dietary essential nutrient intake is shown along the *y*-axis, and the top 30 genera associated with Japanese patients with type 2 diabetes mellitus are shown along the *x*-axis. Blue font denotes genera with reduced abundance in patients with type 2 diabetes mellitus. Red font denotes genera with elevated abundance in patients with type 2 diabetes mellitus. (a) Hierarchical clustering. Red shows a positive association between essential habitual dietary intake and diabetes specific gut microbial genera (Spearman's correlation coefficient). Blue shows a negative association between essential habitual dietary intake and diabetes specific gut microbial genera (Spearman's correlation coefficient). (b) *P*-values (Spearman's correlation coefficient). Red indicates a *P*-value <0.05.

the adipose tissue, and improves the translocation of glucose transporter-4 and insulin-stimulated glucose uptake[5,47], in which dietary sucrose intake might be associated with insulin sensitivity.

A previous study showed that glycolysis/gluconeogenesis is associated with the degradation of carbohydrates into short-chain fatty acids[48], and is prevalent in patients with type 2 diabetes mellitus. In addition, we showed that sucrose intake was associated with the functional profiles. Hence, it is possible that sucrose intake leads to altered short-chain fatty acid profiles in the gut, and that the source of carbohydrate was important for development of type 2 diabetes mellitus. In addition, other functional profile pathways, including other glycan degradation and biotin metabolism, of patients with type 2 diabetes differ from healthy individuals. Other glycan degradation included N-glycans biosynthesis. It has been reported that N-glycans biosynthesis is associated with diabetes through a link to an asparagine residue of a polypeptide chain and O-GlcNAc[49,50]. Furthermore, biotin metabolism is also associated with diabetes through regulation of pancreatic cells[51]. In contrast, the pathogenesis of diabetes is really complicated, and thus, there is a possibility that there are important habitual dietary intakes other than sucrose intake. However, the association between these pathways and type 2 diabetes mellitus was still unknown, and further studies are required to clarify the role of these pathways in type 2 diabetes mellitus.

Furthermore, we showed that intake of medium-chain fatty acids, which have been reported as exerting an anti-inflammatory effect[52], was associated with the impairment of the gut microbiota or the functional profiles of the gut microbiota. Whereas the mechanism(s) underpinning the relationship between the medium-chain fatty acids and impairment of the gut microbiota or the functional profiles of the gut microbiota in patients with type 2 diabetes mellitus remain unknown. There is a possibility that this is a unique feature of the gut microbiota within the Japanese population[8]. Thus, further

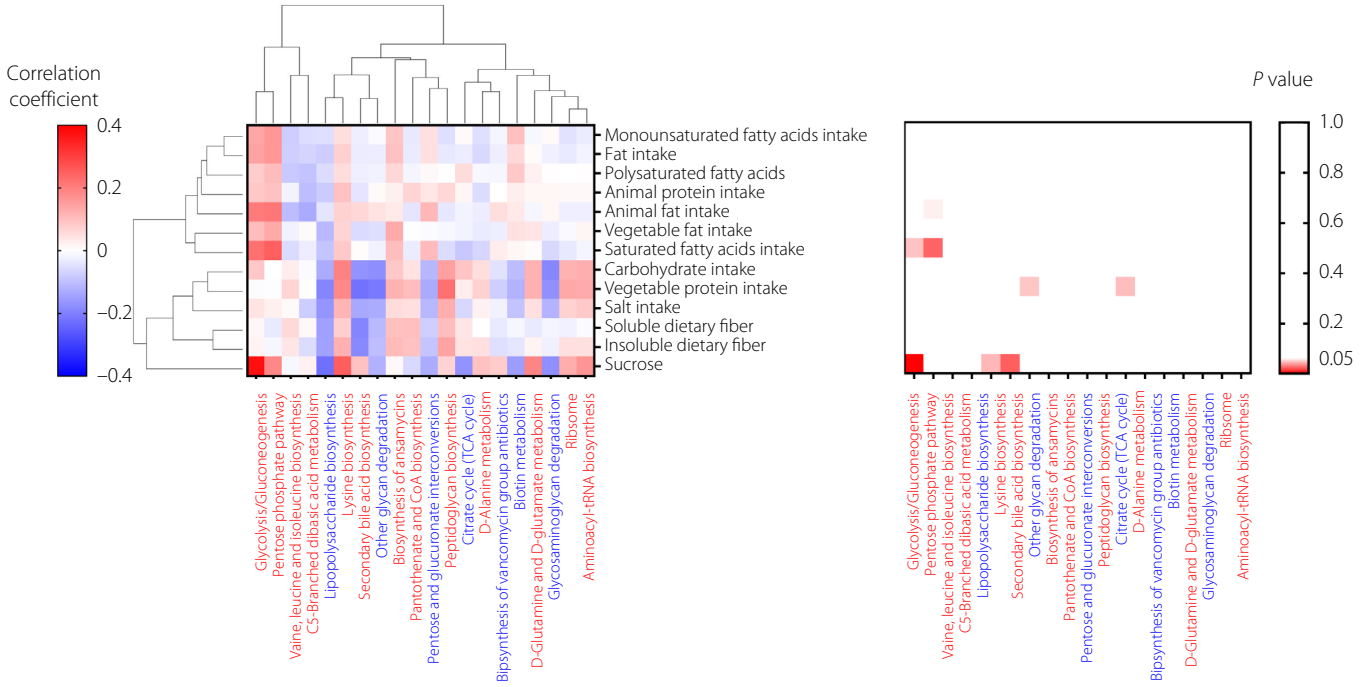


**Figure 5** | Cluster analysis for the association between habitual dietary intake and the metabolism profiles for the gut microbiota of Japanese patients with type 2 diabetes mellitus. The effects of essential habitual dietary intake, including carbohydrate, animal protein, vegetable protein, fat, animal fat, vegetable fat, saturated fat, polyunsaturated fat, monounsaturated fat, soluble dietary fiber, insoluble dietary fiber, salt and sucrose or fatty acid intake, on gut microbiota metabolism profiles of Japanese patients with type 2 diabetes mellitus was investigated by Spearman's correlation coefficient. Furthermore, hierarchical clustering was carried out to investigate the impact of habitual dietary essential nutrient intake on gut microbiota metabolism profiles of Japanese patients with type 2 diabetes mellitus. The habitual dietary essential nutrient intake is shown along the *y*-axis, and the top 20 metabolism profiles of Japanese patients with type 2 diabetes mellitus annotated using the Kyoto Encyclopedia of Genes and Genomes orthology are shown along the *x*-axis. Blue font denotes metabolism profiles less prevalent in Japanese patients with type 2 diabetes mellitus than in healthy individuals. Red font denotes metabolism profiles more prevalent in Japanese patients with type 2 diabetes mellitus than in healthy individuals. (a) Hierarchical clustering. Red shows a positive association between essential habitual dietary intake and metabolism profiles of gut microbiota (Spearman's correlation coefficient). Blue shows a negative association between essential habitual dietary intake and the metabolism profiles of gut microbiota (Spearman's correlation coefficient). (b) *P*-values (Spearman's correlation coefficient). Red indicates a *P*-value <0.05.

**Table 1** | Association between sucrose intake and *Bacteroides* or *Bifidobacterium*

| | *Bacteroides* | | *Bifidobacterium* | |
|---|---|---|---|---|
| | β | *P* | β | *P* |
| Age (years) | −0.05 | 0.649 | −0.37 | 0.001 |
| Men | −0.03 | 0.808 | −0.13 | 0.218 |
| Duration of diabetes | −0.21 | 0.075 | 0.15 | 0.175 |
| Exercise | 0.11 | 0.297 | −0.03 | 0.716 |
| Smoking | 0.13 | 0.241 | −0.11 | 0.288 |
| BMI (kg/m$^2$) | 0.005 | 0.967 | 0.03 | 0.788 |
| Hemoglobin A1c (%) | 0.16 | 0.142 | 0.16 | 0.108 |
| Total energy intake (kcal/ideal bodyweight) | 0.09 | 0.469 | −0.24 | 0.040 |
| Sucrose intake (g/day) | −0.30 | 0.015 | 0.44 | <0.001 |

Exercise status was defined as non-regular exerciser (=0) or regular exerciser (=1); smoking status was defined as non-smoker (=0) or smoker (=1). BMI, body mass index;

studies need to clarify whether the effect of the medium-chain fatty acids might differ in other populations.

The current study had several limitations. First, this was a cross-sectional study. Hence, a causal relationship between the gut dysbiosis or function profiles for the gut microbiota and diabetes was unknown. In addition, there is a possibility that hypoglycemic drugs, including metformin and α-glucosidase inhibitors, have changed the microbiota composition. Second, although we predicted the function of gut microbiota by 16S rRNA metagenomic sequencing, we did not obtain accurate data for the functioning of gut microbiota. In addition, the pathobionts in type 2 diabetes mellitus, such as *Clostridium ramosum* in type 2 diabetes mellitus through upregulation of small intestinal glucose and fat transporters[53], are important. Therefore, further studies, such as metabolome analysis, are required in order to understand the pathobionts of gut microbiota in type 2 diabetes mellitus. Third, we only analyzed data for the dietary habits from type 2 diabetes mellitus patients, as diet was part of their detailed background information. Therefore, we have not been able to rigorously assess the effects of dietary habits on gut bacteria in healthy Japanese individuals. Fourth, there is a possibility that the sample size of the present study was not large enough. However, previous studies carried out this analysis with approximately ≤100 participants[54,55], thus, the sample size of the present study might not be too small to evaluate. Finally, the universality of the findings of the study (e.g., relationship to other races) is unknown.

In conclusion, the presented data showed that the gut microbiota and functional profiles for the gut microbiota in patients with type 2 diabetes mellitus were significantly different from those in healthy individuals. Furthermore, we showed that habitual dietary intake, particularly sucrose intake, was closely associated with these differences. Although further studies are still required, there is a possibility that reducing sucrose intake could help prevent the onset of type 2 diabetes mellitus through prevention of gut dysbiosis in Japanese individuals.

## ACKNOWLEDGMENTS

We thank Editage (www.editage.jp) for English language editing. This work was supported by Grants-in-Aid for Scientific Research (B) (KAKENHI) to YN (no. 16675576) from the Japan Society for the Promotion of Science, and by Grant of the Industry-Academia-Government Collaboration in "Field for Knowledge Integration and Innovation" (FKII) to YN (no. 16824414) from the Ministry of Agriculture, Forestry and Fisheries of Japan. No funding bodies had any role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The data have been deposited with links to BioProject accession number DRA009950 in the DDBJ BioProject database (available 1 July 2020).

## DISCLOSURE

YH has received grant support from Asahi Kasei Pharma, and honoraria from Mitsubishi Tanabe Pharma Corporation and Novo Nordisk Pharma Ltd. MH has received grant support from Asahi Kasei Pharma, payment for development of educational presentations from MSD K.K., Mitsubishi Tanabe Pharma Corporation, Kowa Company, Ltd. and Sumitomo Dainippon Pharma Co., Ltd., and royalties from US 10,238,714 B2. YN received a scholarship from EA Pharma Co., Ltd., a collaboration research fund from Fujifilm Medical Co., Ltd., and has received lecture fees from Janssen Pharma K.K., Mylan EPD Co., Takeda Pharma Co., Ltd., Mochida Pharma Co., Ltd., EA Pharma Co., Ltd., Otsuka Pharma Co., Ltd., Astellas Pharma Co., Ltd. and Miyarisan Pharma Co., Ltd. The research was partly funded by these funds. Neither the funding agency nor any outside organization has participated in the study design or has any competing interest. These pharmaceutical companies approved the final version of the manuscript. MF has received research support and payment for development of educational presentation from AstraZeneca plc., Astellas Pharma Inc., Nippon Boehringer Ingelheim Co., Ltd., Daiichi Sankyo Co., Ltd., Eli Lilly Japan K.K., Kyowa Hakko Kirin Company Ltd., Kissei Pharmaceutical Co., Ltd., MSD K.K., Sumitomo Dainippon Pharma Co., Ltd. Kowa Company, Ltd., Mitsubishi Tanabe Pharma Corporation, Novo Nordisk Pharma Ltd., Sanwa Kagaku Kenkyusho Co., Ltd., Sanofi K.K., Ono Pharmaceutical Co., Ltd., Taisho Pharmaceutical Co., Ltd., Bayer Yakuhin, Ltd., Mochida Pharmaceutical Co., Ltd., Johnson & Johnson k.k. Medical Company, Nippon Chemiphar Co., Ltd., Terumo Corporation, Teijin Pharma Ltd. and Takeda Pharmaceutical Co., Ltd. The other authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** | Inclusion and exclusion flow chart.
**Figure S2** | Comparative analysis of the taxonomic composition of the microbial community on the phylum level between Japanese patients with type 2 diabetes mellitus and healthy Japanese individuals.
**Figure S3** | Dominant gut microbial genera in Japanese men and women with type 2 diabetes mellitus.
**Figure S4** | The association between sucrose intake and genera *Bacteroides* and genera *Bifidobacterium*.
**Figure S5** | Cluster analysis for the association between habitual dietary free fatty acid intake and the relative quantity of the gut microbial genera of Japanese patients with type 2 diabetes mellitus.
**Figure S6** | Cluster analysis for the association between habitual dietary free fatty acid intake and the metabolism profile for the gut microbiota of Japanese patients with type 2 diabetes mellitus.
**Figure S7** | The association between clinical characteristics of patients with type 2 diabetes mellitus and gut microbial genera.
**Figure S8** | The association between clinical characteristics of patients with type 2 diabetes mellitus and metabolism profile with gut microbiota.
**Table S1** | Characteristics of study participants with type 2 diabetes.
**Table S2** | Correlation between habitual dietary intake and the relative quantity of gut microbial genera of Japanese patients with type 2 diabetes.
**Table S3** | Correlation between habitual dietary intake and metabolism profiles of gut microbiota of Japanese patients with type 2 diabetes.

**Table S4** | Correlation between habitual dietary free fatty acid intake and the relative quantity of gut microbial genera of Japanese patients with type 2 diabetes.
**Table S5** | Correlation between habitual dietary free fatty acid intake and metabolism profiles of gut microbiota of Japanese patients with type 2 diabetes.