Bioscience of Microbiota, Food and Health Vol. 41 (3), 103–111, 2022

Full Paper

Association of gut microbiota and inflammatory markers in obese patients with type 2 diabetes mellitus: post hoc analysis of a synbiotic interventional study

Yukiko SUGAWARA[1], Akio KANAZAWA[1*], Masanori AIDA[5], Yasuto YOSHIDA[5], Yuichiro YAMASHIRO[6] and Hirotaka WATADA[1-4]

[1]*Department of Metabolism & Endocrinology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

[2]*Center for Therapeutic Innovations in Diabetes, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

[3]*Center for Identification of Diabetic Therapeutic Targets, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

[4]*Sportology Center, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

[5]*Food Research Department, Yakult Central Institute, 5-11 Izumi, Kunitachi-shi, Tokyo 186-8650, Japan*

[6]*Probiotics Research Laboratory, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

Received December 2, 2021; Accepted January 26, 2022; Published online in J-STAGE February 16, 2022

Chronic inflammation caused by gut dysbiosis is associated with the pathophysiology of metabolic disease. Synbiotics are useful for ameliorating gut dysbiosis; however, it remains unclear what types of bacteria act as key markers for synbiotic-driven improvement of chronic inflammation. Here, we performed a post hoc analysis of a 24-week randomized controlled study using synbiotics to investigate the association between gut microbiota and inflammatory markers. We characterized the responders who showed lower interleukin-6 (IL-6) levels in response to synbiotic supplementation among 86 obese patients with type 2 diabetes mellitus. In our baseline analysis, the relative abundances of *Bifidobacterium adolescentis* and *Alistipes onderdonkii* correlated positively with IL-6, lipopolysaccharide binding protein (LBP), and high-sensitivity C-reactive protein (Hs-CRP) levels. The relative abundance of *Eubacterium rectale* correlated positively with LBP and Hs-CRP levels, and that of *Bacteroides thetaiotaomicron* correlated positively with LBP levels. Based on our responder analysis, patients with higher body mass indices (over 30 kg/m^2 on average), low abundances of *Bacteroides caccae* and *Parabacteroides merdae* at baseline and 24 weeks, and minimal changes in the relative abundance of *E. rectale* and Shannon index from baseline showed decreased IL-6 levels compared with baseline. However, glycemic control in responders was unchanged. In conclusion, we identified four bacterial species (*B. adolescentis*, *A. onderdonkii*, *E. rectale*, *and B. thetaiotaomicron*) related to chronic inflammation and predictive markers (*B. caccae, P. merdae,* and severity of obesity) in responders to synbiotic supplementation among obese patients with type 2 diabetes.

Key words: diabetes mellitus, chronic inflammation, gut microbiota, synbiotic, obesity

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease. Multiple factors, including genetics, lifestyle, diet, and aging, contribute to either the onset or progression of this disease, if not both [1]. Among these factors, diet is an important environmental factor affecting the gut microbiota, and diet-induced changes in microbial composition are involved in human physiology and disease processes [2]. We previously demonstrated that obese patients with T2DM had chronic inflammatory states accompanied by gut dysbiosis and bacterial translocation [3]. Therefore, the gut microbiota is a new therapeutic target for treating chronic inflammation in T2DM.

To date, probiotics, prebiotics, and synbiotics (the combination of one or more probiotics and prebiotics) have been reported to be useful for inhibiting bacterial translocation [4] and improving the

*Corresponding author. Akio Kanazawa (E-mail: akana@juntendo.ac.jp)

(Supplementary materials: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2480/)

doi: 10.12938/bmfh.2021-081

intestinal environment in metabolic disease [5] and other diseases [6]. Therefore, we previously performed a 24-week randomized controlled study to investigate the effects of daily intake of a synbiotic comprising *Lacticaseibacillus paracasei* (previously known as *Lactobacillus casei*) strain Shirota YIT 9029, *Bifidobacterium breve* strain Yakult YIT 12272, and galacto-oligosaccharides on chronic inflammation and gut microbiota in 86 obese patients with T2DM [7]. This synbiotic did not reduce plasma interleukin-6 (IL-6) levels as the primary outcome, although numerous bacterial species that showed significant changes in response to synbiotic supplementation were identified by using 16S rRNA amplicon gene analysis [7].

The presence of responders and non-responders to diet and probiotics in relation to cholesterol metabolism and insulin sensitivity has already been reported in obese individuals [8] and T2DM [9]. However, little is known about the clinical characteristics of responders with improvement of chronic inflammation in response to synbiotic supplementation among obese patients with T2DM. Therefore, it is important to identify clinical or microbial biomarkers for predicting responders to synbiotic supplementation in order to pave the way for personalized nutrition.

Recently, the associations between specific bacterial species and inflammation have been reported in some diseases, such as inflammatory bowel diseases [10] and autoimmune diseases [11]. However, bacterial species relating to inflammatory markers, such as IL-6, high-sensitivity C-reactive protein (Hs-CRP), and lipopolysaccharide binding protein (LBP), have yet to be investigated in obese patients with T2DM. Based on this background information, we performed a post hoc analysis of a randomized controlled study using synbiotics to investigate gut microbiota related to chronic inflammation. We also sought to identify clinical and microbial markers among responders to synbiotic supplementation in obese patients with T2DM.

MATERIALS AND METHODS

Study participants

In this post hoc analysis, inclusion and exclusion criteria were applied as previously described [7]. Briefly, the main inclusion criteria were 1) age ≥30 but <80 years, 2) HbA1c (National Glycohemoglobin Standardization Program, NGSP) ≥6.0 but <9.0%, and 3) body mass index (BMI) ≥25.0 kg/m^2. The exclusion criteria were 1) serious kidney disease (serum creatinine level ≥1.5 mg/dL and/or hemodialysis), 2) serious liver disease excluding fatty liver, and 3) inflammatory bowel disease. A total of 86 patients with T2DM who met the requirements of the above inclusion and exclusion criteria were recruited into the interventional study examined [7]. As we aimed to perform the analysis by having as many subjects as possible, the baseline data of these subjects (n=86) before synbiotic supplementation were used to investigate correlations between baseline inflammatory markers (Hs-CRP, LBP, and IL-6) and relative abundances of the gut microbiota obtained by 16S rRNA gene sequencing. Next, we selected the change in IL-6 as an index of responders to synbiotic supplementation because it was the primary outcome in the synbiotic interventional study [7], and we divided the synbiotic recipients (n=44) into responders (n=23) and non-responders (n=21) depending on whether or not they displayed a decrease in IL-6 from baseline to 24 weeks after synbiotic supplementation.

As the synbiotic intervention, the following agents were administered orally: 3.0 g dry powder containing at least 3×10^8 living *L. paracasei* strain Shirota YIT 9029, 3×10^8 living *B. breve* strain Yakult YIT 12272, and 7.5 g galacto-oligosaccharides (GOS) per day (product name: Yakult Super Synbiotics LBG-P, Yakult Honsha Co., Ltd., Tokyo, Japan). Patients were instructed to take the synbiotic twice a day (2.0 g dry powder and 5.0 g GOS at breakfast and 1.0 g dry powder and 2.5 g GOS at dinner). The synbiotic recipients consumed the aforementioned doses every day for 24 weeks. The protocol of the post hoc analysis was approved by the Human Ethics Committee of Juntendo University (approval number: H21-0041). Written informed consent was not obtained from each participant, as this was a retrospective study. Additionally, participants were given the opportunity to opt out of the study (https://www.gcprec.juntendo.ac.jp/kenkyu/6/detail/3357).

Analysis of the gut microbiota and inflammatory markers

We used previously described methods to perform a gut microbiota analysis using 16S rRNA gene sequencing [7]. Sequences generated from the MiSeq platform were analyzed using the open-source software package Quantitative Insights Into Microbial Ecology 2 (QIIME2; 2020.2) [12], and the SILVA138 database (https://www.arb-silva.de/) was used to annotate taxonomic information. Alpha diversities represented as the number of observed operational taxonomic units (OTUs), the Shannon index, and phylogenetic diversity (PD) were estimated for 5,000 randomly selected sequences to account for differences in sampling effort between the samples. Lastly, biochemical assays for HbA1c, lipids, fecal organic acids, and inflammatory markers (Hs-CRP, LBP, and IL-6) were performed as described previously [7]. These data were reanalyzed for the present post hoc analysis.

Statistical analysis

All data are presented as the mean ± standard deviation (SD), and they were analyzed using StatFlex ver. 7 (Artech Co., Osaka, Japan). Comparisons of the groups of responders and non-responders were analyzed by Student's *t*-test. The relationships between microbiota and inflammatory markers were investigated by Pearson's correlation coefficient analysis. $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics of the study participants at baseline

Table 1 shows the clinical characteristics of the study participants at baseline. The average age of the study participants was 58.5 ± 11.1 years, and 21 participants were women. The average BMI was 29.3 ± 4.1 kg/m^2, and only nine patients were not taking any medications. The medications of the participants during the 24-week study period are presented in Supplementary Table 1. A DPP-4 inhibitor or SGLT2 inhibitor was added to the medications taken by each of the non-responders and responders, respectively.

Correlations between gut microbiota and inflammatory markers

At baseline, the relative abundances of seven bacteria phyla and 33 bacterial families assigned based on the SILVA database showed no significant correlations with inflammatory markers

Table 1. Clinical characteristics of the study participants at baseline

n	86
Sex (male/female)	65/21
Age (years)	58.5 ± 11.1
BMI (kg/m^2)	29.3 ± 4.1
HbA1c (%)	7.3 ± 0.8
C-peptide (ng/mL)	2.4 ± 1.3
T-CHO (mg/dL)	192.6 ± 41.0
HDL-C (mg/dL)	50.1 ± 10.0
TG (mg/dL)	183.8 ± 274.5
eGFR (mL/min/1.73m^2)	77.7 ± 20.7
Hs-CRP (ng/mL)	2,157.3 ± 4,530.9
IL-6 (pg/mL)	2.5 ± 1.7
LBP (μg/mL)	6.0 ± 3.5
Medication for diabetes	
No medication	9 (10.5)
Insulin only or with oral therapy	28 (32.6)
Oral therapy only	
SU	8 (9.3)
Metformin	55 (64.0)
Thiazolidine	12 (14.0)
DPP-4 inhibitor	42 (48.8)
Glinide	6 (7.0)
SGLT2 inhibitor	42 (48.8)
GLP-1 receptor agonist	10 (11.6)

Data are expressed as mean ± SD.
Numbers in parentheses are percentages (%).
BMI: Body mass index; T-CHO: Total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; eGFR: estimated glomerular filtration rate; Hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; LBP: lipopolysaccharide-binding protein; SU: sulfonylurea; DPP-4: dipeptidyl peptidase-4; SGLT2: sodium-dependent glucose cotransporter-2; GLP-1: glucagon-like peptide-1.

(Fig. 1). However, among 37 bacterial species (Fig. 2), we found that the relative abundance of *Bifidobacterium adolescentis* was positively associated with IL-6 ($r=0.336$, $p<0.01$, Supplementary Fig. 1A), LBP ($r=0.281$, $p<0.01$, Supplementary Fig. 1B), and Hs-CRP levels ($r=0.263$, $p<0.05$, Supplementary Fig. 1C). Moreover, the relative abundance of *Alistipes onderdonkii* correlated positively with IL-6 ($r=0.230$, $p<0.05$, Supplementary Fig. 2A), LBP ($r=0.404$, $p<0.01$, Supplementary Fig. 2B), and Hs-CRP levels ($r=0.578$, $p<0.01$, Supplementary Fig. 2C). The relative abundance of *Eubacterium rectale* also correlated positively with LBP ($r=0.226$, $p<0.05$, Supplementary Fig. 3A) and Hs-CRP levels ($r=0.256$, $p<0.05$, Supplementary Fig. 3B), while the abundance of *Bacteroides thetaiotaomicron* correlated positively with LBP levels ($r=0.234$, $p<0.05$, Supplementary Fig. 3C).

Clinical characteristics of the non-responders and responders

BMI and plasma IL-6 levels at baseline were significantly higher in patients that were responders compared with non-responders, and BMI at 24 weeks in responders was also higher than in non-responders (Table 2). Plasma IL-6 levels in responders showed a significant negative change from baseline compared with non-responders, and the change in plasma LBP levels in responders was significantly smaller than in non-responders. There were no significant differences between the two groups for the other parameters measured, including HbA1c.

Relative abundances of bacterial phyla and families in the non-responders and responders

Supplementary Table 3 shows the relative abundances of different bacterial families in the responders and non-responders. At baseline, the relative abundance of *Monoglobaceae* in responders was significantly lower than that of non-responders, and it increased from baseline compared with that in non-responders. The changes in the relative abundances of *Bifidobacteriaceae* and *Lachnospiraceae* in responders were significantly smaller than those in non-responders. As shown by Supplementary Table 2, there were no significant differences in the relative abundances of bacterial phyla between the two groups.

Relative abundances of bacterial species in the non-responders and responders

At both baseline and 24 weeks, the relative abundances of *Bacteroides caccae* and *Parabacteroides merdae* in responders were significantly lower than in non-responders. At 24 weeks, the relative abundance of *Collinsella aerofaciens* in responders was significantly higher than in non-responders. In addition, the relative abundance of *E. rectale* in responders showed a significant positive change from baseline compared with non-responders (Table 3).

doi: 10.12938/bmfh.2021-081

Microbial diversity and fecal organic acids in the non-responders and responders

The Shannon index showed a significant positive change from baseline in responders compared with non-responders. However, there were no significant differences in the other indices between the two groups (Table 4). Furthermore, fecal organic acids levels showed no significant changes in comparisons between the two groups (Supplementary Table 4).

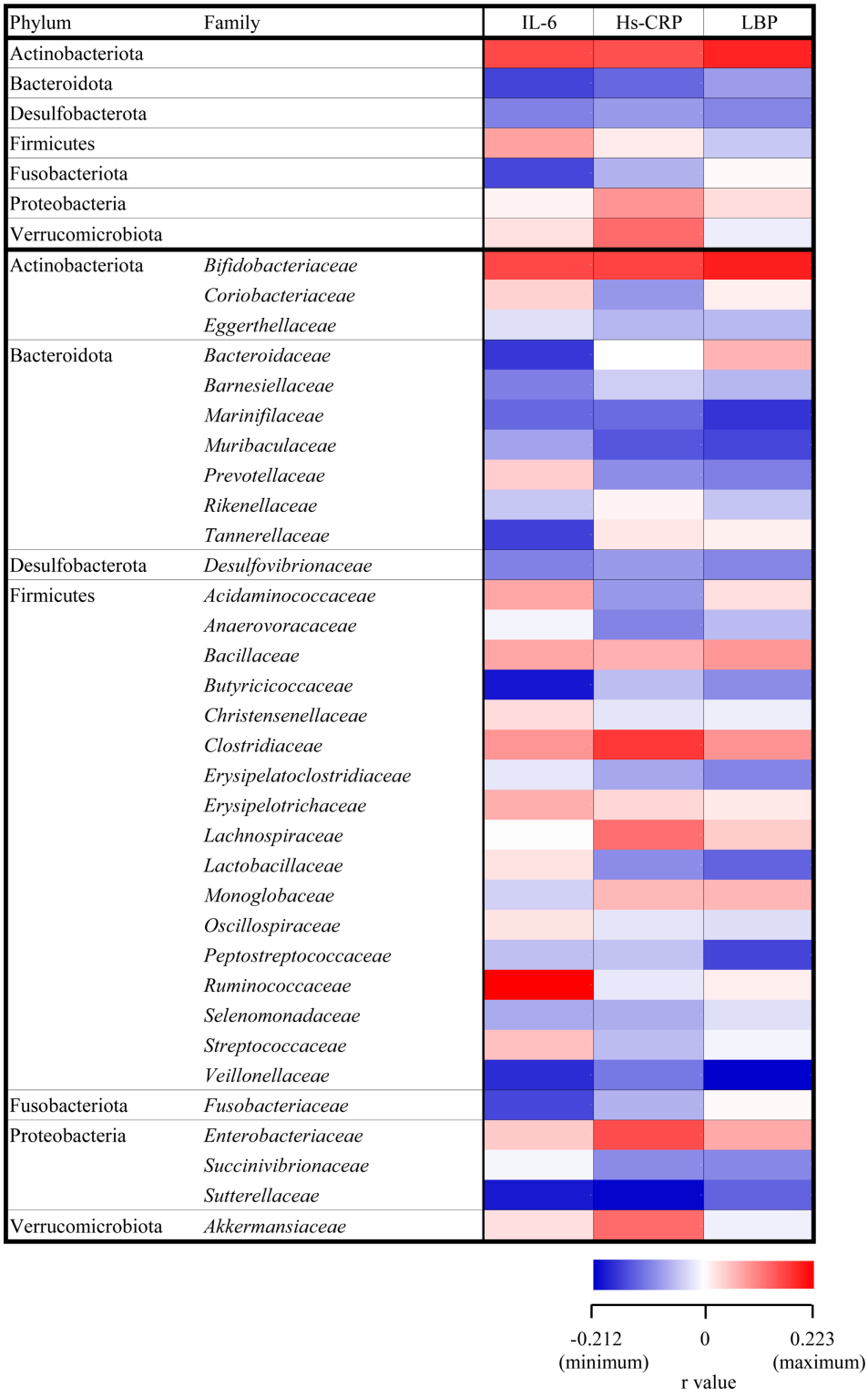

Fig. 1. Heat map of Pearson correlation coefficients between inflammatory markers and relative abundances of gut microbiota from the phylum to family levels. These relative abundances show no significant correlations with inflammatory markers.

doi: 10.12938/bmfh.2021-081

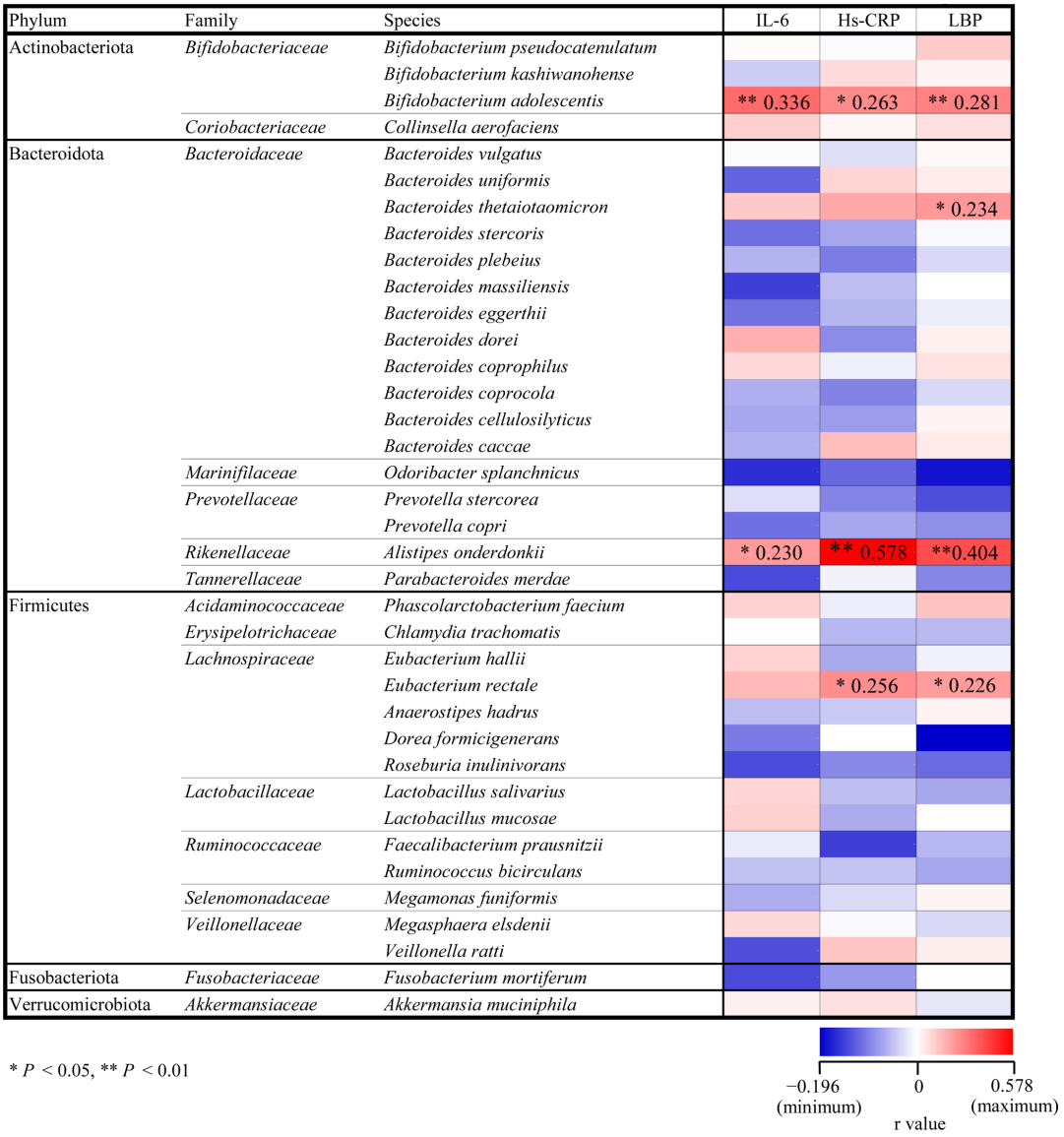

Fig. 2. Heat map of Pearson correlation coefficients between inflammatory markers and relative abundances of gut microbial species. Red and blue columns indicate positive and negative correlations, respectively. Correlation coefficients are visualized by color gradient (blue, minimum < r < maximum, red). Columns with asterisks shows significant correlations (*p<0.05; **p<0.01).

DISCUSSION

This is the first study to investigate associations between inflammatory markers and specific bacterial species and bacterial markers in responders to synbiotic supplementation among obese patients with T2DM. Based on analysis of baseline data, we found that the relative abundances of *B. adolescentis*, *A. onderdonkii*, *E. rectale*, and *B. thetaiotaomicron* correlated positively with plasma inflammatory markers.

Genus *Bifidobacterium* is a predominant bacterium in the human gut and contains more than 50 species, including several subspecies [13]. However, the details of the functional roles of these species in humans remain unclear. To date, some *Bifidobacterium* species have been reported to reduce intestinal endotoxin levels in mice [14] and have anti-inflammatory effects due to inhibition of lipopolysaccharide-induced nuclear factor-κB activation *in vitro* [15]. Furthermore, a previous report showed that *B. adolescentis* supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of metabolic syndrome [16]. Since some beneficial effects of *Bifidobacterium* species on anti-inflammation have been reported, as described above, *B. adolescentis* may be involved in improving chronic inflammation by regulating intestinal barrier function and anti-inflammatory effects.

Genus *Alistipes* is a relatively new bacterial genus that comprises 13 species isolated primarily from human samples [17]. As shown by Table 3, the relative abundance of *A. onderdonkii* in feces is low compared with *Bifidobacterium*. Previous reports indicated that genus *Alistipes* is involved in liver fibrosis [17] and colorectal cancer in Il-10−/− mice [18]. In particular, *A. onderdonkii* tended to be reduced in non-alcoholic fatty liver disease (NAFLD) patients with advanced fibrosis [19]. Although there have been no reports regarding the roles of this species in T2DM, chronic inflammation activation is a characteristic

doi: 10.12938/bmfh.2021-081

Table 2. Clinical parameters before and after synbiotic supplementation in the non-responders and responders

		Baseline	24 weeks	change
n (male/female)	Non-responders	21 (15/6)		
	Responders	23 (16/7)		
Age (years)	Non-responders	59.8 ± 8.9		
	Responders	62.2 ± 13.1		
BMI (kg/m^2)	Non-responders	28.1 ± 2.2	27.8 ± 2.0	−0.25 ± 0.79
	Responders	30.8 ± 5.5*	31.0 ± 5.6*	0.16 ± 0.56
HbA1c (%)	Non-responders	7.3 ± 0.7	7.3 ± 0.6	0.0 ± 0.6
	Responders	7.4 ± 0.8	7.8 ± 1.3	0.4 ± 0.9
C-peptide (ng/mL)	Non-responders	2.0 ± 1.1	1.9 ± 1.2	−0.1 ± 0.6
	Responders	2.6 ± 1.2	2.8 ± 1.6	0.3 ± 1.1
T-CHO (mg/dL)	Non-responders	195.7 ± 31.8	190.3 ± 29.7	−5.4 ± 23.5
	Responders	182.3 ± 35.5	188.8 ± 34.2	6.4 ± 26.8
HDL-C (mg/dL)	Non-responders	52.7 ± 10.2	50.8 ± 9.6	−1.9 ± 6.4
	Responders	49.0 ± 9.0	49.1 ± 9.6	0.1 ± 4.4
TG (mg/dL)	Non-responders	146.2 ± 85.2	185.2 ± 150.4	39.0 ± 104.7
	Responders	137.3 ± 57.5	178.3 ± 117.1	40.9 ± 87.1
eGFR (mL/min/1.73m^2)	Non-responders	75.1 ± 18.4	75.4 ± 23.7	0.3 ± 8.7
	Responders	81.1 ± 26.4	83.6 ± 24.3	2.5 ± 12.3
Hs-CRP (ng/mL)	Non-responders	1,780.8 ± 4,393.3	1,407.4 ± 1,755.8	−373.4 ± 3,816.8
	Responders	2,983.8 ± 6,728.4	1,772.0 ± 2,146.4	−1,211.8 ± 6,736.1
IL-6 (pg/mL)	Non-responders	1.8 ± 0.9	2.7 ± 1.0	0.9 ± 0.7
	Responders	3.5 ± 2.5**	2.3 ± 1.4	−1.3 ± 1.8**
LBP (μg/mL)	Non-responders	5.4 ± 2.3	8.8 ± 2.9	3.4 ± 2.8
	Responders	7.3 ± 5.3	7.9 ± 2.9	0.6 ± 4.8*

Data are expressed as mean ± SD. Change is expressed as the value measured at 24 weeks minus the baseline value. *p<005, **p<0.01 vs. Non-responders.
BMI: Body mass index; T-CHO: Total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; eGFR: estimated glomerular filtration rate; Hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; LBP: lipopolysaccharide-binding protein.

of NAFLD [20], which is highly prevalent in T2DM [21]. Therefore, *A. onderdonkii*, which showed significant correlations with inflammatory markers, may play an important role in either the onset or progression of NAFLD in T2DM, if not both the onset and progression.

Genus *Eubacterium*, which is assigned to the phylum Firmicutes, is a part of the core human gut microbiota and is phylogenetically diverse [22]. *E. rectale*, one of the most extensively studied *Eubacterium* species, was first isolated from the feces of healthy Japanese-Hawaiian males and was identified as a major butyrate producer [23]. To date, this species has been reported to be negatively correlated with IL-6 and IL-1β levels, and the *E. rectale* relatives group has been reported to be correlated with IL-6 and IL-8 in the elderly [24], while the abundance of *E. rectale* and relatives has been reported to be correlated negatively with IL-1β, NLR family pyrin domain containing 3, and CXC motif chemokine ligand 2 in cognitively impaired elderly [25]. In addition, *E. rectale* was reported to be among the gut bacteria that were positively associated with lower postprandial glycemic response [26]. Based on these findings, *E. rectale* may be linked to the pathophysiology of T2DM via various mechanisms.

B. thetaiotaomicron exhibited a considerable increase in obese patients following a weight-loss intervention [27]. Experimentally, *B. thetaiotaomicron* administration protected mice against adiposity [27]. In addition, *B. thetaiotaomicron* modulates the intestinal mucus barrier by modifying goblet cells and mucin glycosylation in rats [28]. As a positive correlation was found between the relative abundance of *B. thetaiotaomicron* and the LBP level in the present study, this species is also considered to be involved in chronic inflammation by regulating mucus barrier function (i.e., goblet cell differentiation, expression of mucus-related genes, and the ratio of sialylated to sulfated mucins).

In our responder analysis, we found that diabetes patients with higher BMIs (over 30 kg/m^2 on average) and IL-6 levels at baseline showed a significant reduction in IL-6 by the end of the study, suggesting that severe obesity is a clinical factor that predicts responders to synbiotics. Furthermore, as shown by the BMI data in Table 2, responders with higher BMIs (over 30 kg/m^2 on average) had higher levels of IL-6. Therefore, the patients with higher BMIs might have had more severe chronic inflammation, suggesting that the patients with higher levels of chronic inflammation at baseline were more sensitive to synbiotic supplementation. The relative abundance of *E. rectale* in responders was stable compared with non-responders. Since *E. rectale* has some antidiabetic effects, as discussed above, the increase in feces in response to synbiotics may be a biological marker for responders. The relative abundances of two bacterial species, *B. caccae* and *P. merdae,* in responders were significantly lower at both baseline and 24 weeks; however, their abundances did not significantly change over time. Although the functional roles of *P. merdae* in chronic inflammation remain unclear, TonB-linked outer membrane protein (OmpW) produced by *B. caccae* is known to be associated with perinuclear anti-neutrophil cytoplasmic antibodies, and the anti-OmpW immunoglobulin A

Table 3. Relative abundances at the species level before and after synbiotic supplementation in the non-responders and responders

Phylum	Family	Species		Relative abundance (%)		Change (%)
				Baseline	24 weeks	24 weeks
Actinobacteriota	*Bifidobacteriaceae*	*Bifidobacterium pseudocatenulatum*	Non-responders	1.3 ± 1.9	6.5 ± 8.5	5.2 ± 7.4
			Responders	2.8 ± 6.8	4.2 ± 4.3	1.3 ± 5.7
		Bifidobacterium kashiwanohense	Non-responders	0.01 ± 0.03	0.01 ± 0.05	0.00 ± 0.02
			Responders	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Bifidobacterium adolescentis	Non-responders	2.3 ± 3.5	7.1 ± 9.2	4.8 ± 7.2
			Responders	4.3 ± 6.4	7.6 ± 8.1	3.4 ± 7.6
	Coriobacteriaceae	*Collinsella aerofaciens*	Non-responders	0.38 ± 0.40	0.46 ± 0.48	0.09 ± 0.29
			Responders	0.78 ± 0.93	0.97 ± 0.64*	0.19 ± 0.76
Bacteroidota	*Bacteroidaceae*	*Bacteroides vulgatus*	Non-responders	0.11 ± 0.40	0.06 ± 020	−0.05 ± 0.21
			Responders	0.65 ± 1.53	0.44 ± 1.24	−0.23 ± 0.49
		Bacteroides uniformis	Non-responders	2.9 ± 2.8	1.9 ± 2.0	−1.0 ± 2.4
			Responders	2.5 ± 3.1	2.2 ± 3.4	−0.4 ± 2.0
		Bacteroides thetaiotaomicron	Non-responders	0.47 ± 0.52	0.49 ± 0.80	0.02 ± 0.67
			Responders	0.58 ± 0.57	0.35 ± 0.48	−0.22 ± 0.40
		Bacteroides stercoris	Non-responders	2.0 ± 4.0	1.8 ± 3.6	−0.3 ± 0.9
			Responders	1.9 ± 3.2	1.9 ± 3.4	−0.1 ± 2.6
		Bacteroides plebeius	Non-responders	1.6 ± 3.8	2.2 ± 4.8	0.6 ± 2.6
			Responders	3.0 ± 7.7	2.3 ± 5.1	−0.9 ± 4.5
		Bacteroides massiliensis	Non-responders	1.00 ± 2.80	0.40 ± 1.41	−0.59 ± 1.47
			Responders	0.66 ± 1.36	0.67 ± 1.57	−0.03 ± 0.70
		Bacteroides eggerthii	Non-responders	0.67 ± 1.53	0.44 ± 1.06	−0.23 ± 0.61
			Responders	0.09 ± 0.38	0.02 ± 0.06	−0.07 ± 0.35
		Bacteroides dorei	Non-responders	0.7 ± 1.9	0.9 ± 2.7	0.2 ± 0.8
			Responders	1.9 ± 4.4	1.5 ± 3.3	−0.4 ± 1.8
		Bacteroides coprophilus	Non-responders	0.18 ± 0.58	0.09 ± 3.2	−0.09 ± 0.36
			Responders	0.82 ± 2.80	0.37 ± 1.59	−0.49 ± 2.54
		Bacteroides coprocola	Non-responders	0.58 ± 2.32	0.69 ± 3.01	0.11 ± 0.72
			Responders	0.14 ± 0.51	0.03 ± 0.08	−0.12 ± 0.49
		Bacteroides cellulosilyticus	Non-responders	0.15 ± 0.32	0.08 ± 0.21	−0.08 ± 0.28
			Responders	0.08 ± 0.19	0.13 ± 0.41	0.06 ± 0.24
		Bacteroides caccae	Non-responders	0.68 ± 0.81	0.78 ± 1.12	0.10 ± 0.59
			Responders	0.24 ± 0.38*	0.25 ± 0.44*	0.01 ± 0.43
	Marinifilaceae	*Odoribacter splanchnicus*	Non-responders	0.16 ± 0.14	0.13 ± 0.16	−0.03 ± 0.12
			Responders	0.13 ± 0.16	0.11 ± 0.13	−0.02 ± 0.17
	Prevotellaceae	*Prevotella stercorea*	Non-responders	0.75 ± 2.30	0.20 ± 0.50	−0.55 ± 1.94
			Responders	0.35 ± 1.34	0.35 ± 1.01	0.02 ± 1.10
		Prevotella copri	Non-responders	0.6 ± 2.3	0.4 ± 1.3	−0.2 ± 1.1
			Responders	1.1 ± 2.9	1.0 ± 1.9	0.2 ± 2.1
	Rikenellaceae	*Alistipes onderdonkii*	Non-responders	0.25 ± 0.52	0.12 ± 0.28	−0.12 ± 0.45
			Responders	0.11 ± 0.26	0.15 ± 0.27	0.05 ± 0.21
	Tannerellaceae	*Parabacteroides merdae*	Non-responders	1.41 ± 1.60	1.29 ± 1.22	−0.11 ± 1.45
			Responders	0.55 ± 0.68*	0.50 ± 0.61*	0.03 ± 0.52
Firmicutes	*Acidaminococcaceae*	*Phascolarctobacterium faecium*	Non-responders	0.30 ± 0.48	0.18 ± 0.38	−0.12 ± 0.38
			Responders	0.29 ± 0.54	0.34 ± 0.67	0.03 ± 0.41
	Erysipelotrichaceae	*Chlamydia trachomatis*	Non-responders	0.16 ± 0.72	0.22 ± 1.01	0.06 ± 0.29
			Responders	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Lachnospiraceae	*Eubacterium hallii*	Non-responders	0.8 ± 1.2	0.5 ± 0.7	−0.3 ± 0.7
			Responders	0.6 ± 1.0	0.4 ± 0.6	−0.2 ± 0.6
		Eubacterium rectale	Non-responders	3.4 ± 4.4	2.0 ± 3.3	−1.4 ± 3.3
			Responders	2.1 ± 3.8	2.5 ± 4.3	0.3 ± 1.6*
		Anaerostipes hadrus	Non-responders	1.7 ± 2.1	1.0 ± 1.3	−0.7 ± 2.1
			Responders	1.5 ± 2.0	1.9 ± 2.7	0.3 ± 1.3
		Dorea formicigenerans	Non-responders	0.19 ± 0.21	0.20 ± 0.23	0.01 ± 0.23
			Responders	0.13 ± 0.17	0.12 ± 0.11	−0.01 ± 0.14
		Roseburia inulinivorans	Non-responders	0.51 ± 0.84	0.21 ± 0.39	−0.30 ± 0.57
			Responders	0.19 ± 0.34	0.14 ± 0.23	−0.05 ± 0.22

Change is expressed as the difference between the value measured at 24 weeks and the baseline value.
*p<0.05 vs. Non-responders.

Table 3. Continued

Phylum	Family	Species		Relative abundance (%)		Change (%)
				Baseline	24 weeks	24 weeks
Firmicutes	*Lactobacillaceae*	*Lactobacillus salivarius*	Non-responders	0.04 ± 0.15	0.08 ± 0.24	0.03 ± 0.11
		(now *Ligilactobacillus salivarius*)	Responders	0.30 ± 0.85	0.20 ± 0.80	−0.12 ± 0.43
		Lactobacillus mucosae	Non-responders	0.04 ± 0.18	0.13 ± 0.40	0.09 ± 0.25
		(now *Limosilactobacillus mucosae*)	Responders	0.36 ± 0.76	0.45 ± 1.17	0.08 ± 1.00
	Ruminococcaceae	*Faecalibacterium prausnitzii*	Non-responders	0.35 ± 0.59	0.25 ± 0.38	−0.11 ± 0.52
			Responders	0.35 ± 0.55	0.31 ± 0.48	−0.05 ± 0.53
		Ruminococcus bicirculans	Non-responders	0.67 ± 1.27	0.94 ± 1.90	0.26 ± 1.00
			Responders	0.42 ± 1.03	0.52 ± 0.92	0.08 ± 0.57
	Selenomonadaceae	*Megamonas funiformis*	Non-responders	0.56 ± 1.19	0.56 ± 1.57	0.00 ± 1.27
			Responders	0.64 ± 2.20	0.18 ± 0.48	−0.49 ± 1.96
	Veillonellaceae	*Megasphaera elsdenii*	Non-responders	0.08 ± 0.20	0.15 ± 0.33	0.07 ± 0.23
			Responders	0.13 ± 0.44	0.18 ± 0.68	0.14 ± 0.54
		Veillonella ratti	Non-responders	0.48 ± 1.01	0.74 ± 2.03	0.26 ± 1.51
			Responders	0.06 ± 0.27	0.06 ± 0.30	0.01 ± 0.03
Fusobacteriota	*Fusobacteriaceae*	*Fusobacterium mortiferum*	Non-responders	0.93 ± 2.51	0.38 ± 1.51	−0.55 ± 1.42
			Responders	0.19 ± 0.88	0.01 ± 0.03	−0.18 ± 0.90
Verrucomicrobiota	*Akkermansiaceae*	*Akkermansia muciniphila*	Non-responders	0.29 ± 0.88	0.03 ± 0.08	−0.26 ± 0.86
			Responders	0.13 ± 0.45	0.16 ± 0.52	0.03 ± 0.71

Change is expressed as the difference between the value measured at 24 weeks and the baseline value.
*p<0.05 vs. Non-responders.

Table 4. Microbial diversity before and after synbiotic supplementation in the non-responders and responders

		Measured values		Change
		Baseline	24 weeks	24 weeks
Phylogenic diversity	Non-responders	26.4 ± 5.3	25.3 ± 5.5	−1.0 ± 2.7
	Responders	26.8 ± 7.2	27.0 ± 6.5	0.2 ± 3.4
Observed OTU	Non-responders	220.7 ± 61.4	209.7 ± 65.8	−11.0 ± 45.7
	Responders	225.8 ± 70.5	225.6 ± 61.6	0.9 ± 40.3
Shannon index	Non-responders	6.12 ± 0.60	5.82 ± 0.62	−0.30 ± 0.39
	Responders	6.06 ± 0.75	6.10 ± 0.66	0.06 ± 0.57*

Change is expressed as the difference between the value measured at 24 weeks and the baseline value. *p<0.05 vs. Non-responders. OTU: observed operational taxonomic unit.

levels were found to be elevated in inflammatory bowel disease [29]. Furthermore, *B. caccae* is enriched in clinical gout [30]. Therefore, the low relative abundances of *B. caccae* and *P. merdae* may be microbial markers for responders to synbiotic supplementation.

The Shannon index accounts for both abundance and evenness of bacterial species, and a higher value is indicative of greater α-diversity, which is known to be lower in obese children [31]. Interestingly, in the present study, the index was stable in responders throughout the study compared with non-responders, suggesting that a stable α-diversity might play some possible roles for responders to synbiotic supplementation.

The present study has several limitations. First, we were unable to confirm a causal relationship between inflammatory markers and the gut microbiota because our study used a retrospective design. Second, the number of participants used in the responder analysis was small. Third, the differences in the daily diets between the responders and non-responders were not evaluated even though they may affect the gut microbiota. Therefore, a large-scale study with a dietary intake assessment is necessary in the future. Finally, species-level identification in this study was based on sequence information for the 16S rRNA gene V1-2 region obtained by MiSeq. Therefore, evaluation by quantitative PCR using species-specific primers is necessary in the future.

In conclusion, we identified the specific bacterial species related to inflammatory markers in obese patients with T2DM. Furthermore, the severity of obesity and presence of two bacterial species (*B. caccae* and *P. merdae*) may be predictive markers for responders to synbiotic supplementation.

FUNDING

None.

CONFLICT OF INTEREST

AK has received lecture fees from Sanofi, Novartis Pharmaceuticals, Daiichi Sankyo Inc., Mitsubishi Tanabe Pharma, Takeda Pharmaceutical Co., and MSD, as well as research funds from Mitsubishi Tanabe Pharma. HW has received lecture fees from Boehringer Ingelheim, Sanofi, Ono Pharmaceutical Co., Novo Nordisk Pharma, Novartis Pharmaceuticals, Eli

Lilly, Sanwa Kagaku Kenkyusho, Daiichi Sankyo Inc., Takeda Pharmaceutical Co., MSD, Dainippon Sumitomo Pharma, and Kowa Co., as well as research funds from Boehringer Ingelheim, Pfizer, Mochida Pharmaceutical Co., Sanofi, Novo Nordisk Pharma, Novartis Pharmaceuticals, Sanwa Kagaku Kenkyusho, Terumo Corp., Eli Lilly, Mitsubishi Tanabe Pharma, Daiichi Sankyo Inc., Takeda Pharmaceutical Co., MSD, Shionogi Pharma, Dainippon Sumitomo Pharma, Kissei Pharma, and AstraZeneca. AM and YYo are employed by the Yakult Central Institute. The other authors declare no conflicts of interest.

REFERENCES

1. Kolb H, Martin S. 2017. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med 15: 131. [Medline] [CrossRef]
2. Gentile CL, Weir TL. 2018. The gut microbiota at the intersection of diet and human health. Science 362: 776–780. [Medline] [CrossRef]
3. Sato J, Kanazawa A, Ikeda F, Yoshihara T, Goto H, Abe H, Komiya K, Kawaguchi M, Shimizu T, Ogihara T, Tamura Y, Sakurai Y, Yamamoto R, Mita T, Fujitani Y, Fukuda H, Nomoto K, Takahashi T, Asahara T, Hirose T, Nagata S, Yamashiro Y, Watada H. 2014. Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. Diabetes Care 37: 2343–2350. [Medline] [CrossRef]
4. Sato J, Kanazawa A, Azuma K, Ikeda F, Goto H, Komiya K, Kanno R, Tamura Y, Asahara T, Takahashi T, Nomoto K, Yamashiro Y, Watada H. 2017. Probiotic reduces bacterial translocation in type 2 diabetes mellitus: a randomised controlled study. Sci Rep 7: 12115. [Medline] [CrossRef]
5. Gonai M, Shigehisa A, Kigawa I, Kurasaki K, Chonan O, Matsuki T, Yoshida Y, Aida M, Hamano K, Terauchi Y. 2017. Galacto-oligosaccharides ameliorate dysbiotic *Bifidobacteriaceae* decline in Japanese patients with type 2 diabetes. Benef Microbes 8: 705–716. [Medline] [CrossRef]
6. Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, Nomoto K, Nimura Y. 2006. Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: a randomized controlled trial. Ann Surg 244: 706–714. [Medline] [CrossRef]
7. Kanazawa A, Aida M, Yoshida Y, Kaga H, Katahira T, Suzuki L, Tamaki S, Sato J, Goto H, Azuma K, Shimizu T, Takahashi T, Yamashiro Y, Watada H. 2021. Effects of synbiotic supplementation on chronic inflammation and the gut microbiota in obese patients with type 2 diabetes mellitus: a randomized controlled study. Nutrients 13: 558. [Medline] [CrossRef]
8. Korpela K, Flint HJ, Johnstone AM, Lappi J, Poutanen K, Dewulf E, Delzenne N, de Vos WM, Salonen A. 2014. Gut microbiota signatures predict host and microbiota responses to dietary interventions in obese individuals. PLoS One 9: e90702. [Medline] [CrossRef]
9. Mobini R, Tremaroli V, Ståhlman M, Karlsson F, Levin M, Ljungberg M, Sohlin M, Bertéus Forslund H, Perkins R, Bäckhed F, Jansson PA. 2017. Metabolic effects of *Lactobacillus reuteri* DSM 17938 in people with type 2 diabetes: a randomized controlled trial. Diabetes Obes Metab 19: 579–589. [Medline] [CrossRef]
10. Prosberg M, Bendtsen F, Vind I, Petersen AM, Gluud LL. 2016. The association between the gut microbiota and the inflammatory bowel disease activity: a systematic review and meta-analysis. Scand J Gastroenterol 51: 1407–1415. [Medline] [CrossRef]
11. Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, Gabay C, Finckh A, Strowig T. 2019. *Prevotella copri* in individuals at risk for rheumatoid arthritis. Ann Rheum Dis 78: 590–593. [Medline] [CrossRef]
12. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37: 852–857. [Medline] [CrossRef]
13. Hidalgo-Cantabrana C, Delgado S, Ruiz L, Ruas-Madiedo P, Sánchez B, Margolles A. 2017. Bifidobacteria and their health-promoting effects. Microbiol Spectr 5: 5. [Medline] [CrossRef]
14. Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, Rossman J, Rich G, Dirienzo D, Ogra PL. 2004. In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. Dig Dis Sci 49: 579–589. [Medline] [CrossRef]
15. Riedel CU, Foata F, Philippe D, Adolfsson O, Eikmanns BJ, Blum S. 2006. Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation. World J Gastroenterol 12: 3729–3735. [Medline] [CrossRef]
16. Chen J, Wang R, Li XF, Wang RL. 2012. *Bifidobacterium adolescentis* supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of the metabolic syndrome. Br J Nutr 107: 1429–1434. [Medline] [CrossRef]
17. Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. 2020. The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol 11: 906. [Medline] [CrossRef]
18. Moschen AR, Gerner RR, Wang J, Klepsch V, Adolph TE, Reider SJ, Hackl H, Pfister A, Schilling J, Moser PL, Kempster SL, Swidsinski A, Orth Höller D, Weiss G, Baines JF, Kaser A, Tilg H. 2016. Lipocalin 2 protects from inflammation and tumorigenesis associated with gut microbiota alterations. Cell Host Microbe 19: 455–469. [Medline] [CrossRef]
19. Rau M, Rehman A, Dittrich M, Groen AK, Hermanns HM, Seyfried F, Beyersdorf N, Dandekar T, Rosenstiel P, Geier A. 2018. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. United European Gastroenterol J 6: 1496–1507. [Medline] [CrossRef]
20. Luo Y, Lin H. 2021. Inflammation initiates a vicious cycle between obesity and nonalcoholic fatty liver disease. Immun Inflamm Dis 9: 59–73. [Medline] [CrossRef]
21. Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. 2016. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut 65: 1359–1368. [Medline] [CrossRef]
22. Mukherjee A, Lordan C, Ross RP, Cotter PD. 2020. Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions to gut health. Gut Microbes 12: 1802866. [Medline] [CrossRef]
23. Moore WE, Holdeman LV. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl Microbiol 27: 961–979. [Medline] [CrossRef]
24. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkïla J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos W. 2010. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS One 5: e10667. [Medline] [CrossRef]
25. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, Ferrari C, Guerra UP, Paghera B, Muscio C, Bianchetti A, Volta GD, Turla M, Cotelli MS, Gennuso M, Prelle A, Zanetti O, Lussignoli G, Mirabile D, Bellandi D, Gentile S, Belotti G, Villani D, Harach T, Bolmont T, Padovani A, Boccardi M, Frisoni GB, INDIA-FBP Group 2017. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol Aging 49: 60–68. [Medline] [CrossRef]
26. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, Haub MD, Walter J. 2013. Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J 7: 269–280. [Medline] [CrossRef]
27. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, Shi J, Zhao S, Liu W, Wang X, Xia H, Liu Z, Cui B, Liang P, Xi L, Jin J, Ying X, Wang X, Zhao X, Li W, Jia H, Lan Z, Li F, Wang R, Sun Y, Yang M, Shen Y, Jie Z, Li J, Chen X, Zhong H, Xie H, Zhang Y, Gu W, Deng X, Shen B, Xu X, Yang H, Xu G, Bi Y, Lai S, Wang J, Qi L, Madsen L, Wang J, Ning G, Kristiansen K, Wang W. 2017. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. Nat Med 23: 859–868. [Medline] [CrossRef]
28. Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, Philippe C, Bridonneau C, Cherbuy C, Robbe-Masselot C, Langella P, Thomas M. 2013. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC Biol 11: 61. [Medline] [CrossRef]
29. Wei B, Dalwadi H, Gordon LK, Landers C, Bruckner D, Targan SR, Braun J. 2001. Molecular cloning of a *Bacteroides caccae* TonB-linked outer membrane protein identified by an inflammatory bowel disease marker antibody. Infect Immun 69: 6044–6054. [Medline] [CrossRef]
30. Guo Z, Zhang J, Wang Z, Ang KY, Huang S, Hou Q, Su X, Qiao J, Zheng Y, Wang L, Koh E, Danliang H, Xu J, Lee YK, Zhang H. 2016. Intestinal microbiota distinguish gout patients from healthy humans. Sci Rep 6: 20602. [Medline] [CrossRef]
31. Chen X, Sun H, Jiang F, Shen Y, Li X, Hu X, Shen X, Wei P. 2020. Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing. PeerJ 8: e8317. [Medline] [CrossRef]

