Research Article

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Biochemical analysis of microbiotas obtained from healthy, prediabetic, type 2 diabetes, and obese individuals

https://doi.org/10.1515/tjb-2022-0110
Received May 15, 2022; accepted September 22, 2022;
published online October 21, 2022

Abstract

Objectives: In this study, we aimed to evaluate the intestinal and urinary microbiota diversity of obese, pre-diabetic, diabetic, and healthy subjects together with their food consumption frequency and investigate the effect on glucose metabolism.

Methods: DNA was isolated from stool and urinary samples of fifteen obese, fifteen prediabetics, fifteen type 2 diabetic, and fifteen lean participants by using the quantitative real-time polymerase chain reaction (qPCR) method. The amounts of *Bifidobacterium*, *Bacteroides*, and *Firmicutes* were measured and food consumption frequency was answered by all participants through a questionnaire.

Results: The levels of *Bifidobacterium* in fecal microbiota were significantly higher in type 2 diabetic patients compared with lean (p=0.034), prediabetic (p=0.009), and obese participants (p=0.012). However, the levels of *Bifidobacterium* in urinary microbiota were decreased in obese, prediabetic, and type 2 diabetic subjects as controls (p=0.048; p=0.038; p=0.015 respectively). Additionally, *Bacteroides*/*Firmicutes* ratio decreased in type two diabetic patients compared with lean subjects and had a negative correlation with BMI in prediabetic subjects. Food consumption frequency illustrates that lean subjects have unhealthy eating habits.

Conclusions: Urinary microbiota could be considered in the future context of a potential biomarker in the progress of type 2 diabetes and obesity.

Keywords: microbiota; obesity; prediabetes; real-time qPCR; type 2 diabetes.

Introduction

Type 2 diabetes and obesity are metabolic disorders has become major public health concerns. Consumption of energy-dense foods, lack of physical activity, and genetic predisposition might lead to diabetes and obesity [1, 2]. Additionally, the microbiota has also been identified as a factor that contributes to obesity and type 2 diabetes [3, 4]. Components of microbiota are mostly bacteria, with a minority of viruses, fungi, and eukaryotic cells. The most abundant phyla in both humans and mice are *Firmicutes*, *Bacteroidetes* (particularly including *Bacteroides*), and *Actinobacteria* (with a predominance of the genus *Bifidobacterium*) [5]. Dysbiosis of gut microbiota is the cause of the low-grade inflammation that plays a major role in the onset of T2D [6, 7]. Recent studies on humans have shown that a higher proportion of *Firmicutes* and a lower proportion of *Bacteroidetes* are related to obesity and diabetes [4]. Lower *Firmicutes: Bacteroidetes* ratio correlated with lower BMI in obesity groups compared to lean groups [4, 8]. On the other hand, the ratio of *Bacteroidetes* to *Firmicutes* in diabetic compared to non-diabetic subjects is not correlated with BMI [1]. Microbiota composition is considerably influenced by factors such as lifestyle, diet, age, seasonal variations, and geography [9, 10]. Food components are the substrates for intestinal microbial metabolism. The end products of bacterial metabolism are predominantly vitamins and short-chain fatty acids that

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have various functions in lipid, glucose, and cholesterol metabolism [11]. The World Health Organization's definition of probiotics is "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [12]. Several recent reviews and meta-analyses, including meta-analyses of randomized controlled trials (RCTs), have suggested the use of probiotics for glycemic benefits in T2D and obesity [13]. *Bifidobacteria* are one of the most numerous probiotics found in the mammalian gut and are a type of lactic acid bacteria. Studies of T2D with *Bifidobacterium* have reported that these microbes are potentially protective against T2D and obesity [14]. The studies of urinary microbiota are mostly carried out on women with urgent urinary incontinence (UUI) [15, 18]. These studies demonstrated, that urinary microbiota diversity changes with age, body mass index, fasting blood glucose (FBG), and urine glucose (UGLU) [15]. Although urinary microbiota studies are mostly on female subjects, we want to demonstrate urinary microbiota dysbiosis independent of gender, identifying the relationship between obesity and diabetes.

According to the American Diabetes Association (ADA), diabetes may be diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG≥126 mg/dL (7.0 mmol/L)) value or the 2-h plasma glucose (2-h PG≥200 mg/dL (11.1 mmol/L)) value during a 75-g oral glucose tolerance test (OGTT), or A1C (A1C≥6.5% (48 mmol/mol)) criteria. People with prediabetes are defined by the presence of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and/or A1C 5.7–6.4% (39–47 mmol/mol) [16]. Moreover, obesity is a BMI greater than or equal to 30 [17]. Microbiota may be the alternative diagnostic biomarker of these criteria in the progress of type 2 diabetes and obesity.

This investigation aimed to characterize fecal and urinary microbiota in obese, prediabetic, and diabetic patients, as well as healthy subjects. *Bifidobacterium*, *Bacteroides*, and *Firmicutes* were tested by real-time PCR to understand changes in gut microbiota in all groups. Food frequency questionnaire results have shown that dysbiosis of microbiota can affect nutrition and glucose metabolism.

Materials and methods

Collection of fecal and urine samples and DNA isolation

The study included newly diagnosed type 2 diabetes (n=15), prediabetes (n=15), obese (n=15), and lean subjects (n=15). The subjects were selected based on the following criteria: female or male aged between 18 and 65 years, body mass index (BMI) 18.5–24.9 for healthy participants, and ≥30 for obese patients, and subjects who were newly diagnosed type 2 diabetes. Exclusion criteria are ınflammatory bowel disease (such as Crohn's disease, ulcerative colitis) and colorectal carcinoma, other chronic diseases other than obesity and diabetes (hypertension, chronic kidney failure, chronic liver disease, hypo/hyperthyroidism, coronary artery disease, polycystic ovarian syndrome causing insulin resistance, acanthosis nigricans, lipoatrophy/lipodystrophy syndromes, etc.), medical treatment including antibiotics and oral contraceptive treatment in the last 3 months, alcohol consumption and the absence of gastrointestinal disease and bowel-related operations in the last 3 months. Moreover, those who were pregnant and breastfeeding were excluded.

Fecal samples were collected by fecal collectors and transferred into sterile tubes. In the same manner, urine samples were collected into sterile tubes. Until analysis, all samples were kept at –20 °C.

For DNA isolation, 150 mg of each fecal sample and 500 µL of each urine sample were taken in tubes separately. Then, total bacterial DNA was isolated from all fecal and urine samples using the Higher Purity Bacterial DNA Extraction kit (Canwax Biotech, Spain) according to the manufacturer's instructions. The DNA concentration in the extracts was determined by a fluorometer (Qubit® 3.0, Thermo Fisher Scientific, USA) with Qubit™ dsDNA HS Assay Kit. Extracted DNAs were stored at –40 °C until real-time qPCR analysis.

Primer and probe designing

Primers and probes for the amplification of *Firmicutes*, *Bacteroides*, and *Bifidobacterium* used in the present study target the 16S rRNA gene. Primer and probes were designed by using the websites ncbi.nlm.nih.gov and https://eu.idtdna.com/calc/analyzer. The specific sequences of primers and probes are shown in Table 1.

Real-time qPCR

Bacterial abundance in fecal and urine samples was quantified by real-time qPCR using the real-time PCR system (Light Cycler 480, Roche,

Table 1: Primers and probes used in the study for real-time qPCR.

Target bacteria	Primer/Probe	Sequence (5′-3′)	Amplicon size, bp
Firmicutes	Fwd primer[a]	GTCAGCTCGTGTCGTGA	173
	Rev primer[a]	CCATTGTAKYACGTGTGT	
Bacteroides	Fwd primer	GGGTTTAAAGGGAGCGTAGG	123
	Rev primer	CTACACCACGAATTCCGCCT	
	Probe	(FAM)TAAGTCAGTTGTGAAAGTTTGCGGCTC (BHQ-1)	
Bifidobacterium	Fwd primer	GCGTGCTTAACACATGCAAGTC	125
	Rev primer	CACCCGTTTCCAGGAGCTATT	
	Probe	(FAM)GGCGAACGGGTGAGTAATGCGTGA (BHQ-1)	

Fwd primer, forward primer; Rev primer, reverse primer; [a]primer (Syber Green); Probe, TaqMan probe.

Germany). To measure the abundance of *Bacteroides* and *Bifidobacterium* TaqMan qPCR was used while SYBR Green qPCR was used for measuring *Firmicutes*. The qPCR reaction mixture (20 µL) of *Bacteroides* and *Bifidobacterium* was composed of 0.8 µL forward and reverse primer, 0.2 µL TaqMan probe, 10 µL TaqMan Probe Mix (Canvax Biotechnology, Spain), 3.2 µL sterile ddH_2O and 5 µL fecal/urine DNA. Real-time PCR was performed by the following cycle conditions respectively for *Bacteroides* and *Bifidobacterium*; initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s, extension at 72 °C for 15 s and initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing/extension at 60 °C for 15 s. The reaction mixture with a total volume of 20 µL of *Firmicutes* was composed of 0.5 µL forward and reverse primer, 10 µL SYBR Green Master Mix (Canax Biotechnology, Spain), 3.5 µL sterile ddH_2O, and 2.5 µL fecal/urine DNA. The amplification program consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and a final extension step at 72 °C for 10 s. The mixture which included water instead of DNA and another component of the PCR reaction mixture was used as the No Template Control (NTC).

Statistical analysis

The statistical analyzes of the findings obtained in the study were performed by the IBM SPSS Statistics Version 22.0 program. Results were expressed as mean value ± standard deviation. As the parameters (abundance of gut microbiota and clinical parameters) belonging to the patients and control groups were not uniformly distributed, the Kruskal-Wallis H test was used to determine the differences between the groups. Pairwise comparison of parameters between the groups was done with the Mann-Whitney U test. Linear correlation between the parameters was evaluated by Spearman's correlation test. A p-value of <0.05 was considered statistically significant.

Results

Characteristics of volunteers

The baseline characteristic of the 60 volunteers is shown in Table 2. The mean age of the 15 obese volunteers was 41.86 ± 8.53 years, 15 prediabetic volunteers were 49.6 ± 8.91 years, 15 type 2 diabetic volunteers with newly diagnosed was 54 ± 7.76 years, and 15 healthy volunteers as control was 41.93 ± 11.3 years.

Fasting blood glucose progressively increased in healthy, obese, prediabetic, and type 2 diabetic groups, respectively. Pairwise comparisons of all groups were statistically significant. The levels of HDL-cholesterol decreased in the prediabetic group compared to the healthy group (p=0.026). The same situation was observed in type 2 diabetic patients when healthy and type 2 diabetic groups were compared (p=0.029). Triglyceride levels were significantly higher in prediabetic and type 2 diabetic groups than in the healthy group (p=0.009; p=0.007 respectively) while there were no significant differences in cholesterol and LDL-cholesterol levels between all groups. Furthermore, the levels of HbA1c were statistically significant found in pairwise comparisons of all groups except the state that was compared with healthy and obese groups. BMI was statistically higher in three groups than in the healthy group while ıt was lower in prediabetic and type 2 diabetic groups in comparison to the obese group.

Quantitative PCR analysis of bacteria

The levels of *Firmicutes*, *Bacteroides*, and *Bifidobacterium* were determined to evaluate the differences in the composition of fecal and urine samples of obese, patients, and healthy groups.

The abundance of *Firmicutes* and *Bacteroides* in fecal and urine was not significantly different between all groups instead for type 2 diabetic and prediabetic groups. The abundance of *Firmicutes* in type 2 diabetic subjects was higher than in prediabetics (p=0.038). However, the levels of *Bifidobacterium* in gut microbiota were significantly higher (p=0.020) in type 2 diabetic group compared with the healthy group (p=0.034), and the same situation was observed in the type 2 diabetic group compared with both the obese group and prediabetic group (p=0.012; p=0.009, respectively) (Table 3). Additionally, the levels of *Bifidobacterium* in urinary microbiota were significantly decreased in the obese, prediabetic, and type 2 diabetic groups compared to the healthy group (p=0.048; p=0.038; p=0.021 respectively) (Table 3). Also, *Bacteroides*/*Firmicutes* ratio was compared with BMI in all groups but there was only a significantly negative correlation in the prediabetic group (Figure 1).

Food consumption frequency

We questioned the food consumption frequency of the subjects by considering the foods such as dairy products, meat, eggs, legumes, vegetables, fruits, bread, cereals, desserts, and drinks frequently consumed in Turkish society. Seven options were given to the subjects for the frequency of consumption. These are daily, 5–6 times a week, 3–4 times a week, once or twice a week, every 15 days, once a month, and never consumed. One bread is accepted as 200 g according to the Turkish food codex (2021). Additionally, yogurt is known according to the Turkish food codex (2021) [19], specifically *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* fermented milk product in which symbiotic cultures are used. On the other hand, there was no standardization of homemade yogurt and homemade yogurt

Table 2: Clinical characteristics of volunteers.

Characteristics	Healthy individuals (n=15)	Obese individuals (n=15)	Prediabetic patients (n=15)	Type 2 diabetic patients (n=15)	p_1-Value	p_2-Value	p_3-Value	p_4-Value	p_5-Value	p_6-Value
Sex, n										
Male	3	3	3	10						
Female	12	12	12	5						
Age, years	41.93 ± 11.3	41.86 ± 8.53	49.6 ± 8.91	54 ± 7.76	0.965	0.105	0.005	0.001	0.060	0.191
Weight, kg	61.57 ± 6.83	91.63 ± 14.03	90.39 ± 16.97	83.93 ± 13.73	0.000	0.000	0.000	0.198	0.901	0.164
Height, cm	163.93 ± 6.51	159.67 ± 5.69	161.33 ± 7.86	166.8 ± 6.49	0.092	0.252	0.205	0.007	0.739	0.059
BMI, kg/m^2	22.85 ± 1.58	35.86 ± 4.72	34.94 ± 7.73	30.27 ± 5.74	0.000	0.000	0.000	0.007	0.693	0.054
HbA1c (%)	5.24 ± 0.24	5.21 ± 0.38	6.01 ± 0.24	7.79 ± 1.42	0.941	0.001	0.001	0.000	0.000	0.000
FBG, mg/dL	86.2 ± 5.72	92 ± 9.32	109.67 ± 15.25	139.92 ± 17.46	0.046	0.000	0.000	0.000	0.001	0.000
HDL-cholesterol, mg/dL	57.71 ± 7.83	49.36 ± 11.11	48.08 ± 8.4	46.75 ± 7.54	0.051	0.026	0.029	0.948	0.884	0.909
Cholesterol, mg/dL	199.5 ± 41.93	205.73 ± 35.65	201.46 ± 41.15	242.75 ± 51.59	0.687	0.895	0.286	0.240	0.664	0.336
LDL-cholesterol, mg/dL	128 ± 36.79	141.92 ± 24.24	135.55 ± 29.53	157.13 ± 21.06	0.280	0.563	0.128	0.123	0.559	0.083
Triglyceride, mg/dL	61.75 ± 10.9	125.89 ± 60.53	155.73 ± 116.19	227.25 ± 147.63	0.064	0.009	0.007	0.054	0.621	0.107

Data are mean ± standard deviation, results of Kruskal Wallis H and Mann-Whitney U test. BMI, body mass index; HbA1c, hemoglobin A1c; FBG, fasting blood glucose; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol. p_1, for obese vs. healthy; p_2, for prediabetic vs. healthy; p_3, for type 2 diabetic vs. healthy; p_4, for obese vs. type 2 diabetic; p_5, for obese vs. prediabetic; p_6, for type 2 diabetic vs prediabetic; $p<0.05$ was considered statistically significant.

Table 3: Statistical results of the microbiota of all groups.

Sample type	Bacteria	Fecal or urine bacterial count (log_{10}copies/g or log_{10}copies/mL)				p-Values					
		Healthy individuals (n=15)	Obese individuals (n=15)	Prediabetic patients (n=15)	Type 2 diabetic patients (n=15)	p_1	p_2	p_3	p_4	p_5	p_6
Fecal	*Firmicutes*	8.11 ± 0.58	8.16 ± 0.97	7.65 ± 1.24	8.48 ± 0.58	0.541	0.407	0.089	0.383	0.163	0.038
	Bacteroides	8.39 ± 0.89	8.36 ± 1.06	7.64 ± 1.13	7.73 ± 1.22	0.965	0.067	0.152	0.214	0.098	0.896
	Bifidobacterium	7.08 ± 0.88	6.92 ± 0.98	6.96 ± 1.03	7.77 ± 0.70	0.724	0.854	0.034	0.012	0.098	0.009
	Bacteroides/ Firmicutes	1.04 ± 0.10	1.04 ± 0.20	0.98 ± 0.17	0.91 ± 0.12	0.727	0.116	0.002	0.061	0.358	0.206
Urine	*Firmicutes*	6.88 ± 1.39	6.11 ±1.13	6.00 ± 1.06	5.97 ± 1.10	0.130	0.057	0.130	0.773	0.729	0.773
	Bacteroides	3,86 ± 0.83	5,79 ± 2.32	4,45 ± 0.42	3.82 ± 0.69	0.157	0.248	1.000	0.071	0.289	0.055
	Bifidobacterium	6.86 ± 1.00	5.53 ± 0.95	5.66 ± 0.25	5.41 ± 1.02	0.048	0.038	0.021	0.728	1.000	0.308

Data are mean ± standard deviation, results of Kruskal Wallis H and Mann-Whitney U test. p_1, for obese vs. healthy; p_2, for prediabetic vs. healthy; p_3, for type 2 diabetic vs. healthy; p_4, for obese vs. type 2 diabetic; p_5, for obese vs. prediabetic; p_6, for type 2 diabetic vs. prediabetic; $p<0.05$ was considered statistically significant.

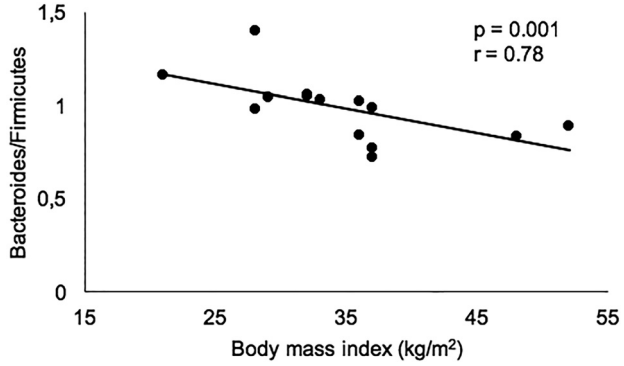

Figure 1: Correlation between BMI and *Bacteroides/Firmicutes* ratio for prediabetic subjects; Spearman's probability (p) and correlation (r), correlation is significant at the 0.01 level.

consumption has become increasingly prevalent in Turkish society. Because of that, we want to research if there was any effect on the microbiota diversity.

When the food frequency consumption of obese, prediabetic, type 2 diabetic and healthy individuals in Turkey were examined. It was seen that 31 of the 60 volunteers included in the study consumed homemade yogurt and the frequency of consumption was highest in healthy individuals (57.1% of healthy individuals consume it daily). While the number of individuals consuming industrial yogurt was close to the number of subjects consuming homemade yoğurt. However, industrial yogurt was consumed most frequently by obese subjects (33.3% of obese subjects consume it daily). Cheese consumption frequency was very high in all groups and 86.7% of obese persons, 93.3% of prediabetics, 93.3% of type 2 diabetics, and all healthy people consumed cheese every day. We observed that the frequency of consumption of potatoes, bulghur, rice, and pasta among subjects with obese, prediabetic, and type 2 diabetes was concentrated 1–2 times a week in consumption. The same situation was observed in the frequency of red and white meat consumption as well. Kefir is known as probiotic food consumed by only six people and has a low frequency of consumption. Additionally, physical activity and smoking knowledge are obtained from the participants (Supplementary Figures S1 and S2 respectively). When Supplementary Figure S1 is examined, It is seen that type 2 diabetic participants are the most sportive group and healthy participants are the least sportive group. Evaluation of smoking data concluded that healthy individuals are the most frequent cigarette users in comparison to all groups. All subjects consume bread every day. Interestingly, it was observed that type 2 diabetic patients consume one bread every day, and daily bread consumption was highest in type 2 diabetic, obese, prediabetic, and healthy individuals, respectively (Supplementary Figure S3).

Discussion

This study focused on the diversity of intestinal and urinary microbiota on obese, prediabetic, type 2 diabetic, and healthy subjects. Bacterial phylum *Firmicutes* and two bacterial groups including *Bacteroides* and *Bifidobacterium* are analyzed in obese, prediabetic, and type 2 diabetes patients which were then compared to healthy groups as well as each other.

In this study, there was no significant difference in abundances of *Firmicutes* and *Bacteroides* in fecal samples of the type 2 diabetic, obese and prediabetic groups compared to the healthy group. Only, *Firmicutes* was

slightly increased in type 2 diabetic patients compared to prediabetics. In addition, we found a significant negative correlation between *Bacteroides*/*Firmicutes* ratio and BMI in the prediabetic group. Similar to previous a study identifying five phyla including *Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia*, and *Actinobacteria* in type 2 diabetic and prediabetic subjects, no differences in the abundance of *Bacteriodetes* and *Firmicutes* were observed [20]. On the other hand, Remely et al. compare type 2 diabetes and healthy individuals and demonstrate type 2 diabetic individuals became more abundant in terms of *Firmicutes*, especially the subgroup *Clostridiales*, *Bacilli*, and *Lactobacillales* while the abundance of *Bacteroidetes* was less more [4]. Also, few studies reported higher concentrations of *Bacteroidetes* and a lower rate of *Firmicutes* and *Bifidobacterium* in the fecal microbiota of patients with diabetes compared with the healthy subject [1, 6]. These studies hypothesize that there is a connection between metabolic diseases and gram-negative bacteria in the gut. It has been indicated that gram-negative bacteria specifically those belonging to the phylum *Bacteroidetes* and *Proteobacteria* are the source of lipopolysaccharide (LPS) which causes metabolic endotoxemia and insulin resistance in obese and type 2 diabetic subjects [1, 20].

Bifidobacterium is a group of bacteria that have a beneficial effect on health, and a decrease in the microbiota composition of beneficial bacteria such as *Bifidobacterium* has been associated with diabetes [6]. The remarkable and interesting result is that the level of *Bifidobacterium* has been increased in type 2 diabetic groups. We observed that the type 2 diabetic group had higher levels of *Bifidobacterium* compared to healthy, obese, and prediabetic groups. Our results are not consistent with the reports that find a reduced abundance of *Bifidobacteria* in type 2 diabetics [6, 21].

In our study, we did not observe any significant differences in abundances of *Firmicutes, Bacteroides*, and *Bifidobacterium* in fecal samples of obese patients compared to the healthy and other groups. Our results following the report by Duncan et al. were no differences between obese and non-obese subjects in the number of *Bacteroide*s measured in fecal samples. Despite applying weight-loss diets, there was no significant change in the percentage of *Bacteroides* in feces, while *Firmicutes* decreased [22]. Our observations differ from previous studies which support the hypothesis that the proportions of *Bacteroidetes* and *Firmicutes* are different between obese and lean subjects.

Many studies that investigate urinary microbiota have shown that urinary microbiota composition is complex and it significantly differs among individuals, in both males and females [23, 24]. The literature has limited studies researching the relationship between urinary microbiota and metabolic diseases such as type 2 diabetes. This present study is the first report analyzing the urinary microbiota in obese subjects, prediabetic and type 2 diabetes simultaneously. According to our results, *Bifidobacterium* abundance was significantly decreased in all groups when compared to healthy controls, while the levels of *Firmicutes* and *Bacteroides* were not significantly different. There are two reports which compared the urinary bacterial diversity profile in women with type 2 diabetes and healthy subjects, demonstrating that urinary microbiota with type 2 diabetes was dominated by *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*. In these studies, They have stated that *Flavobacteriales*, *Actinobacteria*, and *Flavobacteria* could be used as potential distinguishing biomarkers, and *Akkermansia*, and *Bifidobacterium* notably was detected in all four cohorts [15, 25]. *Bifidobacterium* is considered a probiotic bacteria due to its health-promoting beneficial effects in humans [26, 27]. The presence of *Bifidobacterium* in urine may be explained by the idea that *Bifidobacterium* has a protective effect in urine and may play a role in the healthy urine microbiome. However, it has been reported that *Bifidobacterium* may be the pathogenic agent in urinary tract infection, sepsis, and necrotizing pancreatitis [25]. In our study, it is shown that urinary microbiota may affect glucose metabolism with a decrease of *Bifidobacterium* in type 2 diabetes. Moreover, for urinary microbiota dysbiosis, more studies including larger bacterial diversity and parameters such as urinary glucose level, LPS, etc., are required.

The microbial composition is influenced by various factors such as genetics, age, diet, geographic origin, lifestyle, regular medications, and using antibiotics [26]. Growing evidence shows that especially diet impacts microbiota composition and could be related to obesity and metabolic diseases such as diabetes [28, 29]. Carbohydrates, especially non-digestible carbohydrates (such as dietary fiber and resistant starch), have a great influence on the composition and diversity of the gut microbiota [30]. In addition, since these carbohydrates may have prebiotic effects, they can regulate the activity of bacteria such as *Bifidobacterium* and Lactic acid bacteria [31]. When analyzing the yogurt consumption of participants, we observed that 40% of obese individuals, 66% of prediabetic patients, 66% of type 2 diabetic patients, and 46% of healthy individuals consumed homemade yogurt. Considering the results of food consumption frequency, increasing the consumption of probiotic foods such as yogurt and kefir may have a prevention effect on the progression of diabetes and obesity. In addition, the consumption of foods containing carbohydrates such as bread, pasta, potatoes, and rice can be reduced under the control of a dietitian. They may convert their microbial compositions in favor of beneficial bacteria. On the other

hand, healthy participants demonstrate that if their less physical activity and consumption of potatoes, rice, pasta, and desserts like the type 2 diabetic patient could not change, diabetes risk would increase with increasing age. When healthy, obese, and prediabetic participants are compared, with increasing BMI and age, alteration of the *Bacteroides/Firmicutes* ratio is observed. Healthy participants had a high-risk factor for type 2 diabetes with less physical activity, smoking, and unhealthy eating habits.

Conclusions

In conclusion, the results of our study demonstrated that *Firmicutes* and *Bacteroides* did not differ in fecal and urinary microbiota of three different study groups composed of obese, prediabetic, and healthy individuals. The abundance of *Bacteroides* did not change but the abundance of *Firmicutes* increased in type 2 diabetes compared with prediabetic subjects. However, *Bifidobacterium* has undergone different alterations in both fecal and urinary microbiota. Results of food consumption frequency illustrate that obese, prediabetic, and type 2 diabetic individuals have similar dietary habits. Also, healthy subjects have an unhealthy lifestyle and food consumption. If a new treatment is identified by microbiota analysis for prediabetic or obese patients, these results may help develop new strategies to prevent type 2 diabetes in the future.

Research funding: This study was supported by the YTU, Scientific Research Project Coordination with the project no: FBA-2018-3410. The authors thank the YTU Scientific Research Project Coordination and Okmeydanı Training and Research Hospital.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors declare that there is no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study protocol was approved by the Ethical Committee (Decisions numbers: 48670771-514.10) and performed according to the 1964 Helsinki declaration. Both written and verbal consent was obtained from all subjects of the study.

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/tjb-2022-0110).