Relationship Between Fasting and Postprandial Glucose Levels and the Gut Microbiota

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Abstract

Postprandial hyperglycemia increases the risk of mortality among patients with type 2 diabetes and cardiovascular diseases. Additionally, the gut microbiota and type 2 diabetes and cardiovascular disease are known to be correlated. Currently, fasting blood glucose is the primary index for the clinical diagnosis of diabetes; however, postprandial blood glucose is associated with the risk of developing type 2 diabetes and cardiovascular disease and mortality. Therefore, the dynamic change in blood glucose levels under free-living conditions is considered an important and better marker than fasting glucose levels, to study the relationship between glucose levels and microbiota. Here, we investigated the relationship between fasting and postprandial glucose levels and microbiota under free-living conditions, for one week in the older adults. The results revealed a significant correlation between peak glucose levels after dinner and the gut bacteria, particularly, *Bacteroides*, *Clostridiales Clostridiaceae group*, *Anaerostipes*, *Clostridiales [Mogibacteriaceae] group*, *Holdemania*, and *Bilophila*. Together, these findings suggest that the glucose levels after dinner are a better predictor of microbiota conditions than fasting glucose levels.

Introduction

The microbes in our bodies collectively make up to approximately 100 trillion cells, which is 10 times the number of human cells, and they have 100 times more endemic genes than the human genome [1]. Most of the microbes reside in the gut and are collectively called “gut microbiota” [2]. The gut microbiota has a profound influence on human physiology and nutrition and is crucial for human life [3, 4]. Previous studies, using the 16S ribosomal ribonucleic acid (rRNA) gene sequence, showed that two bacterial phyla, *Bacteroidetes* and *Firmicutes*, constitute over 90% of the known phylogenetic categories and dominate the distal gut microbiota [5, 6]. Notably, the gut microbiota is extremely diverse, even among healthy people [6–9]. In recent years, several studies have examined gut microbiota using next-generation sequencing and shown that changes in the gut microbiota may be associated with metabolic diseases, including type 2 diabetes, obesity, and cardiovascular diseases [10–13]. However, reports on the association between diseases and certain taxa are inconsistent. In addition, postprandial hyperglycemia increases the risk of metabolic diseases and mortality, even in people with normal fasting blood glucose levels [14, 15]. Currently, fasting blood glucose is used as a diagnostic marker for diabetes; however, given that postprandial blood glucose is associated with the risk of developing type 2 diabetes and cardiovascular disease and mortality, the blood glucose levels under free-living conditions are considered more important. The association between different types of glucose levels (fasting and/or postprandial blood glucose levels) and the gut microbiota remains largely unknown, and assessing this relationship could aid in predicting the onset of type 2 diabetes and cardiovascular disease.

In this study, we investigated the relationship between fasting and postprandial glucose levels and the gut microbiota under free-living conditions, for one week in the elderly. The purpose of this study was to clarify the time points to focus on the glucose level in order to predict the good association of the gut microbiota.

Results

Characteristics of study participants

The characteristics of the study participants are shown in Table 1. Twenty-two participants stratified by gender (men: *n* = 11; women: *n* = 11) were included in the study.

Peak postprandial glucose levels are more variable than fasting glucose levels

Changes in glucose levels during 24-hour period and fasting period (1 point before breakfast) and peak (after each meal) glucose levels are shown in Figure 1. The results in Figure 1B indicate that the peak glucose levels after each meal had significantly more variance than the glucose levels after fasting (breakfast: $p < 0.001$; lunch: $p < 0.001$; dinner: $p < 0.006$).

Gut bacteria data

In this study, only the gut bacteria found in more than half of the participants were used for analysis. The gut bacteria used in the analysis and their abundance in each participant are presented in Tables 2 and 3. The relative abundance of gut bacteria varied among participants; color intensity increased with increasing abundance. Figures 2 and 3 depict the relative abundances of gut bacteria at the phylum and genus levels in each participant. Notably, *Firmicutes* were highly abundant.

Peak glucose levels after dinner are highly correlated with the gut bacteria compared to other postprandial peak glucose levels

The correlation between gut bacteria and the fasting glucose levels and the peak after each meal is shown in Figure 4. There was a most common statistically significant correlation between the gut bacteria and the peak glucose levels after dinner (20.0%). Six gut bacteria, *Bacteroides*, *Clostridiales Clostridiaceae group*, *Anaerostipes*, *Clostridiales [Mogibacteriaceae] group*, *Holdemania*,and *Bilophila* showed a significant correlation only with the peak glucose levels after dinner (Figure 4). In contrast, the*Bacteroidales s24-7* group showed a negative correlation with fasting glucose levels and the peak glucose levels after each meal (Figure 4).

Discussion

As a result of present study, we observed many of the most significant correlations between the gut bacteria and the peak glucose levels after dinner. In addition, since significant correlations were confirmed mainly at the genus level, it is necessary to look at the level above the genus in order to analyze the gut microbiota.

The six gut microbiota, *Bacteroides*, *Clostridiales Clostridiaceae group*, *Anaerostipes*, *Clostridiales [Mogibacteriaceae] group*, *Holdemania*, and *Bilophila* showed a significant correlation only with the peak glucose levels after dinner. Therefore, these gut bacteria can be used as markers for predicting peak

glucose levels after dinner. Since undetermined gut bacteria such as *Clostridiales Clostridiaceae group* and *Clostridiales [Mogibacteriaceae] group* are also related, it may be possible to predict glucose levels by focusing on undetermined gut bacteria. On the contrary, the *Bacteroidales s24-7* group showed a negative correlation with fasting glucose levels and the peak glucose levels after each meal. In mice, the *Bacteroidales s24-7* group is associated with bacteria that produce short-chain fatty acids (SCFAs) [16]. SCFAs produced in the intestine promote the secretion of hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) by binding to G-protein-coupled receptors (GPR41 and GPR43), which are SCFA receptors present in colon L cells [17]. GLP-1 binds to GLP-1 receptors present on pancreatic β-cells, promotes insulin secretion, and suppresses elevated blood glucose levels [18]. PYY acts on Y2 receptors in the hypothalamus of the brain and suppresses appetite [19]. Based on the above-mentioned studies and our present results, an increase in the abundance of the *Bacteroidales s24-7* group reduced fasting and postprandial blood glucose levels through the production of SCFAs. However, there are other bacteria that produce SCFAs. For example, *Bifidobacterium* stimulates the production of acetic acid and butyric acid [20]. Nevertheless, it is unclear why only the *Bacteroidales S24-7* group specifically showed a negative correlation with fasting and postprandial glucose levels in the present study. In future experiments, when we have a chance to measure other blood hormones such as GLP-1 and PYY, it may be possible to further understand the relationship between gut bacteria and glucose levels.

A previous study reported that the association of diet, gut microbiota, and blood markers is generally stronger with lipid indicators than with blood glucose indicators [21]. However, this previous study investigated the relationship between blood glucose levels after breakfast or lunch and the gut microbiota, but not the relationship between blood glucose levels after dinner and the gut microbiota. Based on our study, it is necessary to measure the glucose levels after dinner while examining the relationship between glucose levels and the gut microbiota. At present, it is not clear why a correlation with more bacterial species was observed after dinner, but it is probably related to the circadian system and the timing of the three meals. Circadian rhythms control the timings of digestion, absorption, and metabolism in the stomach and intestines; additionally, these circadian systems control glucose tolerance to meals [22, 23]. In fact, glucose tolerance is higher in the morning and lower in the evening [24]. Circadian rhythms also influence the composition of the gut microbiota and are controlled by dietary timings. In fact, the composition of the gut microbiota of mice that were restricted-fed during the active phase and those that were restricted-fed during the inactive phase showed opposite rhythms [25]. In addition, the duration of fasting is important for changes in the composition of the gut microbiota [26]. As there are circadian rhythms for changes in the blood glucose levels and changes in the composition of the gut microbiota, it is believed that these factors interact with each other; as a result, a correlation was found between various gut bacterial species and glucose levels, especially after dinner.

The results of this study showed that there was a high correlation between dinner postprandial glucose levels and various gut bacterial species in comparison with the correlation between fasting glucose levels and the gut microbiota. This indicates that postprandial glucose level, especially after dinner, are more important for predicting gut microbiota than the fasting glucose levels. In addition, it may be possible to predict glucose fluctuations after dinner by examining the gut microbiota, and vice versa.

Conclusions

It was suggested that by focusing on the glucose level after dinner, it is highly possible to predict the relationship of glucose levels with the gut microbiota.

Limitations

Despite these findings, our study had several limitations. First, since the study only focused on older people, the results may not be applicable to young people. Hence, the findings of this study may not be generalizable. Future studies should expand the scope of this study. Second, this study was conducted in Japan, and it is unclear whether the same results would be obtained in other countries. Therefore, the number of target country needs to be expanded in future studies. Since the composition of microbiota differs greatly among people, it would be beneficial to confirm these findings in a considerably larger population to obtain more accurate results.

Materials And Methods

Study participants

This study was conducted between July and September 2018 at Tokyo area. The participants were healthy 30 older adults (14 men and 16 women, aged ≥65), and the inclusion criteria were as follows: (1) no drugs and supplements such as antioxidant, anti-obesity, or anti-diabetic; (2) no diagnosis of sleep apnea syndrome, diabetes, dyslipidemia, or hypertension by a physician; and (3) absence of the use of glucose/insulin-lowering or anti-hypertension related medications. This inclusion criteria was similar to our previous paper [27]. Study protocol conformed to the Declaration of Helsinki and was approved by the Ethics Committee for Humans at Waseda University (approval number: 2018-031). We registered this human study at UMIN system (www.umin.ac.jp/ctr/ number: UMIN000032858). After the experimental details had been explained, informed consent was obtained from all the participants. Participants were answered a questionnaire on dietary habits, lifestyle habits, and health and medication status prior to the start of this study. Eight participants were excluded from the study owing to the submersion of their feces during experiments.

Study design

The experiments were conducted for a week, and participants were asked to maintain their lifestyle habits such as diet and exercise throughout experiments. During the experimental period, the physical characteristics of all participants were measured, and all subjects were asked to wear a continuous glucose monitoring (CGM) system as mentioned in our previous paper [27]. Participants were also asked to collect their feces in a tube with 20% glycerol containing phosphate-buffered saline. Tube had been distributed in advance for intestinal microbiota evaluation (morning of Day 8). The collected fecal samples were carried to the university at 4 °C, and were stored at -80 °C until analysis after immediately frozen in liquid nitrogen [27].

Measurements

Anthropometry

According to previous paper [27], body mass was measured using a digital balance (0.1kg minimum, Inbody 230, Inbody Inc., Tokyo, Japan), and height was measured using a wall-mounted stadiometer (0.1cm minimum, YS-OA, As One Corp., Japan). Calculation of body mass index (BMI) was well known as (Kg/ m^2).

Determination of tissue glucose levels

Participants were required to wear a CGM system (FreeStyle Libre Pro; Abbott Laboratories, Chicago, IL, USA) for the continuous monitoring tissue glucose levels throughout experiment. CGM system can continuously measure and store data of tissue glucose levels with 15 min intervals, and this system is considered to be less burdensome than other glucose monitoring systems especially for elderly people. To evaluate CGM, mean (M) and standard deviation (SD), coefficient of variation (CV), and peak glucose levels were calculated for parameters.

Fecal DNA extraction and 16S rRNA gene sequencing

Fecal DNA extraction and 16S rRNA gene sequencing was performed as previously described [27]. 16S rRNA gene sequencing was performed on the Illumina platform. We amplified the V3-V4 variable regions of the 16S rRNA gene with PCR using the following primers:

Forward Primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3';

Reverse Primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC-3'.

Amplicon PCR was performed with 2.5 μL of microbial DNA (5 ng/μL), 5 μL of each primer (1 μM), and 12.5 μL of 2× KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA, USA). We used same protocol of the cycling parameters [27]. The cycling parameters were as follows: denaturation at 95°C for 3 min, followed by 25 cycles of denaturation, annealing, and elongation at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, respectively, and a final extension at 72°C for 5 min. We purified PCR amplicons by AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA).

To perform multiplex sequencing, adapters and barcodes were ligated to amplicons by the following kit, Nextera XT Index Kit v2 (Illumina Inc., San Diego, CA, USA) [27]. Index PCR was performed with 5 μL PCR product, 5 μL of each Nextera XT Index primers, 25 μL of 2× KAPA HiFi HotStart Ready Mix, and 10 μL of PCR-grade water. The conditions of PCR were followed: 1 cycle at 95°C for 3 min, 8 cycles of denaturation, annealing, and extension at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, respectively, followed by a final extension at 72°C for 5 min. We checked the quality of the purified products by an Agilent 2100 Bioanalyzer with a DNA 1000 kit (Agilent Technologies, Santa Clara, CA, USA). Finally, the DNA library was diluted to a concentration of 4 nM and sequenced using MiSeq Reagent Kit v3 (Illumina

Inc.) carrying on an Illumina MiSeq 2×300 bp platform, according to the manufacturer's instructions and our previous paper [27].

Analysis of 16S rRNA gene sequences

Analysis of 16S rRNA gene sequences was performed as previously described [27]. We processed the 16S rRNA sequence reads using the help of quantitative insights into the microbial ecology (QIIME) pipeline version 1.9.1 [28]. The quality-filtered sequence reads were assigned to operational taxonomic units (OTUs) using closed-reference OTU picking at 97% identity with the UCLUST algorithm [29]. And then we compared these reads with reference sequence collections in the Greengenes database (August 2013 version). From 44 samples 1,296,946 reads were obtained in total, and therefore, 22,361±2,721 reads were obtained per sample on an average. At the phylum and genus levels, the taxonomy summary was calculated using the QIIME software (version 1.9.1).

Statistical analysis

Data were analyzed using the Predictive Analytics Software for Windows (SPSS Japan Inc. Tokyo, Japan). Kolmogorov–Smirnov test was applied to check whether data showed normal or non-normal distributions. In this study, the correlation between fasting (1 point before breakfast) and postprandial (breakfast, lunch, and supper) glucose levels and the gut bacteria was investigated. The correlation coefficient was calculated using Pearson's or Spearman's test for the parameters showing either a normal or non-normal distribution, respectively. Gut bacteria found in more than half of the subjects were selected and used for statistical analysis (50 types of gut bacteria from ≥11 persons were used). Levene's test was used to compare the variance in fasting and peak (after each meal) glucose levels. Statistical significance was set at $p < 0.05$.

Declarations

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Author Contribution

Y.M., H.S., and S.S. designed the research, analyzed the data, and wrote the manuscript. H-K.K. reviewed the manuscript. All authors have read and approved the final manuscript.

Ethics declarations

The human trial was registered at UMIN (www.umin.ac.jp/ctr/ number: UMIN000032858).

Competing interests

The authors declare that there are no competing interests.

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Tables

Table 1. Characteristics of study participants.

Physical Characteristics	All participants (*n* = 22)	Men (*n* = 11)	Women (*n* = 11)
Age (years)	74.2 ± 1.13	73.7 ± 1.67	74.7 ± 1.51
Height (cm)	159.2 ± 2.06	167.1 ± 1.62	151.3 ± 1.68
Weight (kg)	57.5 ± 2.24	65.2 ± 2.00	49.8 ± 2.27
BMI (kg/m^2)	22.4 ± 0.461	23.3 ± 0.524	21.6 ± 0.666

All data are presented as mean ± standard error. BMI: body mass index.

Table 2. Composition of the gut microbiota in each participant.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
0.009476	0.17847	0.128834	0.208855	0.022017	0.120324	0.029588	0.021804	0.037065	0.025419	0.082006	0.130338	0.190599	0.133793	0.158384	0.158813	0.085217	0.052596	0.283104	0.411319	0.092044	0.130986	*Actinobacteria*
0.372157	0.300095	0.391323	0.367502	0.241431	0.17339	0.286016	0.141513	0.245892	0.318136	0.286785	0.31188	0.242756	0.050115	0.26683	0.152269	0.290848	0.217896	0.112343	0.24002	0.391056	0.216588	*Bacteroidetes*
0.579083	0.473816	0.467309	0.394748	0.603453	0.696259	0.662205	0.820436	0.702713	0.512407	0.630272	0.445052	0.548616	0.796782	0.56989	0.67801	0.604298	0.510246	0.59257	0.317837	0.50494	0.637089	*Firmicutes*
0.029635	0.02537	0.009904	0.018677	0.118589	0.009101	0.016555	0.015177	0.014329	0.13839	0.000937	0.11273	0.012878	0.001839	0.004896	0.006981	0.009263	0.219262	0.005992	0.029813	0.01196	0.015023	*Proteobacteria*
0.000172	0.000475	0	0.00011	0.001001	0.000463	0	0.002779	0.000191	0.000403	0	0	0	0.002299	0	0.001309	0	0	0.000599	0.003032	0.000347	0.000313	*Actinomyces*
0.001034	0.069801	0.080894	0.178532	0.003252	0.117162	0.008101	0.01646	0.000573	0.003631	0.026242	0.096134	0.142949	0.036322	0.149816	0.035777	0.022601	0.022541	0.171959	0.296109	0.08667	0.108138	*Bifidobacterium*
0.000345	0	0.00184	0	0.000751	7.71E-05	0.002466	0	0.005923	0	0	0	0.003863	0.002299	0.000979	0.00829	0.007781	0.002732	0.025764	0.001516	0.000173	0.001565	*f__Coriobacteriaceae; g__*
0	0.10426	0.039439	0.020105	0.015512	0	0.018669	0	0.028468	0.020173	0.055295	0.033596	0.042498	0.087816	0	0.113002	0.054094	0.02459	0.080288	0.103588	0	0.020188	*Collinsella*
0.007064	0.003799	0	0.009888	0	0.002622	0	0.001496	0.000191	0.000807	0.000469	0.000607	0	0.00092	0.001224	0	0	0	0.001498	0.007074	0.00468	0	*Eggerthella*
0.347174	0.24515	0.033567	0.350472	0.029022	0.170382	0.254315	0.117572	0.191058	0.141416	0.173383	0.268974	0.065679	0.024368	0.134884	0.04363	0.078177	0.012295	0.038945	0.195048	0.339227	0.180751	*Bacteroides*
0.017746	0.009565	0.013848	0.00835	0.015261	0	0.004227	0.008337	0.014329	0.024208	0.025305	0.034406	0.009015	0.004598	0.005875	0.01178	0.016673	0.004098	0.017675	0.044467	0.049575	0.00626	*Parabacteroides*
0	0.033238	0.33567	0.000659	0.178384	0	0	0.000641	0.006496	0.140408	0.079663	0.000405	0.156471	0.001839	0.113097	0.003054	0.178585	0.193306	0.043439	0.000505	0.00052	0	*Prevotella*
0	0.001832	0.000613	0.006482	0.005254	0.002468	0.007397	0.008764	0.002866	0.010087	0.005155	0.007084	0.002576	0.013333	0.001469	0.027051	0.001482	0.002049	0.002097	0	0.000173	0.006416	*f__Rikenellaceae; g__*
0.000345	0.000204	0.000964	0.000439	0.005504	0	0.001761	0.00342	0.000573	0.000202	0.000469	0.000607	0.003863	0.002759	0	0.036213	0	0.000683	0.004793	0	0.00052	0.000156	*f__S24-7; g__*
0	0	8.76E-05	0.00011	0.003252	0	0	0	0.00535	0	0	0	0.001288	0.001379	0.003672	0.001309	0.007781	0	0.001498	0	0	0.002817	*f__[Barnesiellaceae]; g__*
0.006547	0.004545	0.001315	0.000989	0.002002	0.000463	0.002466	0.001924	0.000382	0.001816	0.000469	0	0.001288	0.001839	0	0.000436	0.001112	0.000683	0.000899	0	0.00052	0.003756	*Odoribacter*
0.005341	0.001764	0.001227	0.000659	0.027521	0.000309	0.000704	0.189825	0.001528	0.002824	0.001406	0.002024	0.001288	0.234023	0.000245	0.039267	0.000371	0.028689	0.0003	0	0.001213	0.000626	*Lactobacillus*
0.000861	0.000611	0.011481	0.00033	0.005754	0.000154	0.001057	0.001496	0.000191	0.000403	0.000937	0.001012	0.001288	0.00046	0.000245	0.000436	0.000371	0	0.000599	0.001011	0.00156	0.000313	*Lactococcus*
0.037388	0.002849	0.000876	0.00022	0.070303	0.038257	0.00317	0.141727	0.012228	0.047811	0.044049	0.024691	0	0.204598	0.132436	0.000436	0.002594	0.109973	0.006591	0.004548	0.001907	0.008607	*Streptococcus*
0.056513	0.017637	0.01823	0.013294	0.031524	0.022059	0.023952	0.037837	0.044899	0.044987	0.052015	0.011536	0.052157	0.037701	0.040881	0.167103	0.057799	0.028005	0.059916	0.009096	0.023921	0.022222	*o__Clostridiales; f__; g__*
0.006892	0	0.000175	0.000879	0.001501	0.000309	0.000352	0.003206	0	0.000403	0.000937	0	0.007083	0.007816	0	0.010908	0.000741	0	0.001797	0	0	0	*f__Clostridiaceae; g__*
0.000172	0	0.000351	0.00022	0.00025	0.000926	0	0.000641	0.001146	0.002824	0.007498	0.001012	0.000644	0	0.002203	0.000436	0.001482	0	0.002097	0	0.002947	0.001095	*Clostridium*
0.012922	0.000339	8.76E-05	0	0.00025	0.002005	0.005284	0	0	0	0.000937	0.000202	0.000644	0.00046	0	0.000873	0.002223	0.000683	0.000599	0.000505	0	0	*SMB53*
0.195382	0.094899	0.093339	0.075258	0.078559	0.21558	0.141247	0.098974	0.16603	0.073432	0.156045	0.205829	0.090148	0.022069	0.082742	0.02356	0.093738	0.060792	0.04973	0.023749	0.119258	0.182942	*f__Lachnospiraceae; g__*
0.00224	0.001492	0.001139	0.000989	0.001001	0.002391	0.002113	0.008551	0.009935	0.002421	0.001874	0.005262	0.000644	0	0.026928	0	0.001853	0.003415	0	0.00859	0.002427	0.004851	*Anaerostipes*

Only the gut bacteria used in the analysis in this study are shown. The higher the abundance, the darker the red color.

Table 3. Composition of the gut microbiota in each participant.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
0.056513	0.119319	0.046012	0.022962	0.025269	0.049749	0.05037	0.014964	0.055216	0.020779	0.086223	0.014572	0.03284	0.027586	0.0612	0.013089	0.033716	0.036202	0.046135	0.051541	0.07263	0.053678	*Blautia*
0.000517	0.004545	0.005171	0.002966	0.002252	0.02499	0.005988	0.007054	0.015285	0.004438	0.020619	0.003441	0.006439	0.022529	0.010282	0.031414	0.050389	0.010246	0.031156	0	0.050789	0.020031	*Coprococcus*
0.007753	0.027676	0.01078	0.012854	0.009757	0.011338	0.016203	0.007268	0.023118	0.002219	0.013121	0.002429	0.005151	0.008736	0.010037	0.003927	0.008892	0.009563	0.009587	0.039414	0.001213	0.011737	*Dorea*
0.029118	0.009768	0.007099	0.001868	0.017013	0.025916	0.002818	0.023728	0.022927	0.022594	0.007966	0.009917	0.013522	0.003218	0.000979	0.004799	0.039644	0.010929	0.010485	0.006064	0.015947	0.019249	*Lachnospira*
0.002757	0.002442	0.010079	0.000989	0.006505	0.002854	0.00317	0.031424	0.002675	0.031874	0.005623	0.031573	0.001932	0	0.001224	0.001309	0.01482	0.006148	0.012582	0.006569	0.00104	0.012989	*Roseburia*
0.009649	0.022996	0.002366	0.054274	0.003252	0.011955	0.005636	0.002565	0.017959	0.007666	0.010309	0.007893	0.001288	0.008736	0.003672	0	0.00741	0.000683	0.004494	0.028802	0.007454	0.001565	*[Ruminococcus]*
0	0.000136	0	0.00011	0.001001	0.000154	0	0.003848	0.000764	0.002623	0	0.000202	0.000644	0.00092	0	0	0	0	0	0.001516	0	0	*[Clostridium]*
0.077016	0.02442	0.04645	0.041529	0.018764	0.070729	0.130328	0.061992	0.049102	0.075852	0.082474	0.051407	0.061816	0.078621	0.082252	0.114311	0.08781	0.036885	0.131516	0.009096	0.074536	0.075743	*f__Ruminococcaceae; g__*
0.026878	0.035206	0.050394	0.006482	0.044533	0.148708	0.108489	0.089141	0.05961	0.048416	0.082943	0.038251	0.101095	0.010115	0.015177	0.024433	0.063727	0.065574	0.020971	0.033855	0.082163	0.091236	*Faecalibacterium*
0.023604	0.016484	0.005171	0.035597	0.003252	0.004165	0.027122	0.00513	0.018151	0.009482	0.008435	0.005869	0.008371	0.006897	0.013219	0.012216	0.00741	0.002049	0.009287	0.015664	0.015774	0.009077	*Oscillospira*
0.001895	0.0097	0.00929	0.065041	0.047035	0.021751	0.094752	0.04938	0.097822	0.052249	0.009841	0.001214	0.057952	0.068966	0.013709	0.106457	0.05817	0.003415	0.088975	0.002021	0.010574	0.068858	*Ruminococcus*
0.01275	0.005766	8.76E-05	0.013733	0	0.004782	0	0.001283	0	0.041356	0	0.016191	0	0.001839	0	0.016579	0.029641	0.004098	0	0	0	0	*Dialister*
0	0.022317	0.02603	0.002857	0.016763	0.011492	0.013385	0.000214	0.009553	0	0.030928	0.00081	0.027688	0.001839	0.035985	0.003054	0.011115	0.015027	0.026363	0.006569	0	0.012989	*Phascolarctobacterium*
0	6.78E-05	0	0	0.006255	0.002005	0	0.024583	0	0.005649	0	0	0	0.001379	0	0	0.010004	0.000683	0	0.004042	0.001387	0.001095	*Veillonella*
0	0.001153	0.001139	0.000769	0.001001	7.71E-05	0.001761	0	0.002675	0.000807	0.000937	0.000607	0.001288	0.001839	0.001224	0.006981	0.001482	0.000683	0.006291	0	0.000173	0.001095	*f__[Mogibacteriaceae]; g__*
0.004307	0.04572	0.000438	0.001318	0.003002	0.004936	0.001409	0.001496	0.000382	0.008069	0.002343	0.000202	0.01159	0	0.018605	0.006108	0.009633	0.002732	0.055722	0.009601	0.014734	0.032238	*f__Erysipelotrichaceae; g__*
0.000172	0.000271	0.000526	0.00011	0.00025	0.000231	0	0.002779	0.000573	0	0	0.000607	0	0.001379	0.000245	0.000873	0	0	0	0	0.000693	0	*Allobaculum*
0.002068	0.001425	0.001052	0.000549	0.00025	0.000848	0.001761	0.000214	0.000191	0	0.000469	0	0.000644	0	0.014688	0.000436	0.000371	0	0.0003	0.015664	0	0.000313	*Coprobacillus*
0	0	0.000438	0.000769	0.00025	0.000694	0.001761	0	0.000382	0.001009	0	0.000202	0	0	0	0.000436	0	0	0.000599	0	0	0.000469	*Holdemania*
0.004997	0.001153	0.000526	0.001868	0.042782	0.001697	0.000704	0	0.000955	0.000202	0.000469	0.000405	0.041211	0	0.001224	0.002182	0	0.043033	0.000599	0.011622	0.001907	0.000156	*[Eubacterium]*
0	0.019604	0.002542	0.012085	0.003252	0.005245	0.001761	0.00513	0.010508	0.003833	0	0.011131	0.005151	0.001379	0	0.000436	0.004817	0.008197	0.000899	0	0.006414	0.011268	*Sutterella*
0.005169	0.004274	0.00149	0.001538	0.00025	0.00108	0.000352	0.000428	0.003248	0	0	0.002631	0	0	0	0.000873	0.000741	0	0.001797	0.006064	0.003813	0.001252	*Bilophila*
0.001551	0.001153	0.004996	0.001538	0.063548	0.001774	0.00634	0.001924	0.000191	0.002017	0.000937	0.078122	0.003863	0	0.004896	0.001745	0.001482	0.209016	0.001797	0.011622	0	0.001408	*f__Enterobacteriaceae; g__*
0.016196	6.78E-05	0.000701	0	0.014261	0.000231	0.004579	0.000855	0	0.12326	0	0	0.001932	0	0	0.000873	0.001853	0	0	0	0	0.000626	*Erwinia*
0.009132	0.003799	0.002629	0.00791	0.014511	0.000771	0.004931	0.000641	0	0.004842	0	0	0.004507	0.016092	0	0	0.009633	0	0.005392	0	0	0.000156	*Akkermansia*

Only the gut bacteria used in the analysis in this study are displayed. The higher the abundance, the darker the red color.

Figures

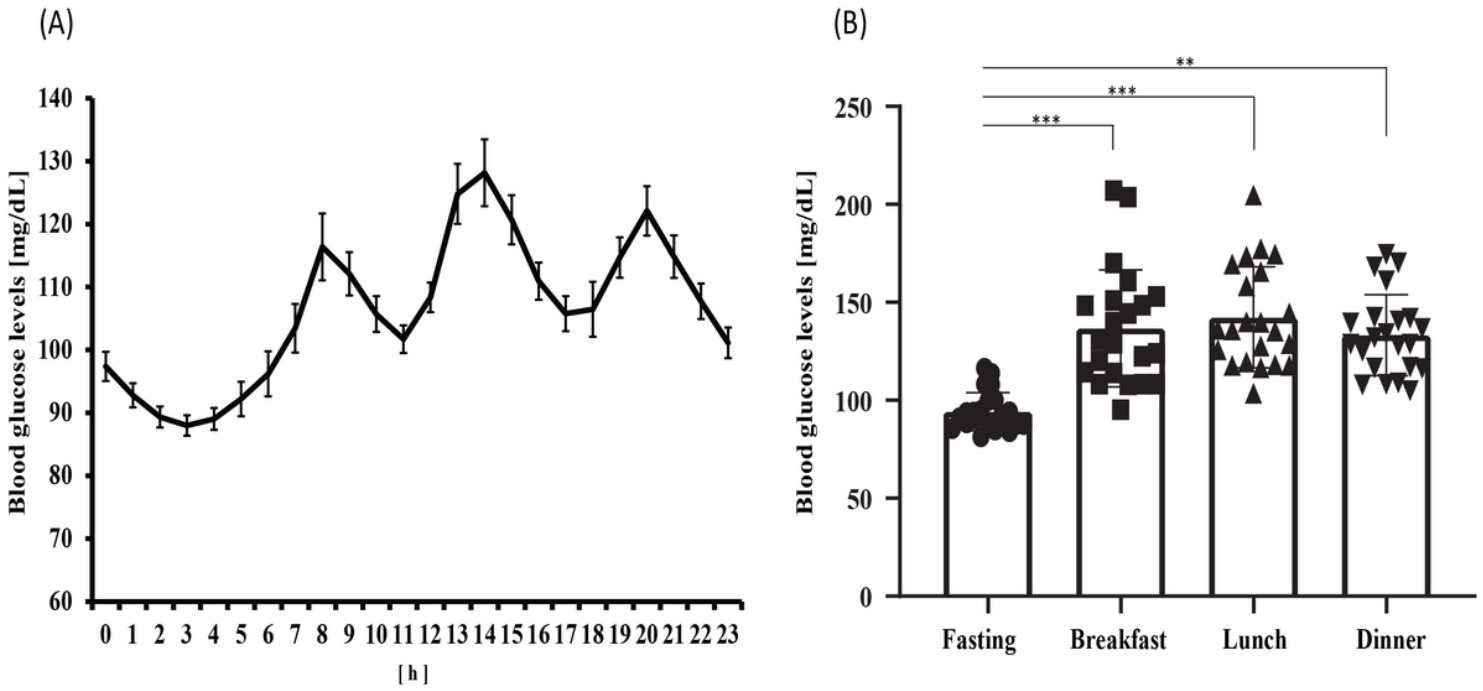

Figure 1

(A) Changes in the glucose levels during 24-h period (n = 22). Values are expressed as mean ± standard error. (B) Comparison of fasting (1 point before breakfast) and peak (after each meal) glucose levels. The average glucose levels of each participant are shown. The coefficient of variation values for fasting, breakfast, lunch, and dinner glucose levels were 0.101, 0.213, 0.177, and 0.151, respectively. **p < 0.01, ***p < 0.001, vs Fasting

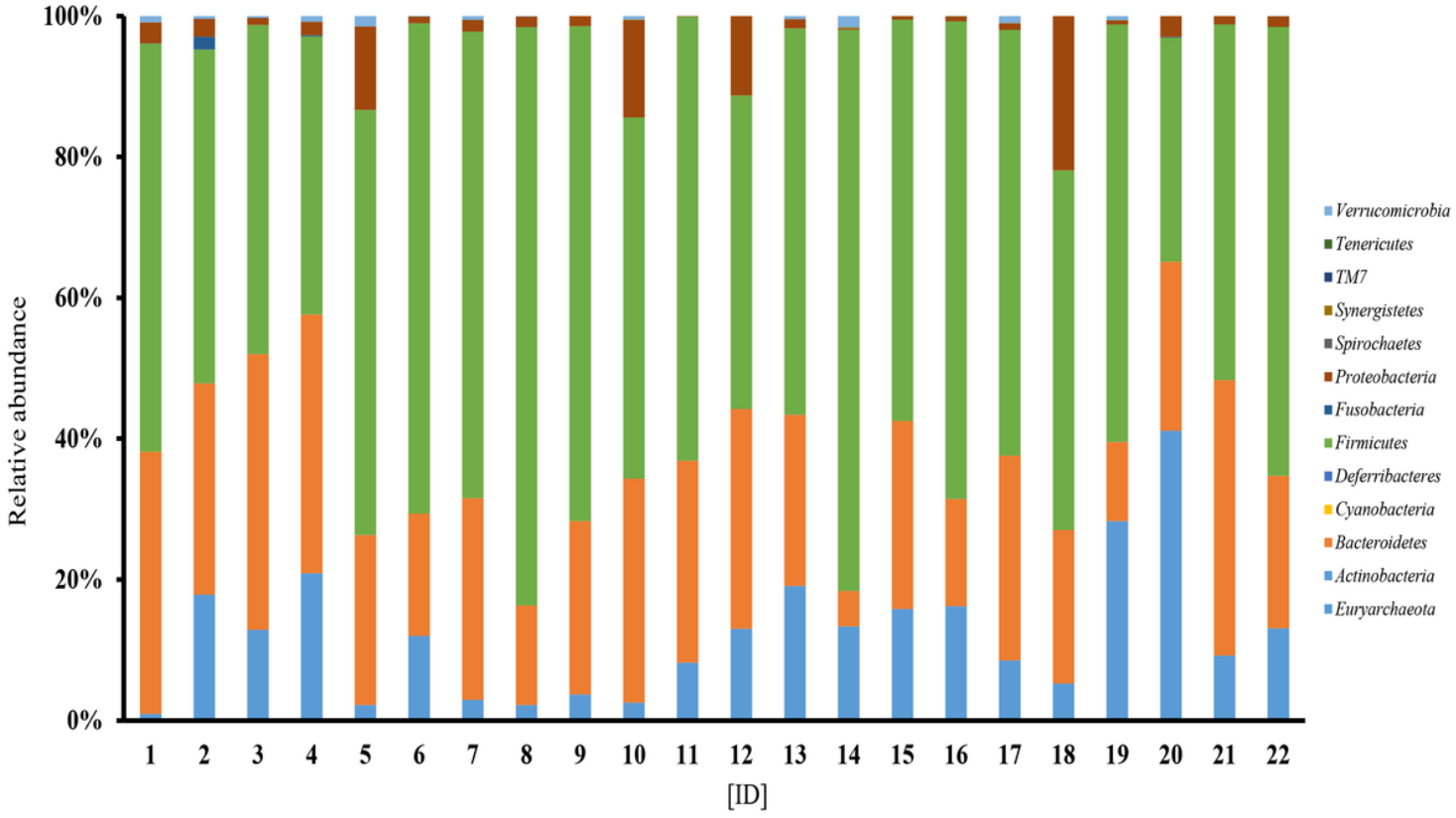

Figure 2

Composition of the gut microbiota at the phylum level in each participant.

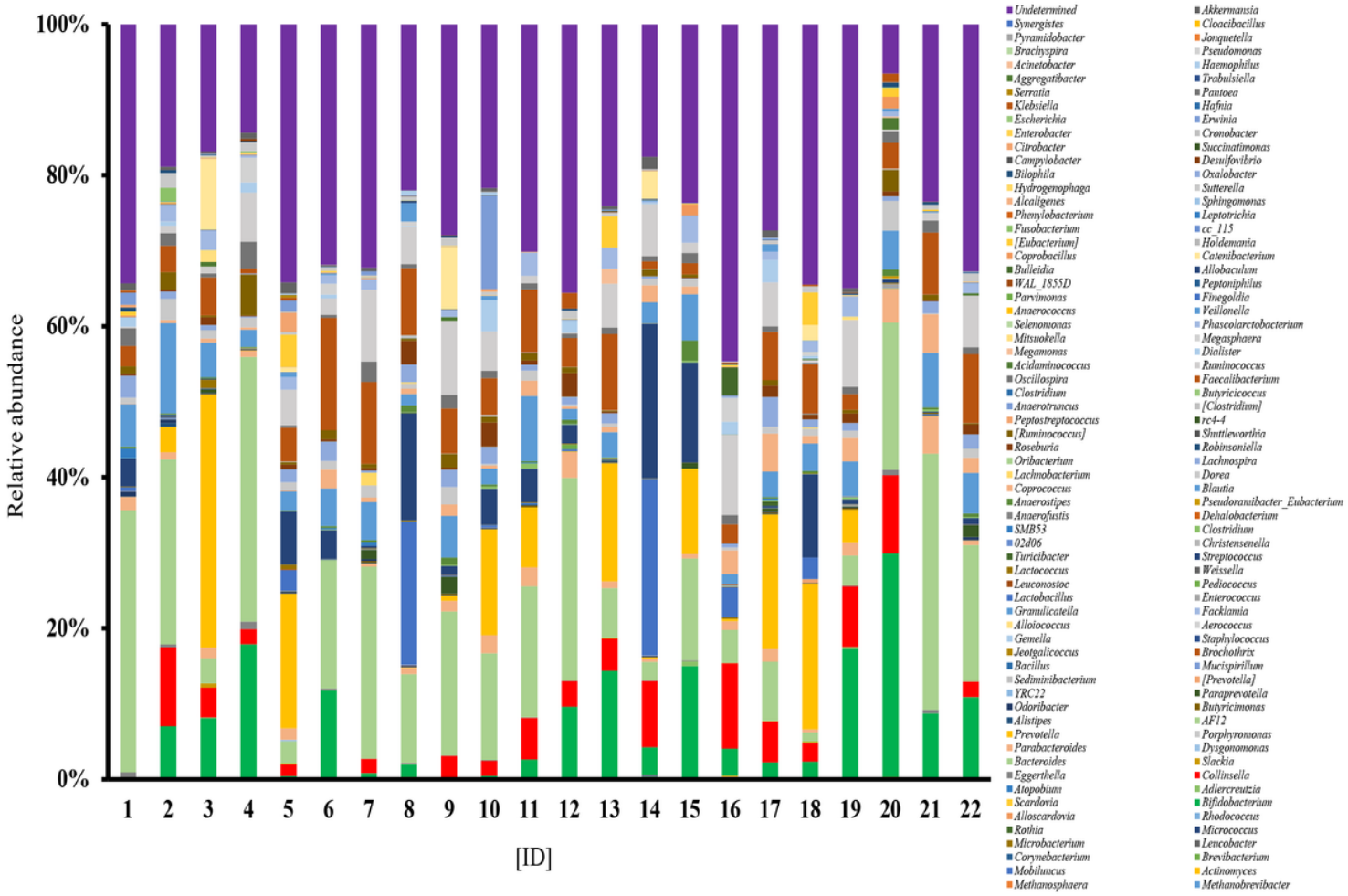

Figure 3

Composition of the gut microbiota at genus level in each participant.

Figure 4

Correlation between the gut bacteria and fasting and peak (after each meal) glucose levels. Positive correlations are shown in red, and negative correlations are shown in green. *p < 0.05, **p < 0.01