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Composition of the intestinal microbiota and its variations between the second and third trimesters in women with gestational diabetes mellitus and without gestational diabetes mellitus

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Objective: This study was designed to explore the composition of the intestinal microbiota and its longitudinal variation between the second trimester (T2) and the third trimester (T3) in women with gestational diabetes mellitus (GDM) and pregnant women with normal glucose tolerance.

Methods: This observational study was conducted at Peking Union Medical College Hospital (PUMCH). Women with GDM and pregnant women with normal glucose tolerance were enrolled in the study, and fecal samples were collected during T2 (weeks 24~28) and T3 (weeks 34~38). Fecal samples were analyzed from 49 women with GDM and 42 pregnant women with normal glucose tolerance. The 16S rRNA gene amplicon libraries were sequenced to analyze the microbiota and QIIME2 was used to analyze microbiome bioinformatics.

Results: The four dominant phyla that *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* which accomplish about 99% of the total relative abundance did not significantly change between the T2 and T3 in the GDM and healthy groups. At the genus level, the relative abundance of *Scardovia* (0 vs. 0.25%, P = 0.041) and *Propionibacterium* (0 vs. 0.29%, P = 0.041) increased significantly in the control group, but not in the GDM group. At the phylum level, the relative abundance of *Firmicutes* and *Actinobacteria* was significantly different between women with GDM and pregnant women with normal glucose tolerance in both T2 and T3. In T2 and T3, the relative abundances of *unidentified_Lachnospiraceae*, *Blautia*, and *Parabacteroides* were significantly higher in the GDM group than in the control group (P<0.05). The relative abundance of *Bifidobacterium* in the GDM group was lower than in the control group in both T2 and T3.

Conclusions: The intestinal microbiota composition was stable from T2 to T3 in the GDM and control groups; however, the intestinal microbiota composition was different between the two groups.

KEYWORDS

gut microbiota, gestational diabetes mellitus, normal glucose tolerance, healthy pregnant normal glucose tolerance women, second trimester, third trimester

Introduction

The intestinal microbiota, which plays an important role in maintaining human health, colonizes the human intestinal tract (1). In general, the gut microbiota participates in various activities, such as metabolism (2). The gut microbiota can play a role by producing short-chain fatty acids, such as butyrate and propionate (3). The alteration of the intestinal microbiota is associated with many diseases, such as type 2 diabetes and obesity (4–8). Some researchers have recently explored the association between gut microbiota and pregnancy (9–11). The gut microbiota is characterized mainly by an increase in *Actinobacteria* and *Proteobacteria*, with a reduction in the diversity of microbiota and butyrate-producing bacteria during pregnancy (9). Gestational diabetes mellitus (GDM) is a common complication during pregnancy, characterized by the incapability of pancreatic beta cells to respond sufficiently to the increased insulin requirements of pregnancy leading to different degrees of hyperglycemia (12). GDM can pose important short- and long-term health risks for both the mother and the offspring. Although insulin resistance and inflammatory processes have been suggested to be involved in the development of GDM, the specific pathogenesis of GDM remains unclear (13). Therefore, researchers have conducted various studies to explore the gut microbiota characteristics in women with GDM and found differences in the gut microbiota compared with pregnant women with normal glucose tolerance. In women with GDM, opportunistic pathogens in the gut microbiota, such as *Bacteroides* and *Firmicutes* increase, and beneficial bacteria decrease (14).

Various factors, such as dietary intervention and probiotics, influence gut microbiota composition (1). Metabolism can change with the progression of trimesters during pregnancy (15). Koren et al. (9) found that the intestinal microbiota changed dramatically from the first to the third trimester, with a general increase in *Proteobacteria* and *Actinobacteria*, and the microbiota in the third trimester induced greater insulin adiposity than in the first trimester. Abdullah et al. (16) showed that lower α-diversity indices in the GDM group than in the control group, higher abundances in the genera *Acidaminococcus, Clostridium, Megasphaera, and Allisonella*, and lower abundances in *Barnesiella* and *Blautia* but no differences by trimester. Sun et al. (17) found that a decrease in the diversity of intestinal microbial species and changes in the composition of intestinal microbiota with advancing gestation was founded in the control group but not

in the GDM group. The gut microbiota in women with GDM may be more stable than that of control group.

To date, the differences in gut microbiota composition between women with GDM and pregnant women with normal glucose tolerance have been explored in various studies, and the conclusions have been similar (18–20). However, a comparison of the intestinal microbiota in women with GDM between different trimesters is lacking. We conducted this prospective observational cohort study to investigate the longitudinal variations of the intestinal microbiota composition from the second (T2) to the third trimester (T3) in women with GDM and pregnant women with normal glucose tolerance.

Methods

Ethical approval

This prospective observational cohort study was conducted at the Peking Union Medical College Hospital (PUMCH) between April 2019 and May 2020. This study was reviewed and approved by the Ethics Review Board at PUMCH (approval number HS-1875). Women who met the inclusion criteria and signed an informed consent form were recruited. This study was registered at clinicaltrial.gov (NCT03916354, 04/12/2019). All the procedures were performed in accordance with the Declaration of Helsinki.

Population and groups

Fifty women with GDM and fifty pregnant women with normal glucose tolerance were enrolled in the study at T2 (24~28 weeks), and basic characteristics such as age, parity, pre-pregnancy body mass index (BMI), height, pre-pregnancy weight and gestational week were collected. Pre-pregnancy BMI was defined as the weight (kg) divided by the square of height (m). The inclusion criteria were as follows: (1) pregnant women, (2) natural pregnancy, (3) singleton pregnancy, and (4) provision of informed consent. Exclusion criteria were: (1) women with pre-pregnancy hypertension, diabetes, and dyslipidemia; (2) severe complications during pregnancy; (3) administration of antibiotics/prebiotics/probiotics during or in the last month before recruitment; (4) any situation of preexisting chronic diseases; and (5) refusal to sign the informed consent.

Definition

GDM was diagnosed using recommendations of the International Association of the Diabetes and Pregnancy Study Groups (IADPSG), based on the result of a 75 g oral OGTT. Pregnant women who exhibited one or more markers of blood glucose levels higher than the cutoff values (fasting venous plasma glucose levels ≥ 5.1 mmol/L and/or 1 h glucose level ≥ 10.0 mmol/L and/or 2h glucose level ≥ 8.5 mmol/L) were diagnosed with GDM.

Fecal sample collection

Participants were asked to collect at least 250 mg of feces into a sterile test tube (PSP® Spin Stool DNA Plus Kit) with preservation solution at 24~28 and 34~38weeks. Researchers would instruct the subjects to store the samples in an environment of 4°C and send the samples to hospital within 24 hours. After that, researchers would store the samples at -80°C for DNA extraction.

Sequencing and analysis of 16S rRNA gene amplicon

DNA was extracted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V4 region of the 16S rRNA bacterial gene was amplified by PCR. A TruSeq® DNA PCR-free Sample Preparation Kit was used for library construction and the Illumina NovaSeq 6000 platform was used for sequencing. According to barcode sequence and the PCR amplification primer sequence, each sample data was separated from disembarkation data. After the amputation of barcode and primer sequences using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) (21) to splice reads of each sample, the splicing sequence for the original tags data (raw tags). Raw tags obtained by splicing need to undergo strict filtering (22) to obtain the high-quality tag data (clean data). According to the QIIME (V1.9.1 http://qiime.org/scripts/split_libraries_fastq.html) (23) tags quality control process, the procedures were as follows: (a) tags to intercept: The raw tags were truncated from the first low-quality base site whose number of consecutive low-quality values (default quality threshold ≤ 19) reached the set length (default length value 3). (b) Tags length filtering: Tags data set obtained by intercepting tags were filtered out tags whose continuous high-quality base length was less than 75% of the length of tags. The tags obtained after the above processing need to be processed to remove the chimeric sequence. The Tags sequence (24) shall be compared with the series annotation database to detect the chimeric sequence, and finally remove the chimeric sequence. Using Uparse software (Uparse v7.0.1001, http://www.drive5.com/uparse/) (25) to cluster all effective tags of all samples. By default, sequences are grouped into operational taxonomic units (OTU) with 97% identification. According to the algorithm principle, the sequences with the highest frequency among OTUs were selected as representative sequences of OTUs. OTU annotation analysis was performed using the Mothur (26) and SSUrRNA databases of SILVA132 (27)

(threshold 0.8–1). The Shannon and Simpson indices were calculated using QIIME (version 1.9.1). Beta diversity was calculated using unweighted UniFrac with QIIME. Principal coordinate analysis (PCoA) was performed to obtain the principal coordinates and visualize the complex multidimensional data, and PCoA plots based on unweighted UniFrac distance analysis were used to evaluate beta diversity.

Statistical analyzes

All statistical analyzes were performed with IBM SPSS 25.0. Clinical baseline characteristics are presented as medians (interquartile range). Continuous variables not normally distributed were reported as medians (interquartile distance), and compared using the Wilcoxon test. The relative abundances of taxa at the phylum and genus levels were compared using the Wilcoxon test. A false discovery rate (FDR)- corrected P < 0.05 was considered statistically significant. All statistical analyzes were performed using two-sided tests.

Results

Clinical characteristics of the participants

The baseline characteristics of the women with GDM and pregnant women with normal glucose tolerance are summarized in Table 1. Fifty women with GDM and fifty controls were enrolled in this study. One person in the GDM group was excluded due to the use of antibiotic drugs. In the control group, two participants were excluded because they experienced serious obstetric complications during pregnancy, four used antibiotic drugs, and two were lost to follow-up (Figure 1). The final sample for analyzes included data from 49 women with GDM and 42 pregnant women with normal glucose tolerance. Fecal samples from all participants in the GDM group (n = 49) were collected in T2 (SGDM) and T3 (TGDM). In the control group, one stool sample in T2 and three stool samples in T3 were not received, and eventually 41 and 39 fecal samples were collected in T2 (SHC) and T3 (THC), respectively,.

Women with GDM were more likely to be older (33 (32–36.5) vs. 32 (29–34), P = 0.018) and deliver at lower gestational age (39 (38–39) vs. 39 (38–40), P = 0.006). Other clinical characteristics were not significantly different between the groups (Table 1).

Dynamics in intestinal microbiota in the GDM and control group from T2 to T3

From T2 to T3 in the GDM group, at the phylum level (Figure 2A), although not statistically significant, the relative abundances of > 1% of the dominant bacteria, *Firmicutes* (60.31% vs. 57.62%, P = 0.772), *Actinobacteria* (5.43% vs. 4.37%, P = 0.772), and *Proteobacteria* (3.47% vs. 3.27%, P = 0.772), showed a downward trend. *Bacteroides* (29.85% vs. 33.53%, P = 0.772)

TABLE 1 Comparison of clinical characteristics in the study groups.

Characteristic	GDM (n=49)	Health women (n=42)	P value
Age (year)	33 (32~36.5)	32 (29~34)	0.018*
Parity (number)	1 (1~2)	1 (1~2)	0.438
Pre-pregnancy BMI (kg/m^2)	22.46 (19.78 ~24.28)	21.05 (19.65~22.68)	0.112
Height(cm)	163.00 (160.00 ~167.00)	163 (162~166.5)	0.592
OGTT-0 hours	4.90 (4.50 ~ 5.25)	4.40 (4.18 ~ 4.60)	< 0.05
OGTT-1 hours	9.80 (8.85 ~ 10.70)	7.55 (6.38 ~8.45)	< 0.05
OGTT-2 hours	8.80 (7.45 ~ 9.40)	6.20 (5.40 ~ 7.23)	< 0.05
Pre-pregnancy Weight (kg)	58 (53.25~64.5)	56.5 (52.5~61.25)	0.217
Gestational week (weeks)	39 (38~39)	39 (38~40)	0.006*

Data presented as median (first quartile, third quartile).
GDM gestational diabetes mellitus, BMI body mass index.
*Statistically significant at P < 0.05.

showed an increasing trend (Supplement File 1). The same trend at the genus level (Figure 2B), among the top 10 dominant bacteria in the GDM group, although not statistically significant, the relative abundances of *Bacteroides* (20.18% vs. 22.68%, P = 0.791), *Faecalibacterium* (8.25% vs. 9.84%, P = 0.392), *unidentified_Lachnospiraceae* (4.88% vs 5.16%, P = 0.820),

Parabacteroides (2.28% vs. 2.73%, P = 0.791) showed an increase from T2 to T3, whereas, the relative abundances of *unidentified_Ruminococcaceae* (4.86% vs. 3.54%, P = 0.392), *Blautia* (4.13% vs. 3.83%, P = 0.520), *Roseburia* (3.61% vs. 3.52%, P = 0.791), *Lachnospira* (3.90% vs. 2.89%, P = 0.502), *Bifidobacterium* (3.72% vs. 2.80%, P = 0.392), *Megamonas* (2.81%

FIGURE 1
The flow chart of the study. * emphasis on exclusion.
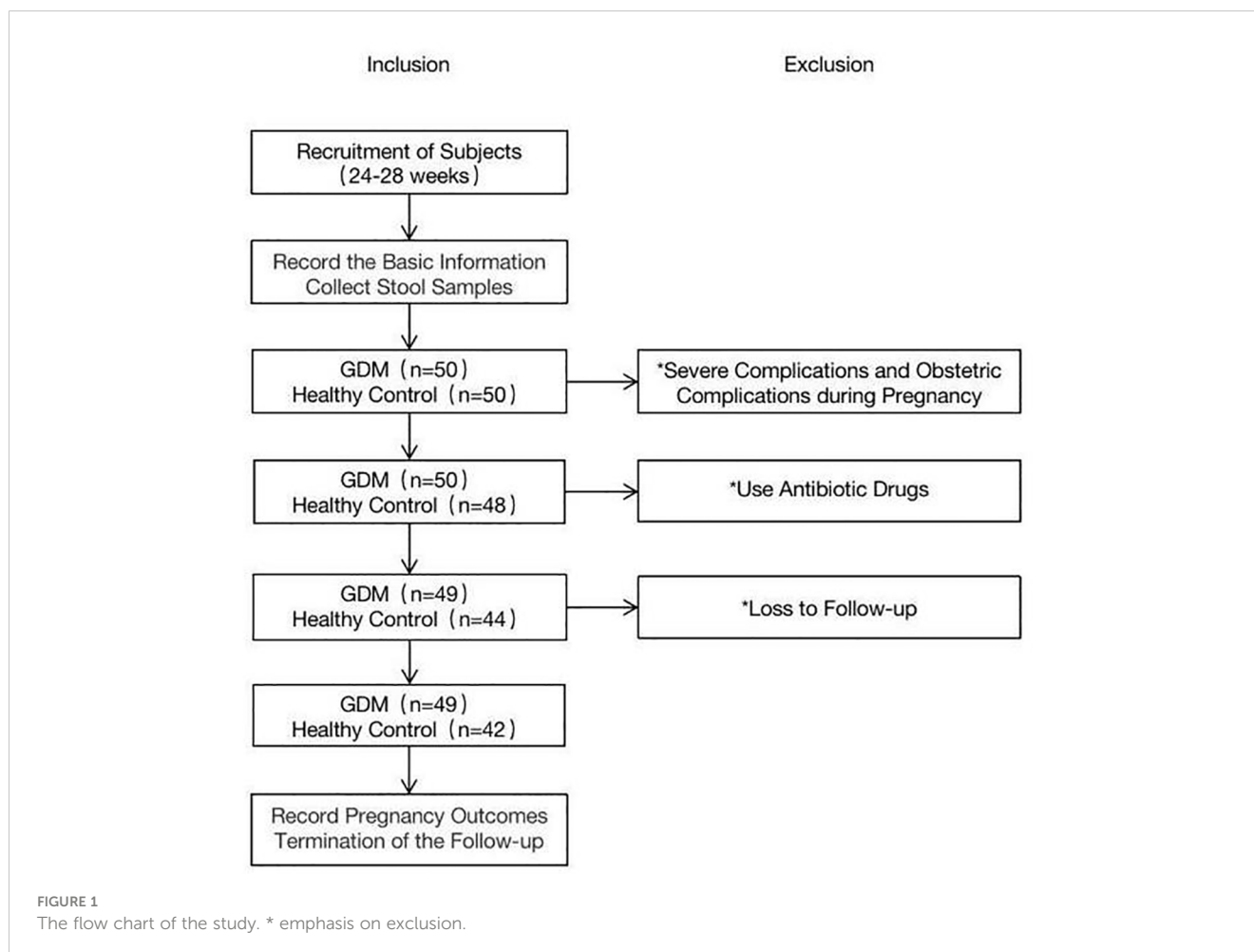
vs. 1.30%, P = 0.502) showed a decrease from T2 to T3, as illustrated in Supplement File 2.

From T2 to T3 in the control group, at the phylum level (Figure 2A), although not statistically significant, the relative abundances of *Firmicutes* (47.84% vs. 49.46%, P = 0.969), *Bacteroides* (29.31% vs. 31.44%, P = 0.969), and *Proteobacteria* (5.35% vs. 5.38%, P = 0.915) showed an increasing trend. The relative abundance of *Actinobacteria* (16.59% vs. 12.63%, P = 0.946) showed a downward trend; however, the differences of other bacteria were not statistically significant (Supplement File 1). At the genus level (Figure 2B), among the top 10 dominant bacteria in the control group, *Bacteroides* (16.31% vs. 16.73%, P = 0.918), *Faecalibacterium* (7.62% vs. 9.70%, P = 0.734), *Bifidobacterium* (10.37% vs. 6.36%, P = 0.637), *Collinsella* (5.52% vs. 5.05%, P = 0.611), unidentified_Ruminococcaceae (4.67% vs. 5.09%, P = 0.833), *Subdoligranulum* (3.19% vs. 3.55%, P = 0.611), *Roseburia* (2.90% vs. 3.20%, P = 0.918), *Lachnospira* (2.15% vs. 2.73%, P = 0.918), *Streptococcus* (2.81% vs. 2.02%, P = 0.820), and unidentified_Lachnospiraceae (2.54% vs. 2.10%, P = 0.637) (Supplement File 2) were both no significant differences from T2 to T3. The relative abundance of *Scardovia* (0 vs. 0.25%, P = 0.041) and *Propionibacterium* (0 vs. 0.29%, P = 0.041) in pregnant women with normal glucose tolerance was significantly higher in T3 than in T2 (Supplement File 2).

In T2, at the phylum level (Figure 2A), the relative abundance of *Firmicutes* in the GDM group was significantly higher than that in the control group (60.31% vs. 47.84%, P < 0.001), and the relative abundance of *Actinobacteria* in the GDM group was significantly lower than that in the control group (5.43% vs. 16.59%, P = 0.009). The abundance of other bacteria is described in Supplement File 1. At the genus level (Figure 2B), the relative abundances of unidentified_Lachnospiraceae (4.88% vs. 2.55%, P < 0.001), *Roseburia* (3.61% vs. 2.90%, P = 0.041), *Lachnospira* (3.90% vs. 2.15%, P = 0.004), *Blautia* (4.13% vs. 2.76%, P = 0), and *Parabacteroides* (2.27% vs. 0.73%, P = 0) in the GDM group were higher than those in the control group. The relative abundance of *Bifidobacterium* in the GDM group was lower than that in the control group (3.72% vs. 10.37%, P = 0.012). The relative abundances of other bacteria were lower in the GDM group than in the control group (Supplement File 2).

In T3, at the phylum level (Figure 2A), the relative abundance of *Firmicutes* (57.62% vs. 49.46%, P = 0.044) in the GDM group was significantly higher than that in the control group. The relative abundance of *Actinobacteria* (4.37% vs. 12.63%, P = 0.007) in the GDM group was significantly lower than in the control group. The relative abundances of other bacteria are detailed in Supplement File 1. At the genus level (Figure 2B), the relative abundances of unidentified_Lachnospiraceae (5.16% vs. 2.11%, P = 0), *Blautia* (3.83% vs. 1.46%, P = 0), *Parabacteroides* (2.73% vs. 1.18%, P = 0), and *Megamonas* (1.31% vs. 0.21%, P = 0.038) in the GDM group were significantly higher than those in the control group. The relative abundance of *Bifidobacterium* (2.80% vs. 6.36%, P = 0.022) in the GDM group was significantly lower than that in the control group (Supplement file 2). The relative abundances of other bacteria are detailed in Supplement File 2.

OTUs

Venn diagrams were drawn on the basis of the number of OTUs of samples in the GDM and control groups (Figure 3). As shown in the figure, in the GDM group, the total number of OTUs in T2 and T3 was 3412 and 3806, respectively. The number of common OTUs in T2 and T3 was 2447; the number of unique OTUs in T2 and T3 was 965 and 1359, respectively (Figure 3A). The number of unique OTUs in T2 represented 28.28% of the total OTUs in T2 and 35.71% of the total OTUs in T3. In the control group, the total number of OTUs in T2 and T3 was 4619 and 4618, respectively. The number of common OTUs in T2 and T3 was 2883, and the unique numbers of OTUs in T2 and T3 were 1736 and 1735, respectively (Figure 3B). Unique OTUs in T2 accounted for 37.58% of the total OTUs in T2 and 37.57% of the total OTUs in T3.

The alpha and beta diversity

In the GDM group, there was no significant differences in the Chao index (P=0.123) (Figure 4A) and ACE index (P=0.201) (Figure 4B) were observed from T2 to T3. The same trend in the
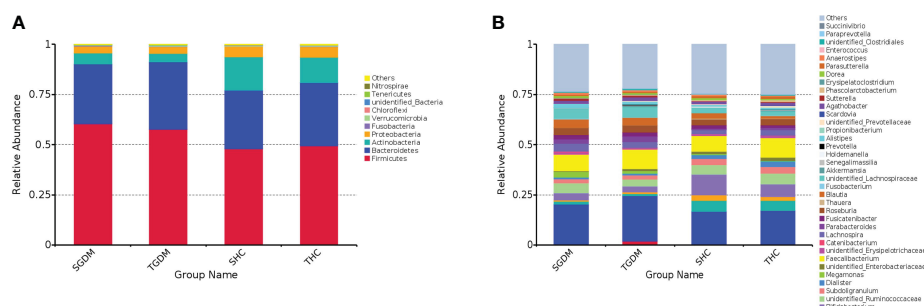

FIGURE 2
The dynamics in intestinal microbiota in the GDM and control group from T2 to T3 at the phylum and genus level. **(A)** Relative abundance of the top 10 bacterial taxa at the phylum level; **(B)** Relative abundance of the top 40 bacterial taxa at the level of bacterial. GDM, Gestational diabetes mellitus; T2, second trimester; T3, third trimester; SGDM, Second trimester in the GDM group; TGDM, Third trimester in the GDM group; SHC, Second trimester in the control group; THC, Third trimester in the control group.
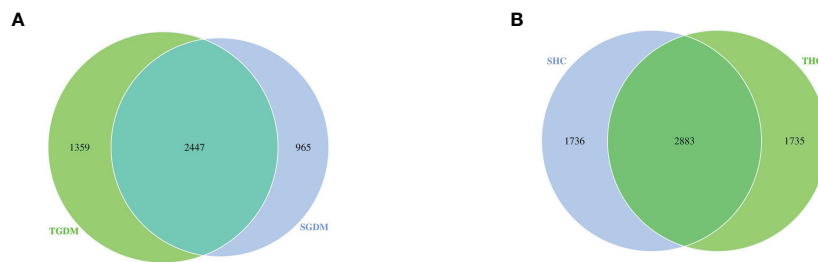
FIGURE 3
Venn diagram among the GDM and control groups. **(A)** The overlaps of OTUs in the GDM group. **(B)** The overlaps of OTUs in the control groups. GDM, Gestational diabetes mellitus; SGDM, Second trimester in the GDM group; TGDM, Third trimester in the GDM group; SHC, Second trimester in the control group; THC, Third trimester in the control group.

control group. In the GDM group, there was no significant difference in changes in the Shannon index (Figure 4C) (6.039 vs 5.822, P = 0.078) and the Simpson index (Figure 4D) was observed from T2 to T3 (0.953 vs 0.937, P = 0.177). The Shannon index (Figure 4C) (5.188 vs. 5.043, P=0.795) and the Simpson index (Figure 4D) (0.904 vs. 0.880, P = 0.824) in the control group from T2 to T3 were not statistically significant. The Shannon index in T2 (6.039 vs 5.188, P = 0) and T3 (5.822 vs 5.043, P = 0) in the GDM group were both significantly higher than those in the control group, and the Simpson index in T2 (0.953 vs. 0.904, P < 0.001) and T3 (0.937 vs. 0.880, P <.001) in the GDM group were both significantly higher than those in the control group.

PC1 was the main coordinate component that caused the largest difference in the samples, with an explanatory degree of 20.74%, followed by PC2, with an explanatory degree of 9.09% (Figure 5). According to the AMOVA analysis, there were no significant differences in the gut composition microbiota in T2 and T3 in the GDM (P = 0.265) and control groups (P = 0.593). However, there was a significant difference in the composition of the gut microbiota between the GDM and control groups (P < 0.001). The distribution of the intestinal microbiota in T2 and T3 was similar in the GDM and control groups; however, the distribution distance of the GDM group was relatively far compared to that of the control group.

Discussion

This study explored the composition of the intestinal microbiota and its alternative characteristics from T2 to T3 in women with GDM and pregnant women with normal glucose tolerance. The results showed that *Scardovia* and *Propionibacterium* were significantly higher in T3 than in T2 in the control group, but not in the GDM group. The changes in the relative abundance of the remaining bacteria from T2 to T3 were stable in the GDM and control

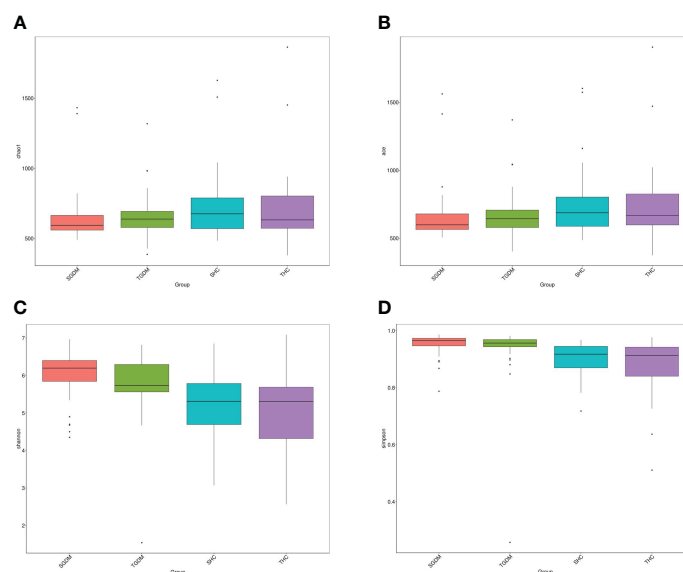FIGURE 4
The alpha diversity of intestinal microbiota in the GDM and control groups. **(A)** Chao1 estimator, **(B)** abundance-based coverage estimator (ACE), **(C)** Shannon, **(D)** Simpson. GDM, Gestational diabetes mellitus. SGDM: Second trimester in the GDM group; TGDM, Third trimester in the GDM group; SHC, Second trimester in the control group; THC, Third trimester in the control group.
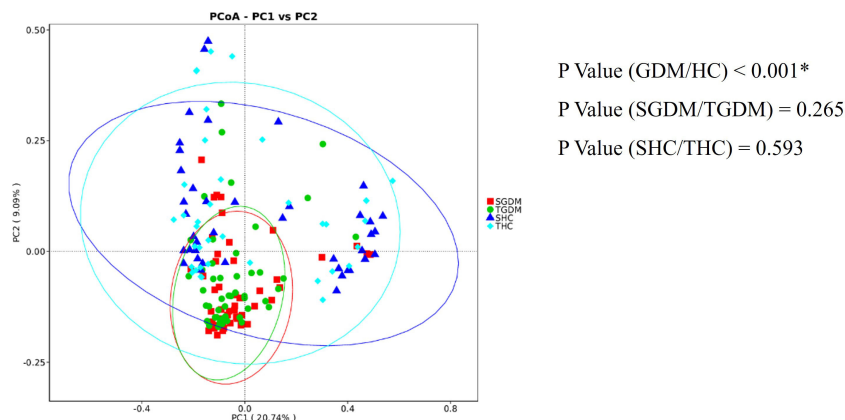
FIGURE 5
PCoA shows the dispersal of gut microbiota between trimesters in the GDM and healthy control groups. Red represents GDM samples in T2, green represents GDM samples in T3, dark blue represents samples of the control group in T2, and light blue represents samples of the control group in T3. SGDM, GDM group in the second trimester; TGDM, GDM group in the third trimester; SHC, control group in the second trimester; THC, control group in the third trimester; PCoA, Principal Coordinate Analysis. *P value < 0.05.

groups. Nevertheless, there were significant differences in the composition of the gut microbiota in the GDM and control groups in both T2 and T3.

We found that the dominant bacteria were composed of four phyla: *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria* at the phylum level in both the GDM and the control groups, which was consistent with the results of Tang et al. (28). Ma et al. (29) found that the four dominant phyla were *Firmicutes, Bacteroidetes, Proteobacteria*, and *Tenericutes. Actinobacteria* (30) act as markers of GDM and are positively correlated with fasting blood glucose levels; however, this association was not present after adjusting for pre-pregnancy body mass index (BMI). *Tenericutes* (31) are the dominant bacteria in the neonatal oral microbiota in babies of women with GDM. In our study, the relative abundance of *Tenericutes* was less than 1%, which may be explained by the type of samples studied and sample size. At the genus level, *Bacteroides, Faecalibacterium, unidentified_Lachnospiraceae, unidentified_Ruminococcaceae, Roseburia, Lachnospira*, and *Bifidobacterium* were the dominant bacteria in both the GDM and control groups. *Blautia, Parabacteroides*, and *Megamonas* were the dominant bacteria in the GDM group, while *Collinsella, Subdoligranulum*, and *Streptococcus* were the dominant bacteria in the control group.These GDM-enriched genus may participate in the development of GDM by influencing host immune status. *Blautia* (32), which is significantly associated with host dysfunctions, such as obesity, diabetes, and various inflammatory diseases, is a genus of biotransformative bacteria with probiotic properties that can regulate host health and alleviate metabolic syndrome. *Parabacteroides* are enriched in overweight women (30) and in women with GDM (18), which is consistent with our findings. *Megamonas* is enriched in obese women (16, 18) and has a positive relationship with glucose tolerance (18). *Megamonas* was the dominant bacterium specific to women with GDM; however, women with GDM were not classified by pre-pregnancy weight class in our study. Women with a history of GDM have a high abundance of *Collinsella* in their postpartum gut microbiota, and *Collinsella* has the potential to be a marker for the

future development of type 2 diabetes in women with a history of GDM (30). However, previous studies have reported that *Collinsella* increases in healthy pregnancies (33, 34). In the present study, the relative abundance of *Collinsella* was higher in the control group. This difference may need the studies that enroll more subjects to explain. *Subdoligranulum*, which produces short-chain fatty acids such as butyrate, is negatively associated with human fat accumulation, insulin resistance, insulin, CRP, IL6, and other markers (35). A study (36) found that the relative abundance of *Streptococcus* in overweight, obese, and diabetic patients was lower than that of healthy controls, and Hajifaraji et al. (37) found that the combination of *Streptococcus* with other probiotics had a positive outcome in the treatment of metabolic diseases.

In our study, we found that the composition of the intestinal microbiota in the GDM and pregnant women with normal glucose tolerance was relatively stable from T2 to T3. Only the relative abundance of *Scardovia* and *Propionibacterium* in T3 was significantly higher than in T2 in pregnant women with normal glucose tolerance. Members of *Scardovia* are one of the seven genera of the *Bifidobacteriaceae* family and recognized as the healthy gut microbiota (38). *Scardovia* can produce acetic acid from glucose, together with small amounts of lactic and formic acid (39). It is reported that *Propionibacterium* can ameliorate insulin resistance by obesity (40).Insulin resistance, which is emphasized in the development of GDM in the late pregnancy, is associated with a reduced abundance of butyrate-producing bacteria (41–43). Ferrocino et al. found that an increase in *Firmicutes* and a reduction in *Bacteroidetes* and *Actinobacteria* from T2 to T3 in women with GDM who adhered to dietary recommendations showed a better metabolic and inflammatory pattern at the end of the study and a clear decrease in *Bacteroidetes* (44). We found that at the phylum level, the *Firmicutes/Bacteroidetes* ratio both decreased in the GDM group and control group from T2 to T3. The increased *Firmicutes/Bacteroidetes* ratio is associated with obesity and inflammation (45), and the decreased *Firmicutes/Bacteroidetes* ratio in our study may be related to factors such as

dietary modifications. However, Sun et al. (17) found a phenomenon that with advancing gestation, decreasing trends in the *Firmicutes/Bacteroides* ratio were observed in the control group but not in the GDM group. In addition, they also found that time-dependent alterations in gut microbiota composition were found in the control group but not in the GDM group. Compared to women with normal glucose, women with GDM tended to have a reduced intestinal microbiota diversity in the first trimester, while differences in intestinal microbiota composition were consistent in T2 and T3. Our research does not observe the composition of the gut microbiota in the first trimester and our study also observed the stable composition of the gut microbial in T2 and T3 in women with GDM. Women who develop GDM may have alterations in intestinal microbial composition from early pregnancy, explained by metabolic status. *Bacteroides*, a Gram-negative bacterium, can produce large amounts of LPS, leading to inflammation. LPS mainly activates inflammation via the Toll-like receptor 4 signaling pathway (46). From the first to the third trimester, women gain adiposity and have higher circulating levels of insulin (9). In women with GDM, two main inflammatory pathways, nuclear factor kappa B (NF-kB) and signal transducers and activators of the transcription 3 (STAT3) pathways, have been identified (13). The findings of this study provide evidence to explain the stable status of GDM.

In this study, the Shannon and Simpson indices of the GDM and healthy pregnancy groups both decreased from T2 to T3; however, the Shannon and Simpson indices of the GDM women were significantly higher than those of pregnant women with normal glucose tolerance. Our study was consistent with previous researches, showing the decreased microbial diversity with advancing gestation (9). This phenomenon might be due to the metabolic modifications occurring pregnancy, including changes of blood glucose and hormone. Higher α diversity values were associated with a lower incidence of type 2 diabetes, which was not affected by energy intake, exercise, education, smoking, or medication (47). Insulin resistance and elevated blood glucose levels can increase the risk of type 2 diabetes (48). With increasing gestational age, the level of insulin resistance increases to meet the nutritional supply of the mother and child (49). A lower Shannon index significantly correlated with blood glucose levels in patients with diabetes (19). The high Shannon and Simpson indices of the GDM group in this study could be explained by the inherent differences between the GDM and control groups. According to previous studies, β diversity is associated with insulin resistance and plasma OGTT levels (19, 47). Different methods to investigate beta diversity can influence the results. Unweighted UniFrac is sensitive to the absence and presence of low abundant bacteria, while both weighted Unifrac and Bray Cruits are more sensitive to the more abundant bacteria. In our study, unweighted Unifrac is used to investigate beta diversity. More methods should be used to claim beta diversity.

Our study explored the alterations of gut microbiota with the increasing gestational age in women with GDM and pregnant women with normal glucose tolerance. So far, few studies have explored the changes of intestinal microbiota composition in women with GDM during different trimesters. The longitudinal study will contribute to the understanding of the association between gut microbiota and GDM and provide the thinking way to predict the occurrence of GDM during early pregnancy. There are also some limitations in our study. First, this study was conducted at a single center with a limited sample size, and larger studies are needed in the future to verify the results of the study. Second, our study is an observational study and data may lack causality. There need more randomized control tests to research the association of dynamic gut microbiota composition between different trimesters in women with GDM. Third, lifestyle management is the first-line treatment for GDM but the diet patterns of the participants in this study were lack.

Conclusion

Our study indicated that the composition of the gut microbiota was stable with advancing gestation in women with GDM compared with the control group and gut microbiota composition was obviously different between women with GDM and controls. These findings may help explore the etiology of GDM from new perspective of the relationship between gut microbiota and glucose metabolism.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: [https://www.ncbi.nlm.nih.gov//PRJNA937449].

Ethics statement

The studies involving human participants were reviewed and approved by Peking Union Medical College Hospital (PUMCH). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study design: YS and LM. Sample and data collection: SZ and HL. Analysis and interpretation of data: NL and YW. Drafting the manuscript: NL. Critical revision of the manuscript for important intellectual content: YS. Statistical analysis: NL and YW. Obtained funding: LM. Study Supervision: YS and LM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1126572/full#supplementary-material

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