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Association between gut microbiota and prediabetes in people living with HIV

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ABSTRACT

The prevalence of prediabetes is rapidly increasing in general population and in people living with HIV (PLWH). Gut microbiota play an important role in human health, and dysbiosis is associated with metabolic disorders and HIV infection. Here, we aimed to evaluate the association between gut microbiota and prediabetes in PLWH. A cross-sectional study enrolled 40 PLWH who were receiving antiretroviral therapy and had an undetectable plasma viral load. Twenty participants had prediabetes, and 20 were normoglycemic. Fecal samples were collected from all participants. The gut microbiome profiles were analyzed using 16S rRNA sequencing. Alpha-diversity was significantly lower in PLWH with prediabetes than in those with normoglycemia ($p<0.05$). A significant difference in beta-diversity was observed between PLWH with prediabetes and PLWH with normoglycemia ($p<0.05$). Relative abundances of two genera in *Firmicutes* (*Streptococcus* and *Anaerostignum*) were significantly higher in the prediabetes group. In contrast, relative abundances of 13 genera (e.g., *Akkermansia* spp., *Christensenellaceae* R7 group) were significantly higher in the normoglycemic group. In conclusion, the diversity of gut microbiota composition decreased in PLWH with prediabetes. The abundances of 15 bacterial taxa in the genus level differed between PLWH with prediabetes and those with normoglycemia. Further studies on the effect of these taxa on glucose metabolism are warranted.

1. Introduction

Type 2 diabetes mellitus (T2DM) prevalence is increasing and has led to higher rates of diabetes-related morbidity and mortality in adults worldwide. In Thailand, T2DM prevalence was 8.3% in 2020, increasing from 7.5% in 2009 (Aekplakorn et al., 2011). Prediabetes, a state of abnormal glucose homeostasis with blood glucose levels not yet reaching the diabetes diagnosis criteria, is associated with diabetes complications, including early nephropathy, sensory neuropathy, retinopathy, and cardiovascular diseases (Brunner et al., 2006; Nathan et al., 2007; Plantinga et al., 2010; Sumner et al., 2003; Xu et al., 2009). Prediabetes prevalence is also rapidly increasing worldwide, and up to 10% of people with prediabetes progress to T2DM yearly (Tabák et al., 2012).

Because of the increased access to antiretroviral therapies (ART) for people living with HIV (PLWH), a significant reduction in acquired immunodeficiency syndrome (AIDS)-associated morbidity and extension of the predicted lifespan have been observed (Palella et al., 1998). Nonetheless, the non-AIDS events have become an increasing burden. ART, HIV itself, and the aging process increase the risk of non-communicable diseases, including insulin resistance, hypertension, metabolic disorders, and cardiovascular diseases (Aekplakorn et al., 2011; Chantrathamachart et al., 2006; Prioreschi et al., 2017). Compared to the general population, prediabetes and T2DM in PLWH are more prevalent (Brown et al., 2005; Phuphuakrat et al., 2020; Srivanich et al., 2010).

Gut microbiota, as intestinal microorganisms (bacteria, archaea, viruses, and eukaryotic microbes), play an essential role in human health by influencing cellular metabolism, immune regulation, and the inflammatory process (Feng et al., 2018; Valdes et al., 2018). Gut dysbiosis or adverse change in microbiome contributes to insulin resistance,

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Table 1
Characteristics of participants with normoglycemia and prediabetes

Characteristics	Normoglycemia (N=20)	Prediabetes (N=20)	P-value
Demographic and anthropometric parameters			
Age (years)	51.8±6.6	50.9±5.6	0.625
Male, N (%)	13 (65.0)	13 (65.0)	>0.999
History of smoking, N (%)	10 (50.0)	7 (35.0)	0.337
History of alcohol drinking, N (%)	16 (80.0)	12 (60.0)	0.168
Underlying diseases, N (%)			
Hypertension	4 (20.0)	3 (15.0)	0.677
Dyslipidemia	9 (45.0)	7 (35.0)	0.519
NAFLD	0 (0.0)	1 (5.0)	0.311
Body weight (kg)	62.7±12.8	63.7±12.4	0.814
Body mass index (kg/m^2)	23.1±4.6	24.1±4.0	0.501
SBP (mmHg)	130.5±15.5	129.5±14.5	0.842
DBP (mmHg)	81.3±8.1	82.1±10.6	0.790
Waist circumference (cm)	84.8±10.8	85.4±10.7	0.878
Waist hip circumference ratio	0.91±0.07	0.91±0.06	0.825
HIV-related parameters			
Duration of HIV infection (years)	15.2±6.1	14.4±5.4	0.682
Type of ART regimen, N (%)			
NNRTI-based	13 (65.0)	13 (65.0)	>0.999
PI-based	7 (35.0)	7 (35.0)	>0.999
Duration of ART (years)	11.5±6.1	11.9±5.1	0.834
CD4 cell counts (cells/mm^3)	559.8±264.0	475.0±204.5	0.263
Biochemical parameters			
FPG (mg/dL)	91.8 ±7.9	101.4±13.6	0.009
2h PG (mg/dL)	113.5±37.0	138.7±37.3	0.041
HbA1c (%)	5.32±0.21	5.99±0.22	<0.001
Triglycerides (mg/dL)	130.5 (81.8 – 185.3)	134.0 (99.5 – 198.5)	0.379
HDL cholesterol (mg/dL)	51.2±11.6	46.2±11.4	0.181
LDL cholesterol (mg/dL)	141.3±36.0	130.0±33.4	0.308
Total cholesterol (mg/dL)	219.0±42.0	203.4±36.3	0.215

Data were presented as mean±SD or median (interquartile range).
2h PG = 2-h plasma glucose; ART = antiretroviral therapy; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HbA1c = hemoglobin A1c; HDL = high-density lipoprotein; LDL= low-density lipoprotein; NAFLD = non-alcoholic fatty liver disease; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; SBP = systolic blood pressure

T2DM, obesity, inflammatory bowel diseases, autoimmunity, and carcinogenesis (Sommer et al., 2017). The composition of the gut microbiota differs between people with and without T2DM (Sedighi et al., 2017). Changes in the composition of the gut microbiota play a role in glucose metabolism by alterations in systemic lipopolysaccharide concentrations, bile acid metabolism, short-chain fatty acid production, gut hormone secretion, and circulating branched-chain amino acids (Utzschneider et al., 2016). Likewise, the composition of the gut microbiota and metabolites is altered in PLWH with T2DM as compared to those without T2DM (Moon et al., 2018). Moreover, dietary intervention and physical exercise as well as HIV infection can change the composition of gut microbiota and their metabolism (Chen et al., 2018; Zhao et al., 2018).

A few studies (Hoel et al., 2018; Moon et al., 2018) have investigated the association between gut microbiota and T2DM in PLWH. The data on gut microbiota in PLWH with prediabetes are markedly limited, and the differences of gut microbiota in PLWH with normoglycemia and those with prediabetes are not well understood. The purpose of this study was to evaluate the association between gut microbiota and the prediabetes status in PLWH.

2. Materials and methods

2.1. Study participants and design

This cross-sectional study was conducted at an infectious disease clinic in Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. We included PLWH from a previous cross-sectional study for the prevalence of prediabetes among PLWH (Phuphuakrat et al., 2020), aged 20 years or older, and willing to participate in the study. PLWH using glucose-lowering medications, having a history of diabetes, and those who were pregnant were excluded. Patients were screened for prediabetes and consecutively enrolled with a goal of 20 participants with prediabetes (prediabetes group) and 20 participants with normoglycemia (normoglycemia group). All participants had received ART for more than 6 months, had HIV viral loads less than 50 copies/mL, and had CD4 counts more than 200 cells/mm^3. Their medical histories were retrieved. Anthropometric parameters were measured by physicians, and all clinical samples, including feces and blood, were collected under standard techniques. The protocol was approved by the Institutional Review Board, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (COA. MURA2020/1203). Written informed consent was obtained from each participant. All methods were performed in accordance with the relevant guidelines and regulations.

2.2. Measurement and laboratory determinations

2.2.1. Prediabetes

According to the American Diabetes Association (ADA) Standards of Medical Care in Diabetes-2019, prediabetes was defined as fasting plasma glucose (FPG) levels of 100-125 mg/dL or 2-h plasma glucose (2h PG) levels 140-199 mg/dL during a 75-g oral glucose tolerance test (OGTT) or hemoglobin A1c (HbA1c) 5.7- <6.5% (American Diabetes Association, 2019). All participants had fasted at least 12 h before the test. At baseline, blood samples were collected for FPG and HbA1c. After a 75-g glucose solution was taken, blood samples were collected at 120 min for 2h PG.

2.2.2. Fecal sample collection

Fecal samples were self-collected by participants using a clean disposable spatula and a plastic container over the toilet seat. Participants washed their hands and cleaned the perianal area before sample collection. Fecal samples were immediately refrigerated at -80°C.

2.2.3. RNA extraction and high-throughput sequencing

Genome DNA was extracted with the QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) following the manufacturers' instructions. Polymerase chain reaction (PCR) amplification was performed on the 16S rRNA gene using 341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAGCCTACGGGNGGCWGCAG) and 805R (GTCTCGTGGGCTCGGA-GATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) primers, targeting the V3-V4 variable regions and sparQ HiFi PCR master mix (Quanta bio, Beverly, MA, USA). The amplification condition consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles of 98°C for 20 s, 55°C for 30 s, 72°C for 30 s, and a single final extension step at 72°C for 5 min. Additionally, an internal transcribed spacer of nuclear ribosomal DNA amplification was performed using the primers ITS-1F and ITS-2R (Gardes and Bruns, 1993) with PCR conditions as follows: an initial denaturation step at 94°C for 3 min, followed by 25 cycles of 98°C for 20 s, 60°C for 30 s, 72°C for 30 s, and a single final extension step at 72°C for 5 min. Subsequently, both metagenomic marker amplicons were purified using AMPure XP beads and indexed using 5 µl of each Nextera XT index primer (Illumina, San Diego, CA, USA) in a 50-µl PCR reaction, followed by 8-10 cycles of PCR conditions, as above. The final PCR products were cleaned, pooled, and diluted to the final loading concentration at 6 pM. Cluster generation and 250-bp

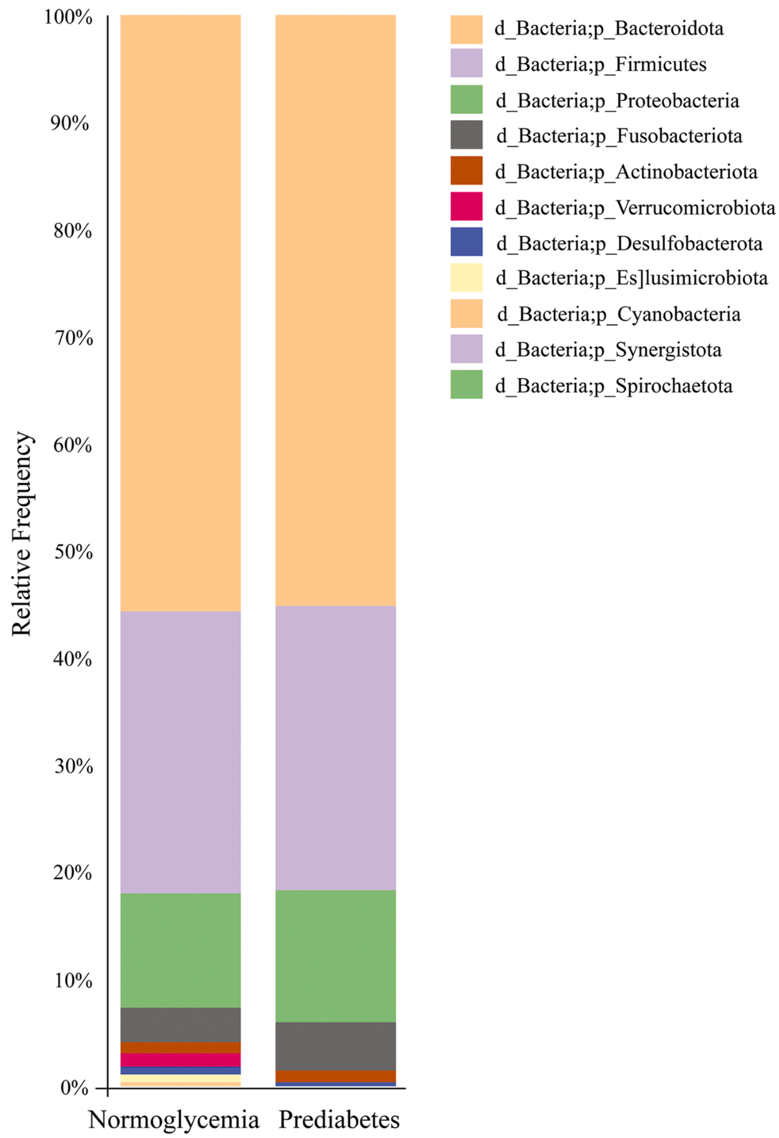

Fig. 1. Taxonomic profile at the phylum level between participants with prediabetes and normoglycemia

paired-end read sequencing were performed on an Illumina MiSeq (Illumina).

2.2.4. Bioinformatics analysis

Microbiome bioinformatics was performed with QIIME 2 2019.10 (Bolyen and Rideout, 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (via q2-dada2) (Callahan et al., 2016). A phylogeny tree was constructed using the SEPP q2-plugin, placing short sequences into a sepp-refs-gg-13-8.qza phylogenetic reference tree for the 16S marker gene (Janssen et al., 2018). Alpha-diversity metrics [Shannon diversity, Faith's Phylogenetic Diversity (Faith, 1992), and observed operational taxonomic units (OTUs)], beta-diversity metrics (weighted UniFrac, unweighted UniFrac, Jaccard distance, and Bray-Curtis dissimilarity) (Lozupone and Knight, 2005; Lozupone et al., 2007), and Principle Co-ordinate Analysis (PCoA) were performed using q2-diversity after samples were rarefied (subsampled without replacement) to the minimum number of sequences. Taxonomy was assigned to ASVs using the q2-feature-classifier (Bokulich et al., 2018) to classify sklearn naive Bayes taxonomy classifier against the SILVA database (Quast et al., 2013).

2.3. Statistical methods

Baseline demographics of the participants are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)] for continuous variables and as frequency (%) for binary or categorical variables. Chi-square tests were used to analyze categorical variables. Student's t-test was used to compare means, and the Mann–Whitney U test was used to compare medians between the prediabetes and normoglycemic groups, depending on the data distribution. The alpha-diversity of microbiota in OTU levels between the prediabetes and normoglycemic groups was determined using four measures: Shannon diversity, Faith's phylogenetic diversity, observed OTUs richness, and Evenness and presented in box-and-whisker plots. The dissimilarity of microbial community compositions (beta-diversity) between participants in the prediabetes and normoglycemic groups were estimated using weighted UniFrac, unweighted UniFrac, Jaccard distance, and Bray-Curtis dissimilarity. Alpha and beta diversity were analyzed using Kruskal-Wallis and PerMANNOVA (number of permutations=999), respectively. Significantly differential taxa abundances between condition groups were tested using linear discriminant analysis (LDA) effect size (LEfSe). Statistical significance was considered as p-value <0.05, and all reported probability tests were two-sided. Statistical analysis was

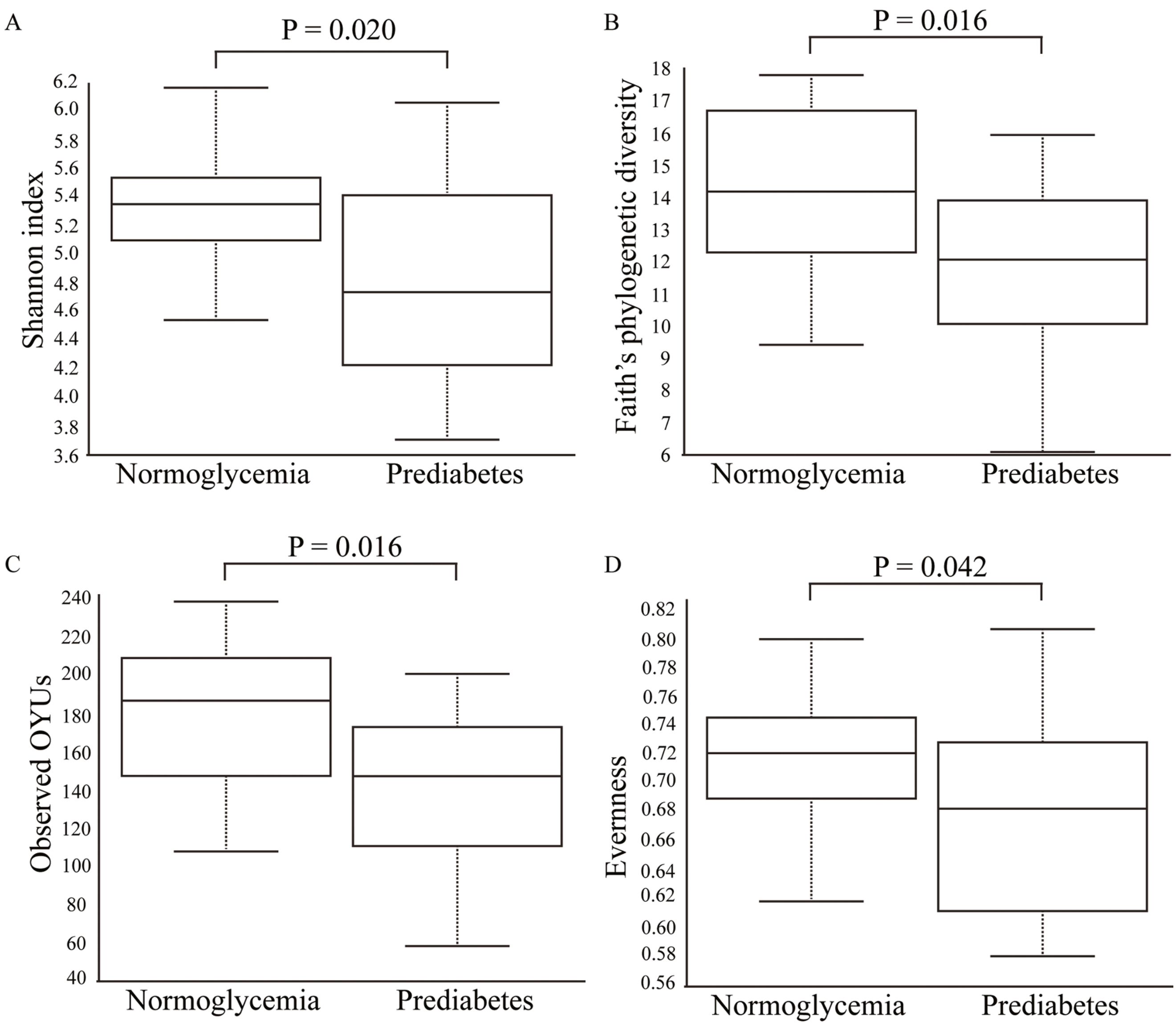

Fig. 2. Alpha diversity of microbial composition in participants with normoglycemia and prediabetes measured by (a) Shannon index, (b) Faith's phylogenetic diversity, (c) observed OTUs, and (d) Evenness Box-and-whisker plots represented median and IQR.

conducted using SPSS statistical software package, version 18.0 (SPSS, Chicago, IL, USA).

3. Results

Of the 40 PLWH, 20 participants were in the prediabetes group and 20 participants were in the normoglycemia group. The mean age was 51.3 ± 6.0 years, and 65% of the participants were male. The duration of HIV infection and ART was 14.8 ± 5.7 and 11.7 ± 5.5 years, respectively. As expected, FPG, 2h PG, and HbA1c levels were significantly higher in the prediabetes group than in the normoglycemia group. Demographic, anthropometric, HIV-related, and other biochemical parameters did not significantly differ between the two groups (Table 1).

Mean relative abundance of fecal samples showed that *Bacteroidota* was the most abundant at the phylum level in both groups. This was followed by *Firmicutes, Proteobacteria*, and *Fusobacteriota* in both groups. However, *Elusimicrobiota, Synergistota*, and *Spirochaetota* were not found in the prediabetes group (Fig. 1 and Supplementary Table 1).

Alpha-diversity (within-sample microbial diversity for each measurement) was significantly lower in the prediabetes group than in the normoglycemia group: Shannon index (p=0.020), Faith's phylogenetic diversity (p=0.016), observed OTUs richness (p=0.016), and Evenness (p=0.042) (Fig. 2). Beta-diversity (similarity or dissimilarity between the two groups) in the fecal microbiome was evaluated by unweighted UniFrac at OTU levels. Principal coordinates analysis illustrated the clustering of fecal samples between gut microbiomes of the prediabetes group and the normoglycemia group (Fig. 3a). PerMANOVA showed a significant difference between samples obtained from the prediabetic group and the normoglycemia group by unweighted UniFrac (p=0.001) (Fig. 3b), but no significant difference between the two groups by Bray-Curtis (p=0.315), Jaccard (p=0.065), and weighted UniFrac (p=0.584) (Supplementary Fig. 1).

Differential analysis in taxa between fecal microbiome between the prediabetes and normoglycemia groups was conducted by LDA effect size (LEfSe) (Fig. 4). At the genus level, the differential abundance analysis demonstrated 15 genera were associated with prediabetes in the PLWH (Supplementary Table 2). Two genera in *Firmicutes* (*Streptococcus* and *Anaerostignum*) became significantly more abundant in the prediabetes group than in the normoglycemia group. Interestingly, *Firmicutes*, together with *Bacteroidota, Cyanobacteria, Desulfobacerota, and Verrucomicrobiota* were significantly more abundant in the normoglycemia group than in the prediabetes group. *Akkermansia, Gastranaerophilales,*

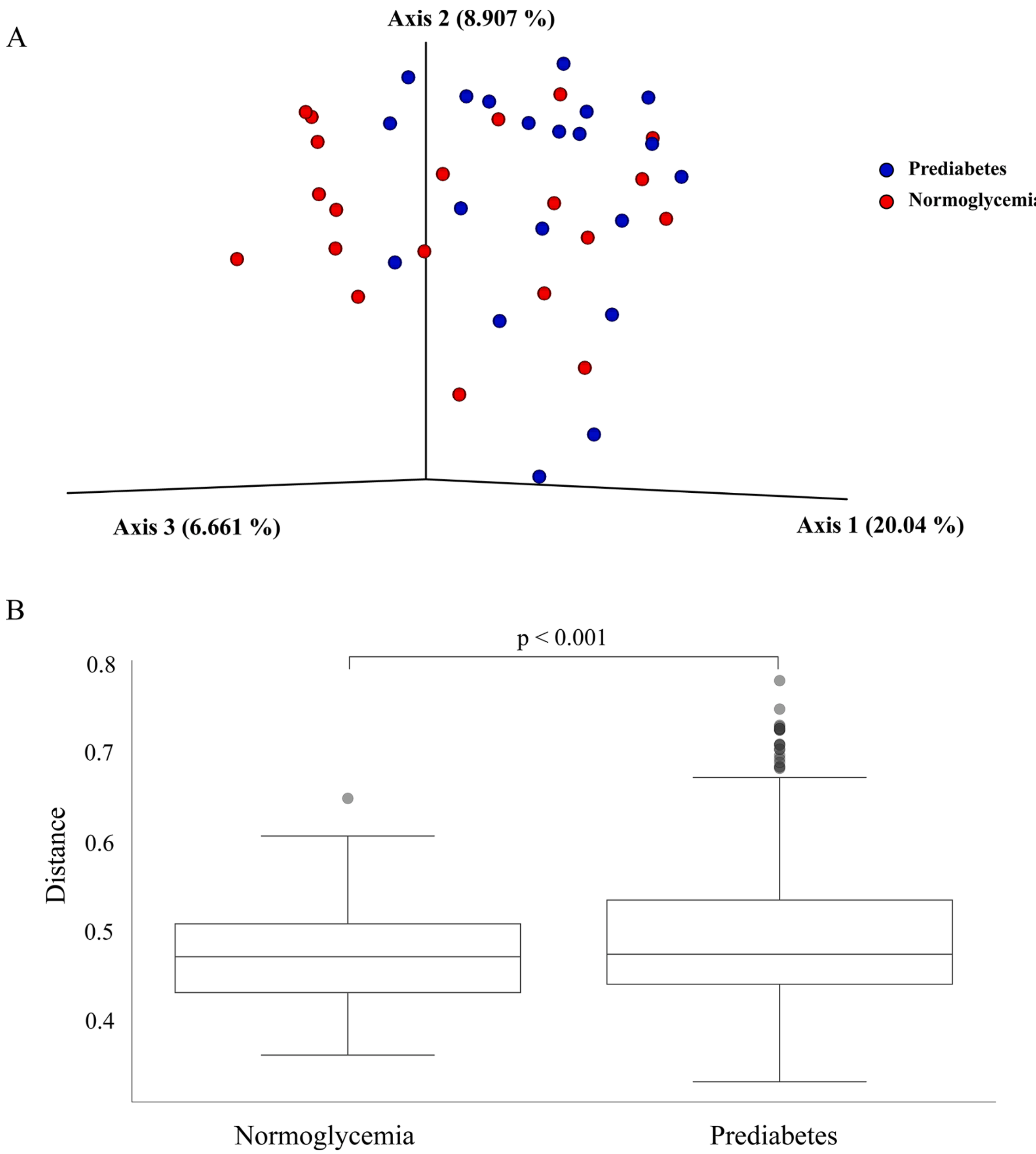

Figure 3. Beta-diversity of microbial composition in participants with normoglycemia and prediabetes by (a) principal coordinates analysis (PCoA) of unweighted UniFrac and (b) perMANOVA-observed differences of unweighted UniFrac.

Desulfovibrio, Butyricimonas, Colidextribacter, Christensenellaceae R 7 group, *Victivallis*, Uncultured *Bacteroidota*, Uncultured phylum *Firmicutes, Holdemanella*, UCG-005, *Eubacterium ruminantium* group, and family *Oscillospiraceae*-associated group were more abundant in the normoglycemia group.

4. Discussion

Gut microbiota has been known to be associated with various metabolic syndromes, especially T2DM (Wu et al., 2020). Nonetheless, this association has not been well established in prediabetes, particularly among PLWH. This cross-sectional study evaluated the association between gut microbiota and prediabetes in PLWH. We found that both the diversity and composition of microbiomes between PLWH with prediabetes and those with normoglycemia were significantly dissimilar. Compared to PLWH with normoglycemia, those with prediabetes had less genus and diversity of gut microbiomes. Additionally, the percentages of abundance were higher in two particular genera and lower in 13 other genera among PLWH with prediabetes.

We have demonstrated that alpha diversity and beta diversity were significantly different between PLWH with prediabetes and those with normoglycemia. The alpha diversity was significantly lower in PLWH with prediabetes. In the general population, the diversity of gut microbiota composition was changed in individuals with hyperglycemia (Larsen et al., 2010). Previous works reported that the alpha diversity of gut microbiota was lower in patients with T2DM and prediabetes (Lambeth et al., 2015; Li et al., 2020). However, alpha diversity was not significantly different in patients with T2DM and without diabetes in a study conducted in Mexican Americans (Kitten et al., 2021). Another study showed a decreased alpha diversity in patients with newly diagnosed diabetes, but not in those with prediabetes, when compared with those without diabetes (Gaike et al., 2020). The diversity of gut microbiota composition could be affected by multiple factors (Lozupone et al., 2012), including the types of diet and health status (Senghor et al.,

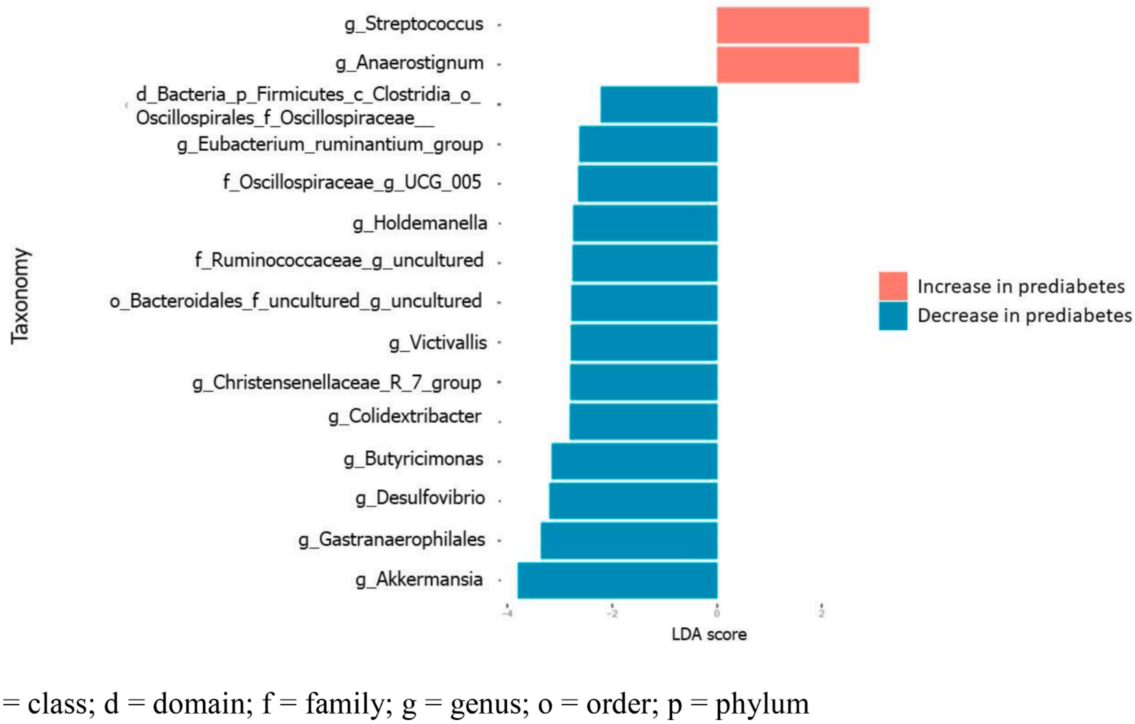

Fig. 4. Differentially abundant bacterial taxa of participants with prediabetes and normoglycemia illustrated by linear discriminant analysis (LDA) effect size (LEfSe) plot c = class; d = domain; f = family; g = genus; o = order; p = phylum

2018). Regarding the diversity of microbiota composition in PLWH, a previous study in women with or at high risk for HIV infection showed no significant differences in the diversity of microbial communities between those with and without diabetes; nonetheless, relative abundances of genus *Finegoldia, Anaerococcus, Sneathia*, and *Adlercreutzia* were decreased in those with diabetes (Moon et al., 2018).

Our study revealed that *Akkermansia* spp. was significantly reduced in PLWH with prediabetes. This finding is consistent with several previous studies showing that *Akkermansia* spp. could mainly contribute to reducing the risk of diabetes and other metabolic syndromes (Ouyang et al., 2020b; Xu et al., 2020; Zhou et al., 2021). A purified membrane protein of *Akkermansia muciniphila* has been shown to reduce the expression of hepatic flavin monooxygenase 3 (Fmo3) (Plovier et al., 2017). A knockout of this gene prevented the development of hyperglycemia in the mouse model (Miao et al., 2015). Regarding glucose metabolism, *Akkermansia muciniphila* increases thermogenesis by induction of uncoupling protein 1 in brown adipose tissue and regulates appetite by stimulating L-cells (enteroendocrine cells) to release glucagon-like peptide-1 (GLP-1). However, the data of bioactive molecules involving GLP-1 secretion are lacking (Derrien et al., 2017; Yoon et al., 2021). *Akkermansia muciniphila* also played a role in maintaining a healthy gut barrier and reducing inflammation in mice (Schneeberger et al., 2015). The increased gut permeability increased gram-negative bacteria-derived lipopolysaccharide leakage into the systemic circulation with subsequent inflammation and metabolic dysfunction, including insulin resistance (Utzschneider et al., 2016). This may emphasize that *Akkermansia* spp. can be a potential probiotic for T2DM. Metformin was also shown to increase the abundance of *Akkermansia muciniphila* in PLWH (Isnard et al., 2020; Ouyang et al., 2020a), thus metformin might be a potential treatment for modifying the progression to diabetes in PLWH.

In addition to *Akkermansi*a, our findings revealed the significantly reduced abundance of *Christensenellaceae* in PLWH with prediabetes compared to those with normoglycemia. It has been assumed that the appropriate abundance of *Christensenellaceae* can improve metabolic syndrome. The reduction in *Christensenellaceae* abundance was observed in prediabetes individuals (He et al., 2018), while the normal abundance of *Christensenellaceae* was associated with healthy glucose metabolism (Lippert et al., 2017). Furthermore, *Christensenellaceae* was enriched following healthy lifestyle behavior, including regular consumption of fruits and vegetables (Bowyer et al., 2018; Klimenko et al., 2018). This change could also be observed when feeding rodents with dietary fiber (Ferrario et al., 2017). Interestingly, *Christensenellaceae* significantly increased in normal body mass index (BMI) (18.5-24.9 kg/m^2) individuals as compared to people with obesity (BMI >30 kg/m^2) (Goodrich et al., 2014; Waters and Ley, 2019). A clinical trial to improve metabolic syndrome using *Christensenellaceae* has been conducted (clinical trial.gov: NCT04663139). However, our findings did not show a difference in BMI and other body component analysis between the prediabetes and normoglycemia groups. It remains unclear how *Christensenellaceae* is involved in the hyperglycemic status. One possible mechanism is that *Christensenellaceae* can produce short-chain fatty acids, which are known to reduce the risk of diabetes by various mechanisms, for example, increased insulin sensitivity and suppression of appetite (Lau and Vaziri, 2019; Waters and Ley, 2019; Zhou et al., 2021). Additionally, inflammation has been considered one of the causes of both type 1 and type 2 diabetes (Tsalamandris et al., 2019). An *in vitro* study showed that the supernatant obtained from *Christensenellaceae* culture can maintain the integrity of intestinal epithelia and suppress inflammatory response (Kropp et al., 2021). Taken together, this suggests that a decreased abundance of *Christensenellaceae* may lead to a hyperglycemic/prediabetic status.

Our study in PLWH revealed a decrease in the abundance of *Akkermansia* and an increase in the abundance of *Streptococcus* in those with prediabetes. A recent systematic review on bacteria involved in T2DM reported that the genera of *Ruminococcus, Fusobacterium,* and *Blautia* were positively associated with T2DM, while the genera of *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia,* and *Roseburia* were negatively associated with T2DM (M et al., 2020). Another case-control study showed a decreased abundance of the genus *Clostridium*, but an increased abundance of *Dorea (Ruminococcus), Suterella,* and *Streptococcus* in prediabetics as compared to age- and sex-matched normoglycemic persons (Allin et al., 2018). A damage of gut epithelial barrier in PLWH is a potential factor of microbial translocation and inflammation in PLWH (Ellis et al., 2021). This might contribute to the different abundance of bacterial taxa between PLWH and the general population.

To the best of our knowledge, the present study is the first to reveal the association between gut dysbiosis and prediabetes in PLWH. None of the participants were receiving diabetic treatment that could have affected the results. The study was conducted under a well-designed protocol and standard technique. Nevertheless, our study has some limitations that should be considered when interpreting these results: (1) the sample size is relatively modest; (2) since the study design is a cross-sectional study, the causal relationship between gut microbiota and prediabetes in PLWH cannot be evaluated directly; (3) as the participants are only PLWH who received ART with an undetectable plasma viral load, we might not be able to apply the results to the ART-naïve PLWH or those without successful ART; and (4) some potential factors such as route of HIV infection, sex preferences, other sexually transmitted infections, history of antibiotics and antacid usage, and dietary intakes can affect gut microbiota change; nonetheless, we did not include these factors as covariates in our study data.

In conclusion, our study demonstrated the association between gut microbiota and prediabetes in PLWH receiving ART with an undetectable plasma viral load. Diversity of gut microbiota composition decreased in PLWH with prediabetes. The abundances of *Akkermansia* spp. and *Christensenellaceae* R 7 group were also decreased in PLWH with prediabetes. We also found that PLWH with prediabetes had increased abundances of two genera in *Firmicutes* (*Streptococcus* and *Anaerostignum*). Further studies on the mechanism that contributed to the development of dysglycemia by these two genera in PLWH are warranted.

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CRediT authorship contribution statement

KJ, AP, SR, SS conceived and designed the study. SS obtained funding. KJ, AP, PPo, PPr analyzed and interpreted the data. HN, SS contributed to data collection. KJ, AP, PPo drafted the manuscript. HN, SR, SS revised the manuscript. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript before submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2022.100143.

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