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Examining the composition of gut microbiota in a South African population: a comparative study between type 2 diabetes mellitus patients and non-diabetic individuals

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Abstract

Background

Literature has highlighted the gut microbiota's role in metabolic functions, suggesting a potential link between gut microbiota composition and T2DM. The purpose of the study was to identify microbial signatures unique to T2DM patients and non-diabetic individuals, compare microbial profiles between the two groups and investigate how gut microbiota may be related to inflammation associated with T2DM.

Methods

A cross-sectional study was conducted involving 51 T2DM patients and 99 non-diabetic South African individuals. Faecal samples were collected and analysed using 16S rRNA gene sequencing to characterize the gut microbiota. Blood samples were obtained to perform

HbA1c, CRP and ferritin tests. Bioinformatic and statistical analyses were performed to identify differences in microbial composition and diversity between the two groups.

Results

The gut microbiota in T2DM patients was predominantly composed of Firmicutes (47.7%), Bacteroidota (37.5%), and Proteobacteria (11.4%), while the non-diabetic group showed a slightly different microbial profile with higher Bacteroidota (41.9%) and a notable presence of Actinobacteriota (4.5%). Abundant families in the T2DM group included Bacteroidaceae (22.8%), Prevotellaceae (7.4%), Enterobacteriaceae (7.4%), Erysipelotrichaceae (6.0%) and Lachnospiraceae (5.2%). The non-diabetic group exhibited dominant families such as Lachnospiraceae 26.7%, Prevotellaceae (25.3%), Bacteroidaceae (12.7%), Ruminococcaceae (9.5%) and Oscillospiraceae (3.8%). At the genus level, Bacteroides (22.8%), Escherichia-Shigella (5.0%), Holdemanella (4.8%), Phascolarctobacterium (3.2%) and Blautia (2.8%) were prevalent in the T2DM group, while Prevotella_9 (22.1%), Bacteroides (12.7%), Agathobacter (6.7%), Blautia (6.3%) and Faecalibacterium (5.1%) were dominant in the non-diabetic group. Differential abundance testing revealed 5 phyla, 16 families, and 25 genera that were either enriched/depleted in T2DM patients relative to non-diabetic individuals. The comparison of alpha diversity metrics between the two groups revealed significant differences across all four measures ($P < 0.001$), with non-diabetic individuals showing higher values than T2DM patients. HbA1c and CRP levels showed mostly slight/ moderate correlations with the relative abundance of various gut microbes at various phyla, family, and genus levels, as well as with all alpha diversity metrics.

Conclusion

The study revealed distinct differences in gut microbiota composition between T2DM patients and non-diabetic individuals, with T2DM patients showing a higher prevalence of certain phyla, families, and genera linked to metabolic dysregulation. Non-diabetic individuals displayed greater microbial diversity and beneficial taxa, highlighting a potential protective microbial profile.

Keywords: Gut microbiota; Gut microbiome; Intestinal flora; Dysbiosis; Type 2 diabetes mellitus; Inflammation

1. Introduction

The human gastrointestinal tract is an intricate ecosystem inhabited by trillions of microorganisms collectively known as the gut microbiota [1]. The complex relationship between human health and the gut microbiota has attracted remarkable attention in recent years, as emerging research explains the important role these microbial communities play in metabolic and immune processes [2]. Among numerous health conditions influenced by gut microbiota, type 2 diabetes mellitus (T2DM) stands as an alarming global health challenge [3], affecting approximately 530 million individuals [4] and posing a substantial burden on healthcare systems worldwide [5].

The burden is growing rapidly in Sub-Saharan Africa, which is projected to experience the largest relative increase globally, estimated at 129% by 2045 [4]. The rising prevalence of T2DM in South Africa is a growing concern, particularly in the context of rapid urbanisation, changing dietary patterns, and genetic diversity [6]. These factors highlight the need to better understand what drives the development and progression of T2DM. One area that has received increasing attention is the role of gut microbiota in the development and progression of T2DM, particularly through its influence on glucose metabolism and systemic inflammation.

The gut microbiota plays an important role in regulating energy balance, gut integrity, and glucose metabolism [7]. Dysbiosis, characterised by reduced microbial diversity, loss of beneficial bacteria, and an increase in harmful microbes, can disrupt these metabolic processes [8]. Specifically, this can increase intestinal permeability, allowing bacterial products to enter the systemic circulation [9]. These products, including lipopolysaccharides (LPS), can activate toll-like receptors and trigger an inflammatory response [10]. Consequently, persistently elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and CRP have been observed in patients with T2DM [11] . This chronic inflammation is closely associated with insulin resistance [12] impaired glucose metabolism [13] and pancreatic β-cell dysfunction [14].

Beneficial gut bacteria, including *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Eubacterium hallii*, ferment indigestible carbohydrates to produce short-chain fatty acids (SCFAs), which have anti-inflammatory and immunomodulatory effects [15,16]. In T2DM, reduced levels of these bacteria and an increase in harmful bacteria may lower SCFA production [17]. This may affect intestinal immune balance and interfere with GLP-1 and PYY signalling through GPR41/GPR43 receptors [7].

These mechanisms are supported by several clinical studies reporting significant differences in gut microbial profiles between individuals with T2DM and non-diabetic controls [18–21]. Despite growing research on the role of the gut microbiome in metabolic disease, evidence from Africa remains limited [21,22]. Gut microbiota composition is influenced by genetics, ethnicity, diet, and environment, hence findings from high-income countries may not directly apply to African populations.

This study therefore aimed to compare gut microbiota composition between South African adults with T2DM and non-diabetic individuals to identify microbial patterns linked to T2DM and better understand its pathophysiology. Furthermore, we also examined the relationship between inflammatory markers (C-reactive protein- CRP and ferritin) and gut microbiota profiles in this population. Identifying microbial patterns associated with these markers will help identify inflammatory microbial signatures.

To our knowledge, this is the first study to characterise the gut microbiota within a South African population. The findings have the potential to contribute significantly to the global understanding of the role of gut microbiota in metabolic disorders and offer crucial insights into the specific microbial determinants of T2DM. Ultimately, mapping distinct microbial profiles will provide a framework for developing targeted population-specific interventions and personalized management strategies for patients with T2DM.

2. Materials and Methods

Sample collection

Blood and stool samples were obtained from all the participants. Samples from patients with diabetes (*n*= 51) were collected at the diabetic clinic of DGMAH. Samples from non-diabetic individuals (*n*= 99) were collected at the Sefako Makgatho Health Sciences University's Chemical Pathology Department. Although the samples were collected in batches, the same sample collection protocol was adhered to for all participants in the study. Blood was drawn using EDTA and BD Vacutainer™ venous blood collection tubes, specifically SST™ Serum Separation tubes, in both groups. Stool samples were obtained using OMNIgene•GUT OM-200 kits (DNA Genotek Inc., Canada), as per the manufacturer's instructions. Specimens did not have to be frozen after collection and while being transported to Inqaba Biotechnical Industries (Pty) Ltd laboratory for 16S rRNA gene sequencing analysis. This is because the

collection kits included a solution with a chelating agent to effectively stabilise DNA present in faecal samples, making collection, prolonged storage, and transportation at ambient temperature possible.

Diabetic patients who could not provide stool samples at their clinic visit were supplied with faecal collection kits and guided to collect them at their convenience in their homes. The researchers used the provided home addresses to trace the patients and collected the samples once the patients signalled by phone call that they had collected the stool sample. All non-diabetic participants managed to provide stool samples promptly as per the recruitment instruction to visit the department only when they were prepared to pass stools.

Data collection and biochemical tests

We collected demographic data including age, gender, location, diabetes mellitus status, physical activity, diet, type of diabetes medical treatment, antibiotic use, probiotic use, and presence of gastrointestinal disorders. Additionally, participants' whole blood samples were assessed for glycated haemoglobin (HbA1c), while serum samples were used to evaluate Ferritin and CRP levels. All biochemical assays were performed using the Atellica Solutions autoanalyser (Siemens Healthineers, Germany).

Bacterial genomic DNA extraction

Genomic DNA was extracted using ZymoBIOMICS™ DNA Miniprep kit (Zymo Research, USA) following the manufacturer’s protocol. To determine the purity and estimate the concentration of the extracted DNA, the NanoDrop Spectrophotometer (NanoDrop Technologies, USA) was used. The resulting amplicons were also checked for quality on agarose gel electrophoresis and quantified with Qubit® dsDNA HS (High Sensitivity) Assay kits.

Library preparation for amplicon sequencing

Polymerase chain reaction was performed using universal primer pair 27F and 1492R, targeting the V1 -V9 region of the bacterial 16S rRNA gene. These primers were tagged with PacBio M13 adaptor sequences on the 5’- end of the forward and reverse sequences to allow barcoding of each amplicon. Resulting amplicons were barcoded with PacBio M13 barcodes for multiplexing through limited cycle PCR. These barcoded amplicons were then quantified, pooled equimolarly, and purified using AMPure PB beads.

PacBio SMRTbell library preparation, metagenomic analysis of full length 16S gene amplicons and bioinformatics analysis

Samples were sequenced on the Sequel II system by PacBio (www.pacb.com). Raw sub-reads were processed through the SMRTlink (v11.0) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These reads were then analysed in a reproducible manner using the Nextflow [23] based nf-core community [24] pipeline ampliseq (v2.6.1, https://nf-co.re/ampliseq/2.6.1) [25] with both DADA2 (https://benjjneb.github.io/dada2/index.html) and QIIME2 v2023.7 (https://qiime2.org/) options enabled for quality control assessment, taxonomic classification as well as relative abundance analysis. Clustering, denoising and filtering was performed using DADA2 with the default parameters and truncating reads at mean quality < Q10. Cutadapt was used to remove primers sequences. The SILVA 138.1 full-length prokaryotic SSU database was used as reference for taxonomic classification. The approach also enabled the assessment of microbial community diversity (alpha and beta diversity metrics). Alpha diversity was determined using the Shannon's diversity index, Faith's PD, Pielou's evenness index and observed features. Beta diversity (microbial community dissimilarity) investigation was conducted with principal coordinate analysis (PCoA) based on the Bray-Curtis metric, weighted and unweighted UniFrac distance. Permutational multivariate analysis of variance (PERMANOVA) was conducted using distance matrices. Adonis was employed to assess the significant differences between the group with T2DM and the non-diabetic group. Furthermore, differential abundance testing was performed using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) plugin in QIIME2 v2023.7. The versions of individual software and exact parameters, as well as the taxonomic databases used in the pipeline are documented in the supplementary (Table S1) MultiQC version 1.18 report [26]. Finally, potential confounding associations between bacterial features and covariates were explored with MaAsLin2 at the lowest taxonomic level reported in this study (genus), which was most sensitive to change. The genus level counts produced by the pipeline described above were transformed to relative abundance, whereafter a compound Poisson linear model (CPLM) was applied with covariates as fixed effects.

Quality and contamination control

The ZymoBIOMICS Microbial Community DNA standard (Zymo Research, USA) served as an ideal positive mock control and ensured the quality of microbiome profiling. It allowed for benchmarking and validation of microbial composition profiling workflows and facilitated the assessment of bias in DNA isolation.

Statistical analysis

Statistical analyses were conducted using STATA statistical software. The three taxa levels (Phyla, Family and Genera) were skewed, so the Mann–Whitney U test was used to investigate differences in their abundance levels between the two groups of participants. Alpha diversity metrics (Shannon index, Faith's PD, Pielou's evenness index and Observed features) as well as Firmicutes/Bacteroidota (F/B) ratios were also compared using the same test. The observed significant values were adjusted for multiple testing. The ANCOM-BC plugin computed an internal statistic (W), utilized for generating p-values with two-sided z tests. Subsequently, these p-values were adjusted for multiple hypothesis correction. Differentially abundant taxa with adjusted p-values (q-values) < 0.05 were shown in the visualizations. In addition to employing STATA and QIIME2, the visual representations in this study were also created using RStudio (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.) and GraphPad Prism version 10.1.2. (specifically, for generating pie charts and heat maps). A binary logistic regression analysis was performed to assess the association between various taxa and alpha diversity metrics with both T2DM status and the type of T2DM treatment administered. We used the Spearman's correlation test to examine the linear association of HbA1c and inflammatory markers (CRP and ferritin) with relative abundance of predominant taxa and alpha diversity metrics. A P value of < 0.05 was regarded as statistically significant.

3. Results

The study included a total of 150 individuals, comprising 51 T2DM and 99 non-diabetic participants. Among the T2DM group, 49% of patients were on metformin as their primary antidiabetic treatment, while 25% were on insulin therapy. Furthermore, the study included 88 female participants and 59 male participants (Table 1). However, despite this variation, no significant association was found between gender and diabetes status. Age was associated with DM status, $P < 0.001$ (Table 1). Almost half (49%) of the diabetic group displayed poor glycaemic control (HbA1c > 8%) [27], whereas the non-diabetic group (83%) primarily had normal HbA1c levels [27] (Table 1). The diabetic cohort (44%) mostly presented with moderately elevated CRP levels [28], while the non-diabetic group (55%) mostly presented with normal CRP levels [28] (Table 1).

Table 1. Demographic characteristics of study participants

	Variable	T2DM patients (n = 51) *n* (%)	Non-diabetic individuals (n = 99) *n* (%	P
Gender	Male	20 (39)	39 (39)	
	Female	31 (61)	57 (58)	1.000
	Unknown	-	3 (3)	
Age [years]		57 (22)	37.5 (24)	<0.001*
Residence	Township	50 (98)	93 (94)	
	Urban	0 (0)	2 (2)	
	Rural	1 (2)	1 (1)	0.529
	Unknown	-	3 (3)	
Type of treatment	Metformin	25 (49)	-	-
	Insulin	13 (25)	-	
	Metformin + insulin	7 (14)	-	
	Unknown	6 (12)	-	
HbA1c [%]	Normal (≤5.6)	4 (8)	80 (83)	
	Pre-diabetes (5.7- 6.4)	6 (12)	13 (14)	
	Diabetes (≥6.5)	16 (31)	1 (1)	<0.001*
	Uncontrolled diabetes (>8)	25 (49)	2 (2)	
CRP (mg/L)	Normal (< 3)	11 (22)	53 (55)	
	Minor elevation (3- 10)	15 (30)	29 (30)	
	Moderate elevation (10-100)	22 (44)	14 (15)	<0.001*
	Marked elevation (100-500)	2 (4)	0 (0)	
	Severe elevation (> 500)	0 (0)	0 (0)	
Ferritin in females (ug/L)	No hyperferritinaemia (≤ 200)	24 (80)	54 (98)	
	Hyperferritinaemia (> 200)	6 (20)	1 (2)	0.009*
Ferritin in males (ug/L)	No hyperferritinaemia (≤ 400)	18 (90)	40 (98)	
	Hyperferritinaemia (> 400)	2 (10)	1 (2)	1.000

Age is reported as median and interquartile range (IQR) while the rest are reported as number of observations (*n*) and percentage (%). The asterisk (*) denotes P <0.05. Some data points for HbA1c, CRP, and ferritin were missing, leading to calculations being performed based on 147, 146, and 146 observations for each respective variable.

Sequencing results

The sequencing run produced a total of 4542036 reads and after quality control and filtering, 4224201 reads (93 %) remained for downstream analysis through DADA2, retaining between 42.06% and 94.53% reads per sample (average 85.1%).

Phyla composition

The analysis of gut microbiota in patients with T2DM revealed the abundance of 5 major phyla: Firmicutes (47.7%), Bacteroidota (37.5%), Proteobacteria (11.4%), Actinobacteriota (1.5%) and Fusobacteriota (1.3%) (Figure 1A and 2). In contrast to the T2DM group, the composition of gut microbiota in the non-diabetic group demonstrated a slightly distinct profile which was: Firmicutes (48.5%), Bacteroidota (41.9%), Actinobacteriota (4.5%), Proteobacteria (3.4%) and Verrucomicrobiota (1.2%) (Figure 1D and 2).

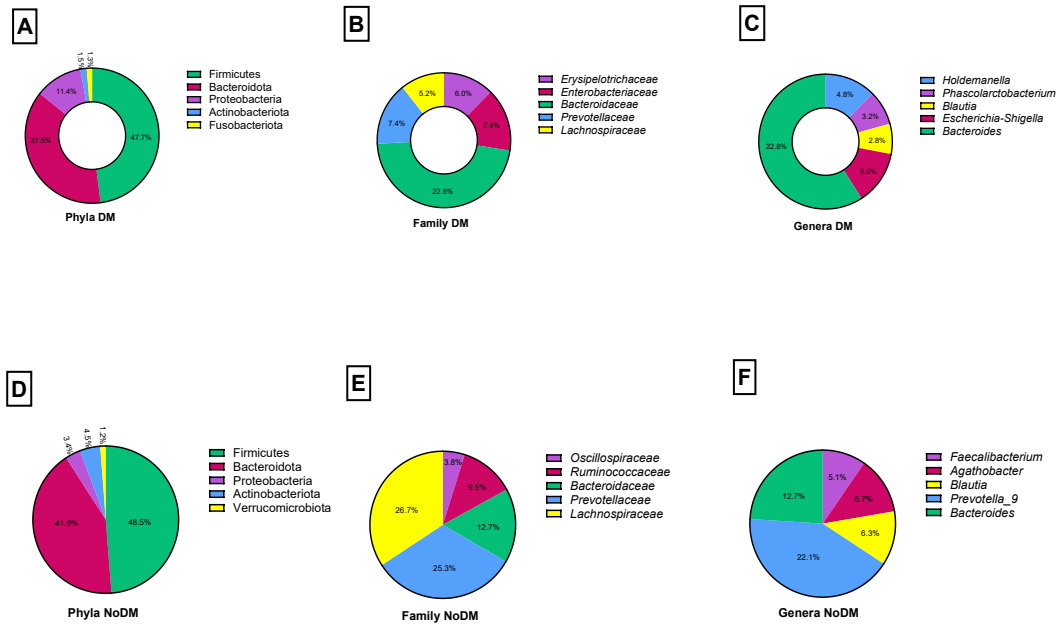


Figure 1. Comparison of relative abundance between T2DM patients and non-diabetic individuals at the phylum, family, and genus level. DM: diabetes mellitus: No DM: no diabetes mellitus.

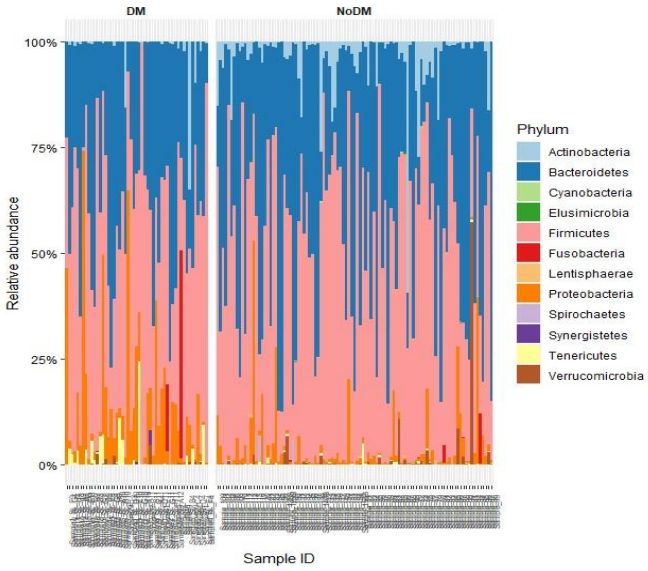


Figure 2. Comparison of relative abundance between T2DM patients and non-diabetic individuals at the phylum level. DM: diabetes mellitus: No DM: no diabetes mellitus.

Family composition

Among the T2DM group, the most abundant families were *Bacteroidaceae*, accounting for 22.8% of the microbial community, followed by *Prevotellaceae* and *Enterobacteriaceae*, each at 7.4%. Other notable families included *Erysipelotrichaceae* and *Lachnospiraceae* representing 6.0% and 5.2% of the community, respectively (Figure 1b and Figure 3). The non-diabetic group exhibited a different composition of dominant families. *Lachnospiraceae* was the most abundant, representing 26.7% of the microbial community, followed closely by *Prevotellaceae* at 25.3%. *Bacteroidaceae, Ruminococcaceae* and *Oscillospiraceae* each represented 12.7%, 9.5% and 3.8% respectively (Figure 1E and Figure 3).

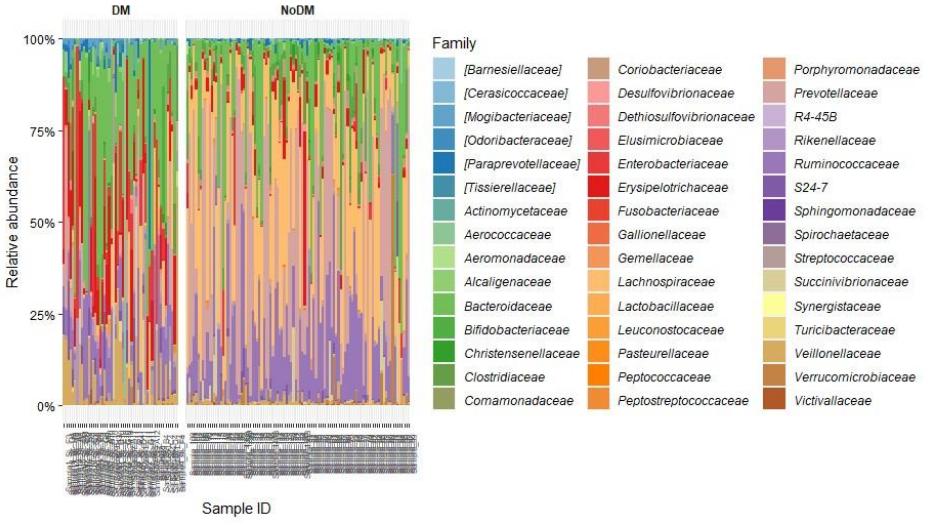


Figure 3. Comparison of relative abundance between T2DM patients and non-diabetic individuals at the family level. DM: diabetes mellitus: No DM: no diabetes mellitus.

Genera composition

The most abundant genera identified in T2DM patients included *Bacteroides*, representing 22.8% of the microbial community. *Escherichia-Shigella* constituted 5.0%, while *Holdemanella* and *Phascolarctobacterium* were both present at 4.8% and 3.2%, respectively. Additionally, *Blautia* were identified at 2.8% (Figure 1c and 4). In contrast, non-diabetic individuals exhibited a different composition of abundant genera within their gut microbiota. *Prevotella_9* was the most prevalent, representing 22.1% of the microbial community. *Bacteroides* constituted 12.7%, while *Agathobacter* accounted for 6.7%. *Blautia* was present at 6.3%, followed by *Faecalibacterium* at 5.1% (Figure 1F and 4). Table 2 presents a comparison of taxa abundance across phylum, family, and genus levels.

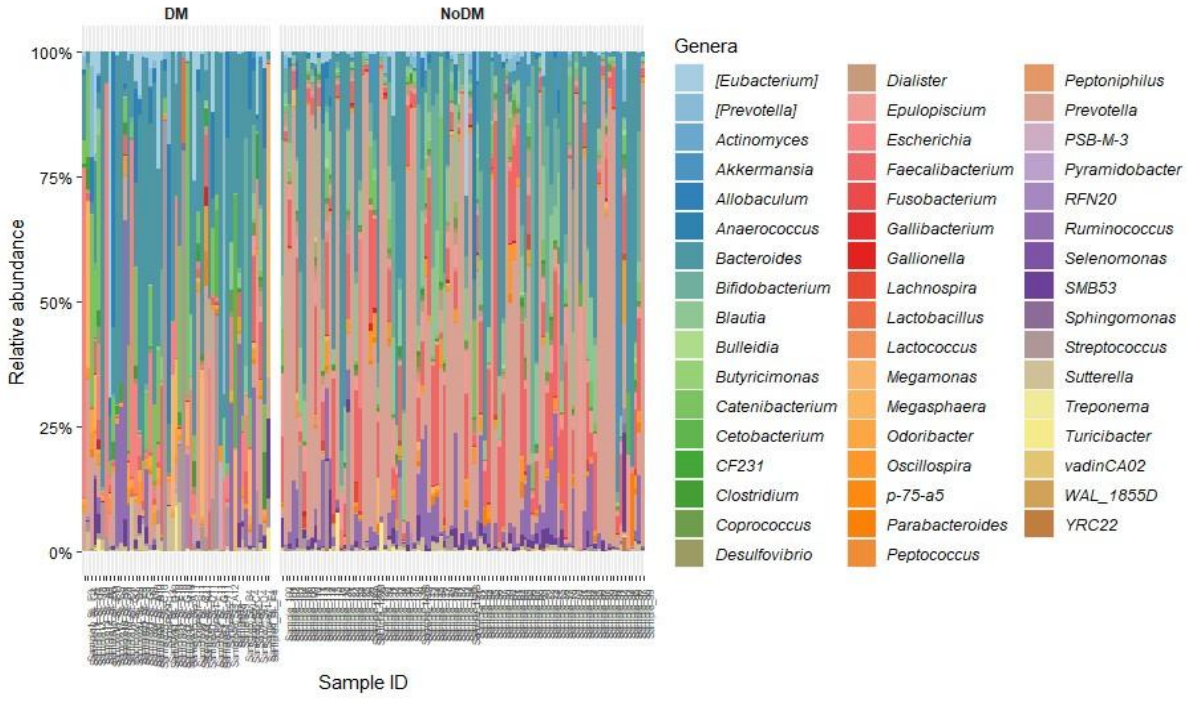


Figure 4. Comparison of relative abundance between T2DM patients and non-diabetic individuals at the genus level. DM: diabetes mellitus: No DM: no diabetes mellitus.

Table 2. Comparison of relative abundance of taxa at phylum, family, and genus level

Taxonomic level	T2DM patients	Non-diabetic individuals	P
Phyla			
Firmicutes	0.48 (0.24)	0.53 (0.39)	0.60
Bacteroidota	0.35 (0.27)	0.37 (0.44)	0.43
Actinobacteriota	0.001 (0.007)	0.02 (0.05)	<0.01
Proteobacteria	0.07 (0.14)	0.01 (0.02)	<0.01
Fusobacteriota	0 (0)	0 (0)	0.82
Verrucomicrobiota	0 (0)	0.0004 (0.0033)	0.01
Family			
Lachnospiraceae	0.006 (0.018)	0.24 (0.27)	<0.01
Oscillospiraceae	0.04 (0.06)	0.02 (0.05)	0.29
Ruminococcaceae	0.01 (0.07)	0.08 (0.10)	<0.01
Prevotellaceae	0.02 (0.02)	0.08 (0.60)	<0.01
Bacteroidaceae	0.17 (0.30)	0.04 (0.21)	<0.01
Erysipelotrichaceae	0.01 (0.10)	0.003 (0.007)	<0.01
Enterobacteriaceae	0.02 (0.11)	0.002 (0.005)	<0.01
Genera			
Faecalibacterium	0.0.3 (0.01)	0.03 (0.06)	<0.01
Bacteroides	0.17 (0.30)	0.04 (0.22)	<0.01
Agathobacter	0 (0)	0.04 (0.08)	<0.01
Prevotella_9	0.001 (0.019)	0.05 (0.54)	<0.01
Blautia	0.001 (0.005)	0.05 (0.08)	<0.01
Holdemanella	0.004 (0.070)	0.002 (0.007)	0.17
Phascolarctobacterium	0.005 (0.060)	0.001 (0.003)	0.01
Escherichia-Shigella	0.005 (0.070)	0.0004 (0.0019)	0.005

Relative abundance presented as median and interquartile range.

Differential abundance testing between T2DM patients and non-diabetic individuals

Differential abundance testing using the ANCOM-BC plug-in in QIIME2 was performed and 5, 16, and 25 differentially abundant features ($p < 0.05$) were identified at phylum, family, and genus level, respectively (Figure 5). The phyla Proteobacteria and Tenericutes were enriched in the T2DM group, and Actinobacteria, Cyanobacteria, and Lentisphaerae were depleted. Similar to what was observed using relative abundance, *Lachnospiraceae* and *Prevotellaceae* were depleted in the T2DM group, compared to non-diabetic individuals. The families *Rikenellaceae* and *Enterobacteriaceae* were observed to be significantly enriched in T2DM patients. At genus level, *Escherichia* and *Odoribacter* were enriched in the T2DM group compared to non-diabetic group, whereas *Epulopiscium* and *Blautia* were depleted. A large number of unique, but unannotated, genera were found to be differentially abundant between the study groups. Additional model-based testing to identify bacterial features associated with

T2DM was performed, which also included age as a fixed effect, to assess the potential bias introduced by the age of T2DM participants, which was statistically higher than the non-diabetic participants. 19 of the 25 features detected by ANCOM-BC were also identified as statistically significant with MaAsLin2 (corrected $p < 0.05$). Eight more statistically significant features were identified, of which 6 were enriched in T2DM (*Butyricimonas*, Bacteroidales S24.7 genus, Clostridiales genus, *Oscillospira*, *Catenibacterium*, and *Enterobacteriaceae* genus) and 2 and were depleted (Barnesiellaceae genus, *Victivallaceae* genus). Only one genus level feature associated with T2DM (*Clostridiaceae* SMB53), was also found to be enriched significantly with increased age, and was excluded.

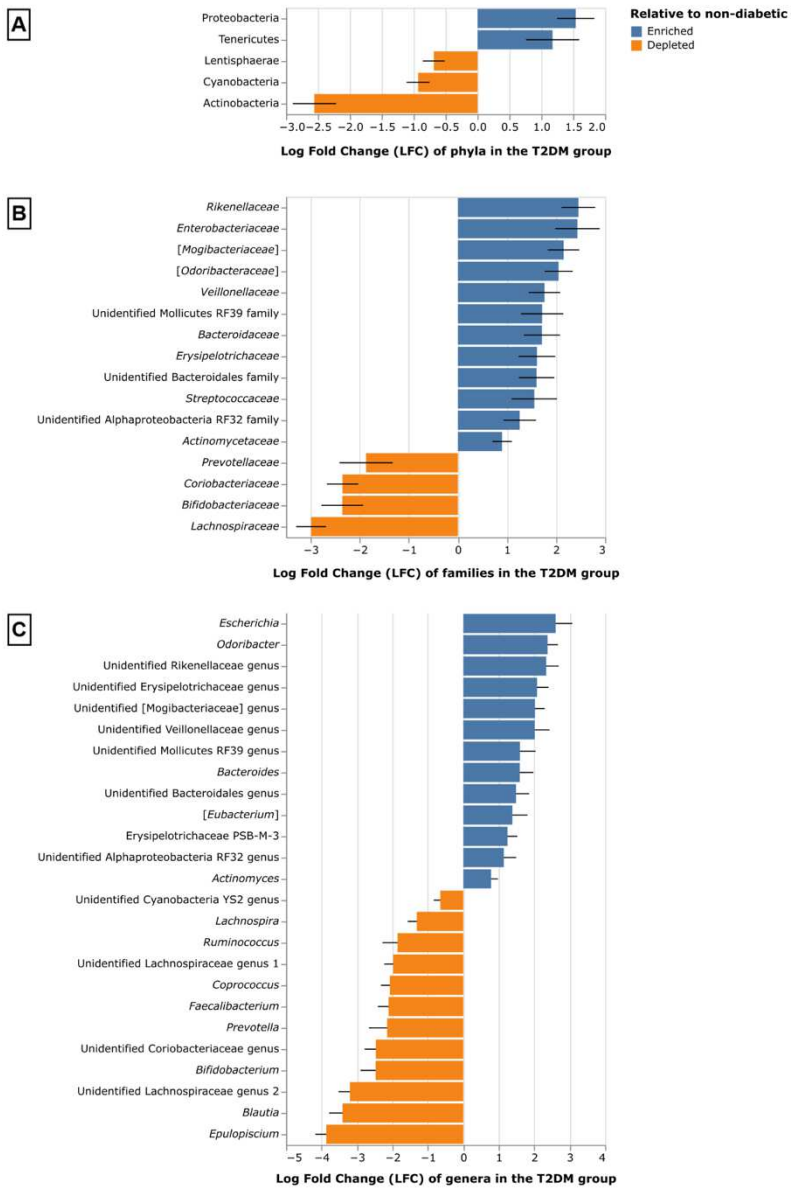


Figure 5. Differentially abundant taxa in T2DM patients compared to non-diabetic individuals at phylum (A), family (B) and genus (C) level. Only taxa with p-values < 0.05 after multiple test correction have been shown. Taxa in square brackets are awaiting reclassification and taxa listed as "Unidentified" were classified to the specified level but had no annotation available in the database.

Alpha diversity metrics in T2DM patients and non-diabetic individuals

The comparison of alpha diversity metrics between the diabetic and non-diabetic groups revealed noteworthy findings. Statistically significant differences were observed between individuals with T2DM and those without diabetes across all alpha diversity metrics, including the Shannon index, Faith's PD, Pielou's evenness index and observed features. All four measures indicated significant differences ($P < 0.001$), with non-diabetic individuals displaying higher median values in comparison to T2DM patients (Table 3). Figure 6 shows the distribution of alpha diversity metrics in T2DM and non-diabetic groups.

Table 3. Comparison of alpha diversity metrics between T2DM patients and non-diabetic individuals

Alpha diversity metrics	**DM group**	**No DM group**	**P**
Shannon index	3.95 (0.98)	4.98 (1.11)	<0.001
Faith's PD	6.26 (2.08)	7.52 (2.82)	<0.001
Pielou's evenness index	0.67 (0.15)	0.77 (0.14)	<0.001
Observed features	54 (33)	88 (31)	<0.001

Alpha diversity metrics presented as median and interquartile range. DM: diabetes mellitus: No DM: no diabetes mellitus.

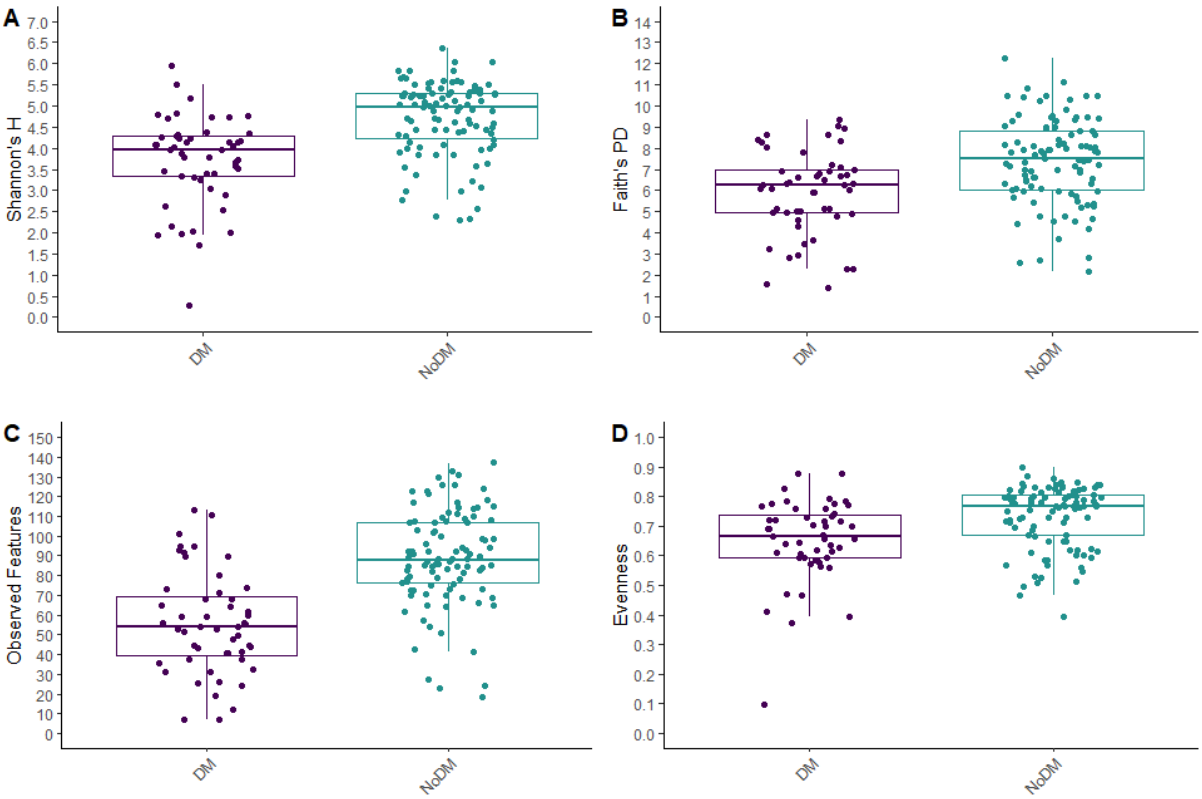
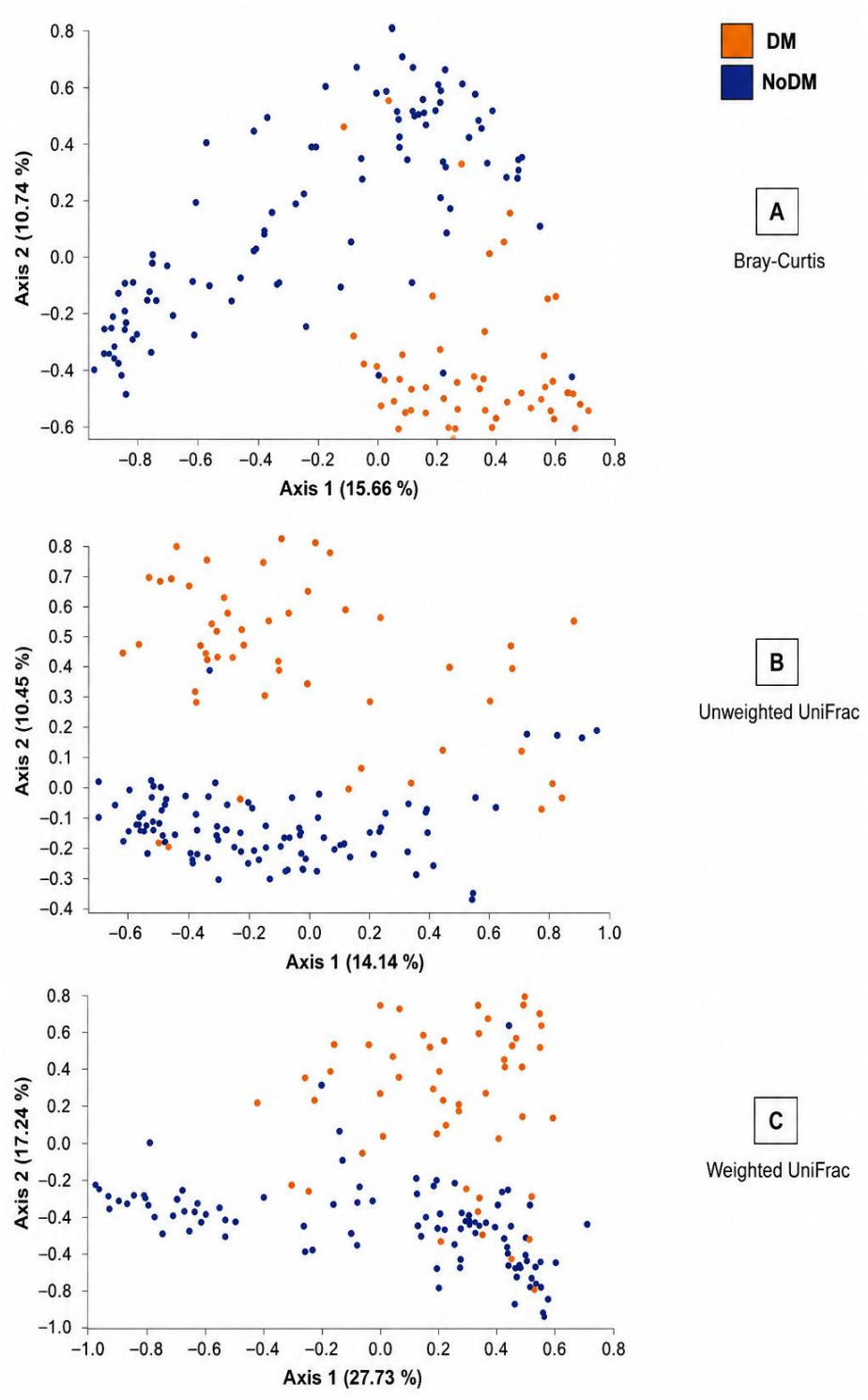


Figure 6. Distribution of alpha diversity metrics in T2DM and non-diabetic groups. DM: diabetes mellitus: No DM: no diabetes mellitus.

Beta diversity metrics in T2DM patients and non-diabetic individuals

The permutational multivariate analysis of variance using the Bray-Curtis distance, unweighted UniFrac distance and weighted UniFrac distance matrices revealed that samples from the T2DM group were significantly different from the ones from the non-diabetic group ($P = 0.001$ for all matrices) (Figure 7). The Bray-Curtis clustering pattern accounted for 15.66% and 10.74% of the total variance along the axis 1 and axis 2, respectively. Similarly, the unweighted UniFrac pattern explained 14.14% and 10.45% of the total variance along axis 1 and axis 2. The weighted UniFrac clustering pattern reflected 27.73% and 17.24% of the total variance along axis 1 and axis 2 (Figure 7).



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332 **Figure 7**. Principal coordinate analysis (PCoA) diagram based on Bray-Curtis (A), unweighted
333 UniFrac (B), and weighted UniFrac (C) distances. Each point on the plots corresponds to a
334 single sample within its respective group. DM: diabetes mellitus: No DM: no diabetes mellitus.

Firmicutes/Bacteroidota (F/B) ratio

The range extended from a minimum ratio of 0.04 to a maximum of 13.4. Specifically, within the T2DM group, the median ratio was 1.4, with an IQR of 1.2. On the other hand, the non-diabetic group displayed a median ratio of 1.5, with an IQR of 2.4 (Table S2). The F/B ratios did not exhibit a statistically significant difference between the T2DM and non-diabetic groups, P = 0.7753.

Relationship of gut microbiota profiles with inflammatory markers, HbA1C, diabetes mellitus status, and type of diabetes mellitus treatment

Binary logistic regression results indicated that Proteobacteria (P = 0.001), *Bacteroidaceae* (P = 0.003), *Erysipelotrichaceae* (P = <0.001), *Enterobacteriaceae* (P = <0.001), *Bacteroides* (P = 0.004), *Holdemanella* (P = 0.001), *Phascolarctobacterium* (P = 0.005), and *Escherichia-Shigella* (P = 0.002) were positively related to the likelihood of falling into the T2DM group. Conversely, the probability of having high levels of *Actinobacteriota* (P = 0.024), *Lachnospiraceae* (P = <0.001), *Ruminococcaceae* (P = 0.003), *Prevotellaceae* (P = 0.001), *Faecalibacterium* (P = <0.001), *Agathobacter* (P = <0.001), *Prevotella_9* (P = 0.002), *Blautia* (P = 0.003), Shannon index (P = <0.001), Faith's PD (P = <0.001), Pielou's evenness index (P = <0.001), and observed features (P = <0.001) was more in the non-diabetic group compared to the T2DM group.

Regarding T2DM treatment type, *Lachnospiraceae* exhibited a positive association with the likelihood of belonging to the metformin-using group (P = 0.043). The probability of having high levels of *Phascolarctobacterium* (P = 0.025), Faith's PD (P = 0.010), and observed features (P = 0.016) was more in the group receiving other T2DM treatments (i.e., insulin, both metformin & insulin, and other unspecified treatments) compared to the metformin-using group.

Correlation analyses indicated a moderate correlation at the phyla level, where Actinobacteriota exhibited a significant negative correlation with HbA1c. Two additional slight associations were noted at the phylum level, consisting of a significant negative correlation between Actinobacteria and CRP, and a significant positive correlation between Proteobacteria and HbA1c (Figure 8).

Lachnospiraceae displayed a moderate negative correlation with both HbA1c and CRP. *Ruminococcaceae* showed a moderate negative correlation with HbA1c and a slight negative correlation with CRP. *Erysipelotrichaceae* and *Enterobacteriaceae* exhibited slight positive correlations with HbA1c and CRP, respectively. Additionally, *Enterobacteriaceae*

demonstrated a further moderate positive correlation with HbA1c. *Faecalibacterium* displayed a moderate negative association with both HbA1c and CRP, and a slight negative association with ferritin (Figure 8).

Agathobacter, *Prevotella_9*, and *Blautia* were moderately negatively correlated with HbA1c. *Escherichia-Shigella* was moderately negatively associated with HbA1c and slightly positively correlated with CRP. All these associations were found to be statistically significant. The Shannon index and Observed features demonstrated moderate negative correlations with both HbA1c and CRP. Faith's PD and Pielou's evenness index showed slight negative correlations with HbA1c and CRP, respectively. Additionally, Faith's PD and Pielou's evenness index revealed moderate negative correlations with CRP and HbA1c, respectively (Figure 8).

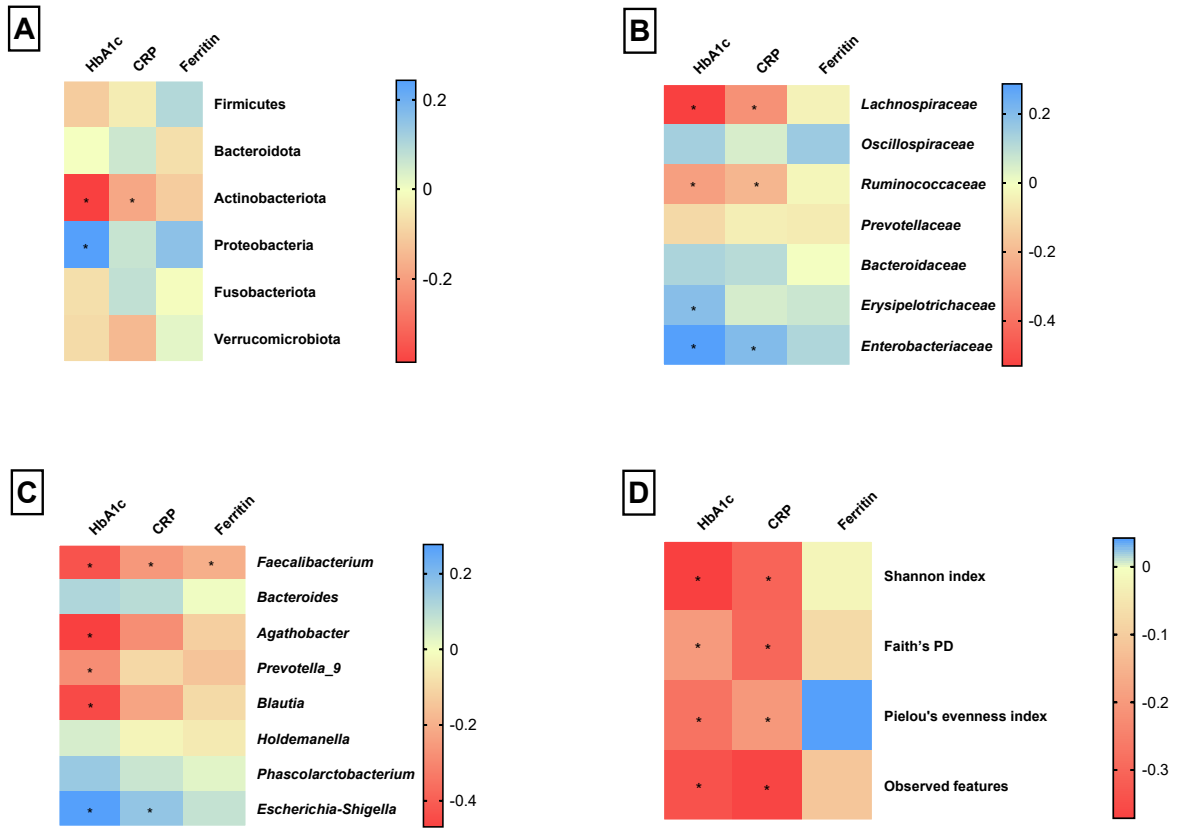


Figure 8: Correlation of HbA1c, CRP and ferritin with relative abundance of the top gut microbes at the phyla, families, and genera and alpha diversity metrics.

4. Discussion

This study characterised the gut microbiota and systemic inflammatory profile of South African adults with and without type 2 diabetes mellitus (T2DM). T2DM was associated with lower alpha diversity, distinct beta diversity, a higher abundance of potentially pro-inflammatory taxa

such as Proteobacteria, Enterobacteriaceae, and *Escherichia-Shigella*, and lower levels of SCFA-producing bacteria, including *Faecalibacterium* and *Prevotella_9*. Higher levels of C-reactive protein (CRP) and ferritin were also associated with lower alpha diversity and reduced abundance of some potentially protective taxa, suggesting a link between gut dysbiosis and systemic inflammation.

Both groups shared four dominant phyla: Firmicutes, Bacteroidota, Proteobacteria, and Actinobacteriota, consistent with previous studies [29–31]. However, differences in their relative abundance were observed. Proteobacteria was more abundant in T2DM, supporting its association with gut dysbiosis, metabolic dysfunction, and low-grade inflammation [18,32]. In contrast, Actinobacteriota was more abundant in non- diabetic individuals and is involved in carbohydrate breakdown, acetate production, and maintenance of the intestinal barrier [33]. Fusobacteriota, which has been associated with mucosal damage and gastrointestinal disease [34], was only detected in T2DM, while Verrucomicrobiota, including *Akkermansia muciniphila*, was only detected in non-diabetic individuals. This bacterium has been linked to mucin integrity and glucose regulation [35,36].

At the family level, Bacteroidaceae, Prevotellaceae, and Lachnospiraceae were among the dominant groups in both cohorts. Bacteroidaceae was more abundant in T2DM and produces metabolites such as succinate, acetate, and propionate [37]. In contrast, Prevotellaceae and Lachnospiraceae were more abundant in non-diabetic individuals, consistent with their roles in SCFA production, anti-inflammatory activity, and intestinal homeostasis [21,38–40]. However, the distribution of Lachnospiraceae has varied across studies [41]. The association between Lachnospiraceae and metformin use in this study also suggests that medication may influence gut microbial composition. T2DM was further associated with higher levels of *Enterobacteriaceae* and *Erysipelotrichaceae*, which have been linked to inflammation [42–45], as well as opportunistic genera such as *Escherichia* and *Odoribacter*. At the same time, potentially beneficial bacteria such as *Faecalibacterium* and *Bifidobacterium* were reduced [46,47]. Non-diabetic individuals had higher levels of SCFA-producing Ruminococcaceae and Oscillospiraceae [48–50].

At the genus level, *Bacteroides* was the most abundant genus in T2DM, consistent with Wu et al., 2010 [51], although other studies have reported lower levels [52,53]. These differences may reflect variation between populations in geography, diet, and lifestyle [54,55]. *Escherichia-Shigella* was more abundant in T2DM, supporting its reported association with dysbiosis and

mucosal damage [52,56,57]. *Holdemanella*, *Phascolarctobacterium*, and *Blautia* showed more complex patterns [58,59]. Phascolarctobacterium has been associated with better insulin sensitivity [60], while Blautia, which was reduced in T2DM in this study, has reported antibacterial and anti-inflammatory effects [61]. In non- diabetic individuals, Prevotella_9 was dominant and may reflect dietary patterns, propionate production, and immune regulation [62–64]. Other SCFA-producing genera, including *Agathobacter* and *Faecalibacterium*, were also more prominent in non-diabetic individulas. The lower abundance of *Faecalibacterium* in T2DM is consistent with previous studies identifying its depletion as a common feature of gut dysbiosis [40,47,65].

The overall diversity findings further supported differences in gut microbial composition between the groups. Non- diabetic individulas had significantly greater microbial richness and evenness, consistent with some previous studies [62,66], although this differs from findings by Doumatey et al. (2020) [21]. Bray-Curtis, unweighted UniFrac, and weighted UniFrac analyses also showed distinct microbial community clustering between the groups [66,67]. No significant difference was observed in the Firmicutes/Bacteroidota (F/B) ratio. Although findings on this ratio in diabetes are inconsistent, changes in the ratio have generally been linked to gut dysbiosis [68,69].

The inflammatory findings were also consistent with a greater inflammatory burden among participants with T2DM. Moderate CRP elevation was observed in 44% of T2DM participants, while 20% of females with T2DM had high ferritin compared with 2% of control females. In contrast, 55% of non-diabetic individulas had normal CRP levels. CRP was also associated with alpha diversity and with SCFA-producing taxa, particularly Lachnospiraceae and Faecalibacterium. These findings support a possible relationship between gut microbial changes, systemic inflammation, and metabolic dysfunction through the gut-liver-immune axis [16,70].

Overall, these findings provide an important baseline description of T2DM-associated gut microbiota changes in a South African population. The relationship between SCFA-producing bacteria and inflammatory markers such as CRP and ferritin suggests that restoring beneficial microbial communities through targeted dietary or probiotic approaches may have potential for reducing inflammation and improving glycaemic control. However, the cross-sectional design does not allow causal relationships between microbial changes and T2DM to be established. The relatively small sample also limited subgroup analyses, including assessment of the

potential effect of metformin on Lachnospiraceae. In addition, 16S rRNA sequencing has limited resolution for species- and strain-level identification and functional analysis.

Future studies should use larger, prospective cohorts to follow changes in the gut microbiota from prediabetes to T2DM. Combining shotgun metagenomic sequencing with metabolomic analysis could provide further information on microbial functions, including SCFA production and circulating lipopolysaccharide levels. Interventional studies investigating dietary modification and targeted prebiotics in Southern African populations are also needed to determine whether modifying the gut microbiota can improve metabolic health.

5. Limitations of the study

No negative controls were sequenced in this study, although a negative control was included during PCR to assess possible contamination. The number of species-level classifications was also lower than expected, likely due to limitations in the databases available for DADA2 and QIIME2 using PacBio CCS data.

The relatively small sample size (n=150), including only 51 participants with T2DM, may limit the generalisability of the findings to the wider South African population and prevented detailed subgroup analyses, such as comparisons between patients taking metformin and those not taking it. In addition, the study was conducted in a specific geographical area, which may limit the applicability of the findings to populations with different genetic, cultural, and environmental backgrounds.

Dietary habits as well as physical activity were not comprehensively assessed and may have contributed to differences in gut microbiota [71,72], therefore confounding the association with T2DM. Limited information on other health conditions and lifestyle factors, including tobacco, alcohol, and drug use, may also have influenced the findings. BMI, an important factor associated with T2DM, was not assessed and may have introduced additional confounding, as differences in BMI could affect gut microbiota composition independently of diabetes status.

Information on the duration of T2DM was not available, so this factor could not be consistently controlled for. This may make it difficult to determine whether differences in gut microbiota were related to diabetes itself or to the duration of the disease. Other medications, in addition to antidiabetic drugs such as metformin, may also affect gut microbial communities and could have influenced the results.

6. Conclusions

The study identified clear differences in gut microbiota between individuals with T2DM and those without diabetes. T2DM patients had a higher abundance of potentially harmful taxa, while non-diabetic individuals had more beneficial taxa. Significant differences were also observed in alpha and beta diversity between the groups, with mostly slight/ moderate associations between inflammatory markers and microbial diversity and richness. These findings add to the understanding of the relationship between gut microbiota and T2DM in the South African population. Although this was an observational study, the findings provide a basis for future experimental research, including studies investigating affordable probiotic-rich dietary approaches to improve gut microbiota and potentially support metabolic health.

Ethics approval and consent to participate

Ethics approval was attained from the Stellenbosch University health research ethics committee (HREC), HREC Reference No: S22/05/009_COVID-19 (PhD). The research adhered to the principles outlined in the Declaration of Helsinki and guidelines established by the HREC. Participants were recruited from the Dr. George Mukhari Hospital (DGMAH) and nearby townships. Before participation, all participants were fully informed about the study and provided written informed consent to participate.

Consent for publication

Not applicable

Availability of data and materials: 16S rRNA sequencing data can be found here: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1064418. Submission ID: SUB14150586, BioProject ID: PRJNA1064418.

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Author's contributions: SMP came up with the study concept and wrote the first draft of the manuscript. PSN, BC-M, and SM helped to develop the study and revise the manuscript. JTN, AS and KN performed bioinformatics analysis and revised the manuscript. All authors approved the last version of the manuscript.

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7. References

[1] Thursby E, Juge N. Introduction to the human gut microbiota. Biochemical Journal 2017;474:1823–36. https://doi.org/10.1042/BCJ20160510.

[2] Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ (Online) 2018;361:36–44. https://doi.org/10.1136/bmj.k2179.

[3] Peter PI, Steinberg WJ, van Rooyen C, Botes J. Type 2 diabetes mellitus patients' knowledge, attitude and practice of lifestyle modifications. Health SA Gesondheid 2022;27. https://doi.org/10.4102/hsag.v27i0.1921.

[4] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract 2022;183. https://doi.org/10.1016/j.diabres.2021.109119.

[5] Ong KL, Stafford LK, McLaughlin SA, Boyko EJ, Vollset SE, Smith AE, et al. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. The Lancet 2023;402:203–34. https://doi.org/10.1016/S0140-6736(23)01301-6.

[6] Chetty L, Govender N, Govender GM, Reddy P. Demographic stratification of type 2 diabetes and comorbidities in district healthcare in Kwazulu-Natal. South African Family Practice 2021;63. https://doi.org/10.4102/safp.v63i1.5218.

[7] Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. Mediators Inflamm 2014;2014. https://doi.org/10.1155/2014/162021.

[8] Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. Cellular and Molecular Life Sciences 2017;74:2959–77. https://doi.org/10.1007/s00018-017-2509-x.

[9] Violi F, Nocella C, Bartimoccia S, Castellani V, Carnevale R, Pignatelli P, et al. Gut dysbiosis-derived low-grade endotoxemia: A common basis for liver and cardiovascular disease. Kardiol Pol 2023;81:563–71. https://doi.org/10.33963/KP.a2023.0115.

[10] Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cellular and Molecular Life Sciences 2021;78:1233–61. https://doi.org/10.1007/s00018-020-03656-y.

[11] Bashir H, Majid S, Khan MS, Bhat MH, Hamid R, Ashraf R, et al. Inter-relationship of Pro- and Anti- inflammatory Biomarkers with the development of Type 2 Diabetes Mellitus. Heliyon 2022;8. https://doi.org/10.1016/j.heliyon.2022.e11329.

[12] Rehman K, Akash MSH. Mechanisms of inflammatory responses and development of insulin resistance: How are they interlinked? J Biomed Sci 2016;23:1–18. https://doi.org/10.1186/s12929-016-0303-y.

[13] Kim MH, Kim HN, Choi WS. The association between subclinical inflammation and abnormal glucose and lipid metabolisms in normal-weight Korean individuals. Nutrition, Metabolism and Cardiovascular Diseases 2018;28:1106–13. https://doi.org/10.1016/j.numecd.2018.06.016.

[14] Dludla P V, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, et al. Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. World J Diabetes 2023;14:130–46. https://doi.org/10.4239/wjd.v14.i3.130.

[15] Deleu S, Machiels K, Raes J, Verbeke K, Vermeire S. Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? EBioMedicine 2021;66. https://doi.org/10.1016/j.ebiom.2021.103293.

[16] Bander Z Al, Nitert MD, Mousa A, Naderpoor N. The gut microbiota and inflammation: An overview. Int J Environ Res Public Health 2020;17:1–22. https://doi.org/10.3390/ijerph17207618.

[17] Lau WL, Tran T, Rhee CM, Kalantar-Zadeh K, Vaziri ND. Diabetes and the Gut Microbiome. Semin Nephrol 2021;41:104–13. https://doi.org/10.1016/j.semnephrol.2021.03.005.

[18] Larsen N, Vogensen FK, Van Den Berg FWJ, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 2010;5. https://doi.org/10.1371/journal.pone.0009085.

[19] Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, et al. Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. Microb Pathog 2017;111:362–9. https://doi.org/10.1016/j.micpath.2017.08.038.

[20] Zhao L, Lou H, Peng Y, Chen S, Zhang Y, Li X. Comprehensive relationships between gut microbiome and faecal metabolome in individuals with type 2 diabetes and its complications. Endocrine 2019;66:526–37. https://doi.org/10.1007/s12020-019-02103-8.

[21] Doumatey AP, Adeyemo A, Zhou J, Lei L, Adebamowo SN, Adebamowo C, et al. Gut Microbiome Profiles Are Associated With Type 2 Diabetes in Urban Africans. Front Cell Infect Microbiol 2020;10. https://doi.org/10.3389/fcimb.2020.00063.

[22] Allali I, Abotsi RE, Tow LAh, Thabane L, Zar HJ, Mulder NM, et al. Human microbiota research in Africa: a systematic review reveals gaps and priorities for future research. Microbiome 2021;9:15. https://doi.org/10.1186/s40168-021-01195-7.

[23] Di Tommaso P, Chatzou M, Floden EW, Barja PP, Palumbo E, Notredame C. Nextflow enables reproducible computational workflows. Nat Biotechnol 2017;35:316–9. https://doi.org/10.1038/nbt.3820.

[24] Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, et al. The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol 2020;38:276–8. https://doi.org/10.1038/s41587-020-0439-x.

[25] Straub D, Blackwell N, Langarica-Fuentes A, Peltzer A, Nahnsen S, Kleindienst S. Interpretations of Environmental Microbial Community Studies Are Biased by the Selected 16S rRNA (Gene) Amplicon Sequencing Pipeline. Front Microbiol 2020;11. https://doi.org/10.3389/fmicb.2020.550420.

[26] Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: Summarize analysis results for multiple tools and samples in a single report. Bioinformatics 2016;32:3047–8. https://doi.org/10.1093/bioinformatics/btw354.

[27] Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomark Insights 2016;11:95–104. https://doi.org/10.4137/Bmi.s38440.

[28] Nehring SM, Goyal A, Patel BC. C Reactive Protein. Nehring SM, Goyal A, Patel BC. C Reactive Protein. [Updated 2023 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: Https://Www.Ncbi.Nlm.Nih.Gov/Books/NBK441843/: 2023.

[29] Cunningham AL, Stephens JW, Harris DA. Gut microbiota influence in type 2 diabetes mellitus (T2DM). Gut Pathog 2021;13:50. https://doi.org/10.1186/s13099-021-00446-0.

[30] Huang Y, Wang Z, Ma H, Ji S, Chen Z, Cui Z, et al. Dysbiosis and Implication of the Gut Microbiota in Diabetic Retinopathy. Front Cell Infect Microbiol 2021;11. https://doi.org/10.3389/fcimb.2021.646348.

[31] Wongsurawat T, Sutheeworapong S, Jenjaroenpun P, Charoensiddhi S, Khoiri AN, Topanurak S, et al. Microbiome analysis of thai traditional fermented soybeans reveals short-chain fatty acid-associated bacterial taxa. Sci Rep 2023;13. https://doi.org/10.1038/s41598-023-34818-0.

[32] Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. Proteobacteria: A common factor in human diseases. Biomed Res Int 2017;2017. https://doi.org/10.1155/2017/9351507.

[33] Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V, Gasbarrini A. Actinobacteria: A relevant minority for the maintenance of gut homeostasis. Digestive and Liver Disease 2018;50:421–8. https://doi.org/10.1016/j.dld.2018.02.012.

[34] Kelly D, Yang L, Pei Z. Gut Microbiota, Fusobacteria, and Colorectal Cancer. Diseases 2018;6:109. https://doi.org/10.3390/diseases6040109.

[35] Bielka W, Przezak A, Pawlik A. The role of the gut microbiota in the pathogenesis of diabetes. Int J Mol Sci 2022;23. https://doi.org/10.3390/ijms23010480.

[36] Li J, Yang G, Zhang Q, Liu Z, Jiang X, Xin Y. Function of Akkermansia muciniphila in type 2 diabetes and related diseases. Front Microbiol 2023;14:1–13. https://doi.org/10.3389/fmicb.2023.1172400.

[37] Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 2014;38:996–1047. https://doi.org/10.1111/1574-6976.12075.

[38] Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The controversial role of human gut lachnospiraceae. Microorganisms 2020;8:1–25. https://doi.org/10.3390/microorganisms8040573.

[39] Iatcu CO, Steen A, Covasa M. Gut microbiota and complications of type-2 diabetes. Nutrients 2022;14. https://doi.org/10.3390/nu14010166.

[40] Siptroth J, Moskalenko O, Krumbiegel C, Ackermann J, Koch I, Pospisil H. Variation of butyrate production in the gut microbiome in type 2 diabetes patients. International Microbiology 2023;26:601–10. https://doi.org/10.1007/s10123-023-00324-6.

[41] Wang Y, Luo X, Mao X, Tao Y, Ran X, Zhao H, et al. Gut microbiome analysis of type 2 diabetic patients from the Chinese minority ethnic groups the Uygurs and Kazaks. PLoS One 2017;12:1–15. https://doi.org/10.1371/journal.pone.0172774.

[42] Kaakoush NO. Insights into the role of Erysipelotrichaceae in the human host. Front Cell Infect Microbiol 2015;5:1–4. https://doi.org/10.3389/fcimb.2015.00084.

[43] Turpin W, Bedrani L, Espin-Garcia O, Xu W, Silverberg MS, Smith MI, et al. Associations of NOD2 polymorphisms with Erysipelotrichaceae in stool of in healthy first degree relatives of Crohn's disease subjects. BMC Med Genet 2020;21:1–8. https://doi.org/10.1186/s12881-020-01115-w.

[44] Baldelli V, Scaldaferri F, Putignani L, Del Chierico F. The Role of Enterobacteriaceae in Gut Microbiota Dysbiosis in Inflammatory Bowel Diseases. Microorganisms 2021, Vol 9, Page 697 2021;9:697. https://doi.org/10.3390/MICROORGANISMS9040697.

[45] Gradisteanu Pircalabioru G, Chifiriuc MC, Picu A, Petcu LM, Trandafir M, Savu O. Snapshot into the Type-2-Diabetes-Associated Microbiome of a Romanian Cohort. Int J Mol Sci 2022;23. https://doi.org/10.3390/ijms232315023.

[46] Hidalgo-Cantabrana C, Delgado S, Ruiz L, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and Their Health-Promoting Effects. Microbiol Spectr 2017;5:10.1128/microbiolspec.bad-0010–2016. https://doi.org/10.1128/microbiolspec.bad-0010-2016.

[47] Martín R, Rios-Covian D, Huillet E, Auger S, Khazaal S, Bermúdez-Humarán LG, et al. Faecalibacterium: a bacterial genus with promising human health applications. FEMS Microbiol Rev 2023;47. https://doi.org/10.1093/femsre/fuad039.

[48] Atzeni A, Bastiaanssen TFS, Cryan JF, Tinahones FJ, Vioque J, Corella D, et al. Taxonomic and Functional Fecal Microbiota Signatures Associated With Insulin Resistance in Non-Diabetic Subjects With Overweight/Obesity Within the Frame of the PREDIMED-Plus Study. Front Endocrinol (Lausanne) 2022;13. https://doi.org/10.3389/fendo.2022.804455.

[49] Palmnäs-Bedard MSA, Costabile G, Vetrani C, Åberg S, Hjalmarsson Y, Dicksved J, et al. The human gut microbiota and glucose metabolism: a scoping review of key bacteria and the potential role of SCFAs. American Journal of Clinical Nutrition 2022;116:862–74. https://doi.org/10.1093/ajcn/nqac217.

[50] Gu X, Sim JXY, Lee WL, Cui L, Chan YFZ, Chang ED, et al. Gut Ruminococcaceae levels at baseline correlate with risk of antibiotic-associated diarrhea. IScience 2022;25:103644. https://doi.org/10.1016/j.isci.2021.103644.

[51] Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, et al. Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol 2010;61:69–78. https://doi.org/10.1007/s00284-010-9582-9.

[52] Zhao X, Zhang Y, Guo R, Yu W, Zhang F, Wu F, et al. The Alteration in Composition and Function of Gut Microbiome in Patients with Type 2 Diabetes. J Diabetes Res 2020;2020. https://doi.org/10.1155/2020/8842651.

[53] Polidori I, Marullo L, Ialongo C, Tomassetti F, Colombo R, di Gaudio F, et al. Characterization of Gut Microbiota Composition in Type 2 Diabetes Patients: A Population-Based Study. Int J Environ Res Public Health 2022;19. https://doi.org/10.3390/ijerph192315913.

[54] Wegierska AE, Charitos IA, Topi S, Potenza MA, Montagnani M, Santacroce L. The Connection Between Physical Exercise and Gut Microbiota: Implications for Competitive Sports Athletes. Sports Medicine 2022;52:2355–69. https://doi.org/10.1007/s40279-022-01696-x.

[55] Wang C, Zhao J, Zhang H, Lee YK, Zhai Q, Chen W. Roles of intestinal bacteroides in human health and diseases. Crit Rev Food Sci Nutr 2021;61:3518–36. https://doi.org/10.1080/10408398.2020.1802695.

[56] Maskarinec G, Raquinio P, Kristal BS, Setiawan VW, Wilkens LR, Franke AA, et al. The gut microbiome and type 2 diabetes status in the Multiethnic Cohort. PLoS One 2021;16:e0250855. https://doi.org/10.1371/journal.pone.0250855.

[57] Yang L, Xiang Z, Zou J, Zhang Y, Ni Y, Yang J. Comprehensive Analysis of the Relationships Between the Gut Microbiota and Fecal Metabolome in Individuals With Primary Sjogren's Syndrome by 16S rRNA Sequencing and LC–MS-Based Metabolomics. Front Immunol 2022;13:1–13. https://doi.org/10.3389/fimmu.2022.874021.

[58] De D, Nayak T, Chowdhury S, Dhal PK. Insights of Host Physiological Parameters and Gut Microbiome of Indian Type 2 Diabetic Patients Visualized via Metagenomics and Machine Learning Approaches. Front Microbiol 2022;13. https://doi.org/10.3389/fmicb.2022.914124.

[59] Zhang C, Liang D, Li X, Liu J, Fan M, Jing M, et al. Characteristics of Gut Microbial Profiles of Offshore Workers and Its Associations With Diet. Front Nutr 2022;9:1–11. https://doi.org/10.3389/fnut.2022.904927.

[60] Naderpoor N, Mousa A, Gomez-Arango LF, Barrett HL, Nitert MD, Courten B De. Faecal microbiota are related to insulin sensitivity and secretion in overweight or obese adults. J Clin Med 2019;8. https://doi.org/10.3390/jcm8040452.

[61] Li W, Li L, Yang F, Hu Q, Xiong D. Correlation between gut bacteria Phascolarctobacterium and exogenous metabolite α-linolenic acid in T2DM: a case-control study. Ann Transl Med 2022;10:1056–1056. https://doi.org/10.21037/atm-22-3967.

[62] Tao S, Li L, Li L, Liu Y, Ren Q, Shi M, et al. Understanding the gut–kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. Acta Diabetol 2019;56:581–92. https://doi.org/10.1007/s00592-019-01316-7.

[63] Zhao H, Lyu Y, Zhai R, Sun G, Ding X. Metformin Mitigates Sepsis-Related Neuroinflammation via Modulating Gut Microbiota and Metabolites. Front Immunol 2022;13. https://doi.org/10.3389/fimmu.2022.797312.

[64] Sun QH, Liu ZJ, Zhang L, Wei H, Song LJ, Zhu SW, et al. Sex-based differences in fecal short-chain fatty acid and gut microbiota in irritable bowel syndrome patients. J Dig Dis 2021;22:246–55. https://doi.org/10.1111/1751-2980.12988.

[65] Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine 2020;51:1–9. https://doi.org/10.1016/j.ebiom.2019.11.051.

[66] Almugadam B, Liu Y, SM C, CH W, CY S, BW R, et al. Alterations of Gut Microbiota in Type 2 Diabetes Individuals and the Confounding Effect of Antidiabetic Agents. J Diabetes Res 2020;2020:7253978. https://doi.org/10.1155/2020/7253978.

[67] Que Y, Cao M, He J, Zhang Q, Chen Q, Yan C, et al. Gut Bacterial Characteristics of Patients With Type 2 Diabetes Mellitus and the Application Potential. Front Immunol 2021;12:1–11. https://doi.org/10.3389/fimmu.2021.722206.

[68] Salamon D, Sroka-Oleksiak A, Kapusta P, Szopa M, Mrozińska S, Ludwig-Słomczyńska AH, et al. Characteristics of gut microbiota in adult patients with type 1 and type 2 diabetes based on next-generation sequencing of the 16S rRNA gene fragment. Pol Arch Intern Med 2018;128:336–43. https://doi.org/10.20452/pamw.4246.

[69] Kusnadi Y, Saleh MI, Ali Z, Hermansyah H, Murti K, Hafy Z, et al. Firmicutes/Bacteroidetes Ratio of Gut Microbiota and Its Relationships with Clinical Parameters of Type 2 Diabetes Mellitus: A Systematic Review. Open Access Maced J Med Sci 2023;11:67–72. https://doi.org/10.3889/oamjms.2023.10478.

[70] Sugawara Y, Kanazawa A, Aida M, Yoshida Y, Yamashiro Y, Watada H. Association of gut microbiota and inflammatory markers in obese patients with type 2 diabetes mellitus: Post hoc analysis of a synbiotic interventional study. Biosci Microbiota Food Health 2022;41:103–11. https://doi.org/10.12938/bmfh.2021-081.

[71] Yin P, Zhang C, Du T, Yi S, Yu L, Tian F, et al. Meta-analysis reveals different functional characteristics of human gut Bifidobacteria associated with habitual diet. Food Research International 2023;170:112981. https://doi.org/10.1016/j.foodres.2023.112981.

[72] Dziewiecka H, Buttar HS, Kasperska A, Ostapiuk–Karolczuk J, Domagalska M, Cichoń J, et al. Physical activity induced alterations of gut microbiota in humans: a systematic review. BMC Sports Sci Med Rehabil 2022;14:1–22. https://doi.org/10.1186/s13102-022-00513-2.