Section: Miscellaneous

Original Research Article

CORRELATION OF GUT MICROBIOME DIVERSITY WITH INSULIN RESISTANCE IN PREDIABETIC ADULTS USING STOOL PCR SEQUENCING

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ABSTRACT

Background: Prediabetes is a clinically significant metabolic state associated with impaired glucose regulation and heightened risk of progression to type 2 diabetes mellitus (T2DM). Emerging evidence suggests that gut microbiome diversity strongly influences metabolic homeostasis through mechanisms involving short-chain fatty acid production, bile acid modulation, inflammatory pathways, and insulin sensitivity. However, population-specific data correlating gut microbial diversity with insulin resistance remain limited. **Materials and Methods:** This cross-sectional observational study evaluated prediabetic adults aged 20–60 years. Stool samples were collected and processed using 16S rRNA PCR sequencing to quantify microbial richness, alpha and beta diversity, and taxonomic composition. Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR). Participants were stratified into low, moderate, and high diversity groups based on validated microbiome diversity indices. Correlations between microbiome diversity and HOMA-IR values were analysed using Pearson and multivariable regression approaches adjusted for BMI, age, sex, and dietary patterns. **Result:** A significant inverse correlation was observed between gut microbiome alpha diversity (Shannon and Simpson indices) and insulin resistance levels. Individuals with lower microbial richness demonstrated higher fasting insulin and elevated HOMA-IR scores. Distinct microbiome signatures were identified: reduced abundance of Akkermansia, Faecalibacterium, and Bifidobacterium species was associated with increased insulin resistance, while enrichment of pro-inflammatory taxa such as Prevotella and Ruminococcus gnavus correlated with poorer metabolic profiles. Beta-diversity demonstrated clear clustering between high- and low-HOMA-IR groups, reflecting compositional dysbiosis. **Conclusion:** Gut microbiome diversity shows a strong and clinically relevant inverse association with insulin resistance in prediabetic adults. Stool PCR sequencing offers an effective approach for early metabolic risk stratification. Enhancing microbial diversity through interventions targeting diet, prebiotics, probiotics, and lifestyle modification may hold potential to delay or prevent progression to T2DM. Further longitudinal and interventional research is warranted.

INTRODUCTION

Prediabetes represents an intermediate metabolic state between normal glucose homeostasis and overt type 2 diabetes mellitus (T2DM), characterised by impaired fasting glucose, impaired glucose tolerance, or elevated glycated haemoglobin levels. Globally, the prevalence of prediabetes has risen sharply, paralleling increases in obesity, sedentary lifestyles, and dietary transitions toward ultra-processed foods.[1] Individuals with prediabetes have a substantially higher lifetime risk of progressing to T2DM and developing cardiovascular and microvascular complications. Despite its clinical relevance, the underlying mechanisms contributing to insulin resistance in this population remain only partially understood, with growing interest in the role of the gut microbiome as a key metabolic regulator.[2] The human gut microbiome comprises trillions of microorganisms, including bacteria, archaea, fungi, and viruses, which contribute to digestion, immune modulation, energy harvesting, and systemic metabolic signalling. A healthy gut microbial ecosystem is characterised by high diversity, stability, and functional redundancy.[3] Alterations in microbial richness often referred to as dysbiosis have been associated with chronic inflammatory disorders,

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obesity, metabolic syndrome, and glucose dysregulation. Studies exploring metabolic disease have demonstrated that specific microbial taxa contribute to pathways affecting insulin sensitivity, including short-chain fatty acid (SCFA) production, intestinal barrier integrity, bile acid metabolism, and systemic endotoxemia.[4]

Mechanistically, SCFAs such as butyrate, propionate, and acetate play vital roles in maintaining epithelial health and regulating host metabolic responses. Reduced abundance of butyrate-producing genera including Faecalibacterium and Roseburia has been consistently observed in individuals with metabolic dysfunction.[5] Conversely, enrichment of pro-inflammatory or mucin-degrading bacteria such as Prevotella and Ruminococcus gnavus has been linked to impaired insulin signalling. Dysbiosis may also increase intestinal permeability, facilitating translocation of lipopolysaccharides, which in turn trigger low-grade systemic inflammation an early driver of insulin resistance.[6]

Advancements in molecular sequencing techniques have refined our understanding of gut microbial ecology. Among these, 16S rRNA PCR sequencing provides a reliable and cost-effective method to quantify microbial diversity, analyse phylogenetic abundance, and identify compositional shifts across disease states. Although several studies have explored microbiome alterations in T2DM, relatively fewer have specifically investigated microbiome–metabolic correlations in prediabetic adults, despite this group representing a crucial window for early intervention.[7,8]

The relationship between microbiome diversity and insulin resistance is particularly relevant in the prediabetic stage, where metabolic dysfunction is still potentially reversible. Early identification of microbial dysbiosis may offer predictive insights into disease progression and enable the development of targeted lifestyle or therapeutic strategies. Population-specific data are essential, as microbiome composition is influenced by dietary habits, ethnicity, environment, antibiotic exposure, and cultural practices. The evaluation of gut microbial diversity using stool PCR sequencing in prediabetic individuals may therefore yield clinically meaningful biomarkers for risk stratification.

Therefore, it is of interest to examine the correlation between gut microbiome diversity and insulin resistance in prediabetic adults using stool PCR sequencing, with the aim of better understanding early metabolic dysregulation and informing potential future interventions.

Objectives

Primary Objective

- To assess the correlation between gut microbiome diversity and insulin resistance in prediabetic adults using 16S rRNA stool PCR sequencing.

Secondary Objectives

- To evaluate differences in alpha diversity (Shannon, Simpson, Chao1 indices) and beta diversity between individuals with varying levels of insulin resistance.
- To identify specific microbial taxa associated with higher or lower HOMA-IR scores in prediabetic adults.
- To analyse the relationship between microbial richness and metabolic parameters including fasting glucose, fasting insulin, BMI, and lipid profile.
- To explore compositional dysbiosis patterns (relative abundance of beneficial vs. pro-inflammatory organisms) across insulin resistance categories.

To generate microbiome-based insights that may support early metabolic risk stratification and future preventive interventions.

MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional observational study conducted among prediabetic adults attending a metabolic health clinic. The study aimed to investigate the association between gut microbiome diversity and insulin resistance using stool PCR sequencing. All participants were enrolled over a defined 12-month period after meeting the eligibility criteria.

Study Population

Inclusion Criteria

- Adults aged 20–60 years
- Diagnosed with prediabetes based on ADA criteria:
    - Impaired fasting glucose (100–125 mg/dL) and/or
    - HbA1c 5.7–6.4%
- Ability and willingness to provide a stool sample and fasting blood sample

Exclusion Criteria

- Known type 2 diabetes mellitus
- Current use of antibiotics, probiotics, prebiotics, or laxatives within the last 3 months
- Chronic gastrointestinal disorders (e.g., inflammatory bowel disease, celiac disease)
- History of gastrointestinal surgery (except appendectomy)
- Pregnant or lactating women
- Known immunosuppressive disorders or active malignancy
- Recent hospitalization or acute infection in the past 1 month

Sample Size Calculation

Sample size was calculated based on detecting a moderate correlation (r = 0.3) between gut microbiome diversity and HOMA-IR with 80%

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power and 5% significance level. The formula used:

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{0.5 \ln\left(\frac{1+r}{1-r}\right)} \right)^2 + 3$$

Where:

- $Z_{1-\alpha/2} = 1.96$
- $Z_{1-\beta} = 0.84$
- Expected correlation $r = 0.3$

This yielded a minimum sample size of 85 participants. Accounting for 10% dropout or unusable samples, a final target of 95 participants was set.

Data Collection Procedure

Clinical and Demographic Assessment

Participants were interviewed and examined using a structured questionnaire to record:

- Age, sex
- Lifestyle habits (dietary pattern, physical activity, sleep duration)
- Anthropometric measurements: height, weight, BMI, waist circumference
- Medical history and medication profile

Biochemical Investigations

After an overnight fast (8–10 hours), venous blood samples were collected to measure:

- Fasting plasma glucose (mg/dL)
- Fasting insulin (µIU/mL)
- Lipid profile (TC, HDL, LDL, triglycerides)
- HbA1c (%)

Assessment of Insulin Resistance

Insulin resistance was calculated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR):

$$\text{HOMA-IR} = \frac{\text{Fasting Glucose (mg/dL)} \times \text{Fasting Insulin (µIU/mL)}}{405}$$

Participants were categorized into tertiles:

- **Low IR**
- **Moderate IR**
- **High IR**

Stool Sample Collection and Processing

Participants provided a fresh stool sample collected in sterile containers. Samples were stored at –80°C until analysis.

DNA Extraction and PCR Amplification

- Microbial DNA was extracted using a standardized commercial kit.
- The V3–V4 region of the bacterial 16S rRNA gene was amplified.
- PCR products were purified, quantified, and prepared for sequencing.

Sequencing and Bioinformatics Workflow

- Sequencing was performed on the Illumina MiSeq platform.
- Quality filtering and denoising were carried out using QIIME2 pipelines.
- Alpha diversity indices (Shannon, Simpson, Chao1) and beta diversity (Bray–Curtis dissimilarity) were computed.
- Taxonomic classification was performed using the SILVA reference database.

Statistical Analysis

- Continuous variables were summarised as mean ± SD; categorical variables as percentages.
- Correlation between microbiome diversity indices and HOMA-IR was analysed using Pearson correlation.
- Multivariate regression was applied to adjust for confounders: age, sex, BMI, dietary habits, and lipid profile.
- Principal coordinates analysis (PCoA) was used to visualise beta-diversity clustering.

A p-value <0.05 was considered statistically significant.

RESULTS

The results of this study demonstrate a clear and consistent inverse relationship between gut microbiome diversity and insulin resistance in prediabetic adults. Across anthropometric, metabolic, diversity, and taxonomic assessments, individuals with higher HOMA-IR values exhibited significantly reduced microbial richness, reduced SCFA-producing capacity, and increased representation of pro-inflammatory, mucin-degrading, and endotoxin-associated microorganisms. These findings collectively indicate that dysbiosis is strongly linked with early metabolic dysfunction and may contribute mechanistically to the progression from prediabetes to type 2 diabetes.

Baseline Characteristics of the Study Population

A total of 95 prediabetic adults were included in the final analysis. The mean age of participants was 48.6 ± 9.4 years, with a slight male predominance (58.9%). The average BMI was 28.1 ± 3.7 kg/m², and nearly two-thirds of the cohort reported predominantly mixed dietary habits. The mean fasting glucose level was 109.4 ± 7.8 mg/dL, and mean HbA1c was 6.02 ± 0.28%, consistent with prediabetes. Lipid parameters showed mildly elevated triglycerides and low HDL levels in a substantial portion of participants, reflecting early metabolic dysregulation.

Prevalence of Insulin Resistance

Using HOMA-IR tertiles, 33 participants each were categorised into low, moderate, and high insulin resistance groups. The overall mean HOMA-IR was 2.98 ± 1.12, with the high IR group demonstrating significantly elevated fasting insulin levels compared with the other groups. BMI, waist circumference, triglycerides, and HbA1c were all higher in individuals within the highest IR tertile.

Gut Microbiome Alpha Diversity

Analysis of alpha diversity revealed a significant inverse relationship between microbial richness and

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insulin resistance. Participants in the high HOMA-IR group demonstrated markedly reduced Shannon, Simpson, and Chao1 diversity scores compared with those in the low IR group. The reduction in diversity was consistent across multiple diversity indices, indicating a genuine narrowing of microbial richness rather than a sequencing artefact. Lower alpha diversity corresponded strongly with higher fasting insulin levels and higher BMI values.

Beta-Diversity and Microbial Community Separation

Beta-diversity assessment using Bray–Curtis dissimilarity displayed clear clustering patterns between low and high IR groups. Principal coordinates analysis (PCoA) plots illustrated distinct separation of microbial communities based on insulin resistance severity, with minimal overlap between the extremes. These findings indicate that not only microbial richness but also overall microbiome composition differed substantially across metabolic states.

Taxonomic Composition and Differential Abundance

Compositional analysis identified significant alterations in taxa associated with metabolic health. Beneficial organisms such as **Akkermansia muciniphila**, **Faecalibacterium prausnitzii**, **Roseburia spp.**, and **Bifidobacterium spp.** were substantially reduced in participants with higher HOMA-IR values. Conversely, pro-inflammatory or dysbiosis-associated taxa—including **Prevotella spp.**, **Ruminococcus gnavus**, and certain **Proteobacteria**—were enriched in the high IR group. These shifts reflected impaired SCFA-producing capacity and increased inflammatory potential in metabolically unhealthy participants.

Correlation of Microbiome Diversity With Insulin Resistance and Metabolic Markers

Correlation analysis demonstrated a **strong inverse association** between Shannon diversity and HOMA-IR ($r \approx -0.41$, $p < 0.01$). Similar negative correlations were observed for Simpson and Chao1 indices. Positive correlations emerged between HOMA-IR and the relative abundance of inflammatory genera (Prevotella, R. gnavus). In multivariable regression models adjusting for BMI, age, sex, and diet, microbial diversity remained an **independent predictor** of insulin resistance. Reduced abundance of SCFA-producing taxa showed the strongest associations with elevated fasting insulin and triglyceride levels.

Patterns of Dysbiosis Across Insulin Resistance Categories

Participants in the high IR tertile exhibited a characteristic dysbiosis pattern marked by reduced gut barrier–protective organisms, lower SCFA-producing capacity, and higher representation of mucin-degrading and pro-inflammatory microbes. Those with low IR showed richer microbial networks, greater phylogenetic diversity, and higher abundance of butyrate-producing taxa. Moderate IR individuals demonstrated intermediate profiles, suggesting a **gradual microbiome shift with worsening metabolic status**.

Table 1. Baseline Demographic Characteristics of the Study Population (n = 95)

Variable	Category	n (%) / Mean ± SD
Age (years)	—	48.6 ± 9.4
Sex	Male	56 (58.9%)
	Female	39 (41.1%)
BMI (kg/m²)	—	28.1 ± 3.7
Waist circumference (cm)	—	94.3 ± 8.1
Dietary pattern	Mixed diet	62 (65.3%)
	Vegetarian	33 (34.7%)

This Table describes the demographic distribution including age, sex, BMI, and lifestyle factors.

Table 2. Glycemic And Metabolic Parameters Of Participants

Parameter	Mean ± SD
Fasting glucose (mg/dL)	109.4 ± 7.8
Fasting insulin (µIU/mL)	11.1 ± 4.3
HOMA-IR	2.98 ± 1.12
HbA1c (%)	6.02 ± 0.28
Total cholesterol (mg/dL)	194.8 ± 34.7
LDL cholesterol (mg/dL)	119.1 ± 28.3
HDL cholesterol (mg/dL)	38.4 ± 6.2
Triglycerides (mg/dL)	168.2 ± 41.6

This Table presents fasting glucose, fasting insulin, HbA1c, and lipid profile values.

Table 3. Distribution Of Participants By Insulin Resistance Categories (HOMA-IR Tertiles)

Category	HOMA-IR Range	n (%)
Low IR	<2.1	33 (34.7%)
Moderate IR	2.1–3.4	32 (33.7%)
High IR	>3.4	30 (31.6%)

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This Table categorizes participants by low, moderate, and high insulin resistance levels.

Table 4. Comparison Of Anthropometric Measures Across Insulin Resistance Groups

Variable	Low IR (n=33)	Moderate IR (n=32)	High IR (n=30)	p-value
BMI (kg/m²)	26.9 ± 3.1	28.4 ± 3.5	29.2 ± 3.9	<0.05
Waist circumference (cm)	90.1 ± 7.3	94.6 ± 8.4	98.2 ± 7.9	<0.01

This Table compares BMI and waist circumference across IR tertiles.

Table 5. Alpha Diversity Indices Across Insulin Resistance Groups

Diversity Index	Low IR	Moderate IR	High IR	p-value
Shannon Index	4.21 ± 0.33	3.89 ± 0.41	3.52 ± 0.38	<0.001
Simpson Index	0.92 ± 0.04	0.88 ± 0.05	0.81 ± 0.06	<0.001
Chao1 Richness	147.3 ± 18.6	132.8 ± 17.5	118.4 ± 16.9	<0.001

This Table presents Shannon, Simpson, and Chao1 diversity across IR categories.

Table 6. Beta-Diversity (Bray–Curtis Dissimilarity) Cluster Separation Statistics

Comparison	F-value	R^2	p-value
Low IR vs High IR	3.42	0.19	<0.01
Low IR vs Moderate IR	2.14	0.11	<0.05
Moderate IR vs High IR	2.78	0.15	<0.01

This Table shows PERMANOVA results supporting group-wise microbial community separation.

Table 7. Relative Abundance Of Key Beneficial Microbial Taxa Across IR Groups

Taxa	Low IR (%)	Moderate IR (%)	High IR (%)	p-value
Akkermansia muciniphila	4.8	3.2	1.9	<0.01
Faecalibacterium prausnitzii	7.1	5.4	3.1	<0.001
Roseburia spp.	5.3	4.1	2.7	<0.01
Bifidobacterium spp.	3.9	2.8	1.6	<0.05

This Table shows taxa associated with metabolic benefits and their reduced abundance in high IR participants.

Table 8. Relative Abundance of Dysbiosis-Associated Taxa Across IR Groups

Taxa	Low IR (%)	Moderate IR (%)	High IR (%)	p-value
Prevotella spp.	6.4	8.7	12.3	<0.01
Ruminococcus gnavus	2.1	3.4	5.6	<0.001
Proteobacteria (overall)	4.2	5.3	7.1	<0.05

This Table describes enrichment of pro-inflammatory taxa in high IR individuals.

Table 9. Correlation Between Microbiome Diversity and Metabolic Parameters

Parameter	Shannon r	Simpson r	Chao1 r	p-value (all)
HOMA-IR	–0.41	–0.39	–0.36	<0.01
Fasting insulin	–0.44	–0.42	–0.38	<0.01
Triglycerides	–0.28	–0.26	–0.23	<0.05
BMI	–0.31	–0.29	–0.24	<0.05

This Table shows correlation coefficients of diversity indices with metabolic markers.

Table 10. Multivariable Regression Analysis Predicting Insulin Resistance

Variable	β Coefficient	95% CI	p-value
Shannon diversity	–0.34	–0.52 to –0.18	<0.001
BMI (kg/m²)	0.27	0.11 to 0.41	<0.01
Triglycerides (mg/dL)	0.19	0.07 to 0.34	<0.05
Age (years)	0.06	–0.04 to 0.16	0.24
Sex	0.03	–0.09 to 0.15	0.61
Dietary pattern	0.08	–0.03 to 0.20	0.18

This Table identifies independent predictors of HOMA-IR after adjusting for confounders.

Table 11. Scfa-Producing Capacity Estimated from Key Microbial Genera

SCFA-Related Taxa	Low IR (%)	Moderate IR (%)	High IR (%)	p-value
Butyrate producers (*Faecalibacterium*, *Roseburia*)	12.4	9.5	5.8	<0.001
Propionate producers (*Bacteroides spp.*)	7.9	6.2	4.1	<0.01
Total SCFA-producing bacterial abundance	20.3	15.7	11.0	<0.001

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This table presents estimated short-chain fatty acid (SCFA) production potential based on abundances of major butyrate- and propionate-producing bacteria.

Table 12. Intestinal Permeability And Endotoxin-Related Taxa Abundance

Taxa/System Marker	Low IR (%)	Moderate IR (%)	High IR (%)	p-value
Ruminococcus gnavus (mucin degrader)	2.1	3.4	5.6	<0.001
Enterobacteriaceae (endotoxin-producing)	1.9	3.2	4.9	<0.01
Gram-negative LPS-rich taxa (overall)	5.1	6.8	9.3	<0.01

This table shows microbiome profiles associated with compromised gut barrier function and endotoxemia.

Table 1: This table highlights that the study population largely consisted of overweight, middle-aged adults with a mixed dietary pattern, reflecting a typical prediabetic risk profile. A male predominance and elevated BMI suggest a population vulnerable to metabolic disturbances. **Table 2:** This table indicates that participants exhibited glycemic and lipid abnormalities consistent with insulin resistance. Elevated triglycerides, low HDL levels, and raised fasting insulin levels support the metabolic risk and inflammatory burden associated with prediabetes. **Table 3:** This table categorizes participants by insulin resistance severity, demonstrating an even distribution across tertiles. This balanced stratification enabled robust comparative analyses of microbiome diversity and composition. **Table 4:** This table shows a significant increase in BMI and waist circumference across ascending IR categories. Central adiposity a driver of systemic inflammation correlates strongly with worsening insulin resistance and likely influences microbiome alterations. **Table 5:** This table demonstrates a sharp decline in Shannon, Simpson, and Chao1 indices with increasing IR. These metrics confirm that microbial richness, evenness, and phylogenetic variety are progressively reduced in metabolically unhealthy individuals. **Table 6:** This table provides PERMANOVA-based evidence of distinct microbial community clustering across IR categories. Clear separation between low and high IR groups indicates that dysbiosis is not random but follows a quantifiable pattern. **Table 7:** This table shows diminished abundance of metabolically protective taxa such as *Akkermansia*, *Faecalibacterium*, *Roseburia*, and *Bifidobacterium*. These bacteria maintain gut barrier integrity and regulate SCFA production, explaining their association with lower insulin resistance. **Table 8:** This table confirms enrichment of dysbiosis-associated organisms (*Prevotella*, *Ruminococcus gnavus*, and Proteobacteria) in high IR individuals. These taxa are linked to inflammation, mucin degradation, and metabolic endotoxemia, all promoting insulin resistance. **Table 9:** This table displays strong negative correlations between microbiome diversity and metabolic markers, especially HOMA-IR and fasting insulin. These findings support diversity indices as potential biomarkers of early metabolic impairment. **Table 10:** This table shows that even after adjusting for BMI, age, sex, and triglycerides, Shannon diversity remains an independent predictor of insulin resistance. This reinforces the physiological relevance of microbial diversity beyond anthropometric factors. **Table 11:** This table illustrates a marked decline in SCFA-producing taxa in the high IR group, indicating impaired metabolic signaling, reduced anti-inflammatory potential, and compromised epithelial health mechanisms known to drive insulin resistance. **Table 12:** This table demonstrates increased abundance of endotoxin-producing and mucin-degrading organisms in participants with high IR. Elevated LPS-rich taxa and mucin degraders suggest compromised intestinal permeability ("leaky gut"), which contributes to systemic inflammation and insulin resistance.

DISCUSSION

This study investigated the association between gut microbiome diversity and insulin resistance in prediabetic adults using stool-based 16S rRNA PCR sequencing. The findings demonstrate a strong inverse relationship between microbial diversity and metabolic dysfunction, with higher HOMA-IR scores consistently linked to lower alpha diversity, distinct beta-diversity clustering, depletion of SCFA-producing taxa, and enrichment of pro-inflammatory and mucin-degrading organisms. Taken together, the results underline the central role of gut microbial ecology in shaping early metabolic homeostasis and highlight dysbiosis as a potentially modifiable determinant in the progression from prediabetes to type 2 diabetes mellitus (T2DM).[9,10]

Reduced microbial diversity emerged as one of the most prominent features in participants with higher insulin resistance. Shannon, Simpson, and Chao1 indices all declined progressively across IR categories, suggesting that individuals at greater metabolic risk have a narrower microbial repertoire, reduced phylogenetic breadth, and a loss of ecological balance.[11] Diversity is a fundamental characteristic of a healthy gut ecosystem because it ensures metabolic redundancy, resilience against perturbations, and broader functional capacity. Reduced diversity has previously been linked to obesity, inflammatory disorders, and metabolic syndrome, and the current findings extend this relationship specifically to prediabetic adults. Importantly, multivariable regression analysis confirmed that microbial diversity independently predicted HOMA-IR even after adjusting for BMI, age, sex, and lipid levels, indicating a direct

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metabolic influence rather than a simple byproduct of obesity.[12]

Distinct separation in community composition between low and high IR groups further reinforces the presence of structured dysbiosis. Beta-diversity analysis showed clear clustering, indicating that insulin resistance is associated not only with reduced richness but also with fundamentally altered microbial community architecture. Such compositional shifts are biologically meaningful because they reflect changes in dominant ecological niches, metabolic pathways, and host–microbe interactions.[13]

One of the central mechanistic insights of this study relates to short-chain fatty acid (SCFA) production. Butyrate- and propionate-producing taxa particularly Faecalibacterium prausnitzii, Roseburia spp., and Bacteroides spp. were significantly reduced in participants with high HOMA-IR. SCFAs play key roles in maintaining epithelial barrier integrity, regulating incretin secretion, improving mitochondrial function, and modulating systemic insulin sensitivity. Their depletion therefore contributes to impaired metabolic flexibility, heightened inflammation, and altered energy utilization. The reduction in SCFA-producing capacity observed here aligns with established evidence linking dysbiosis to metabolic inflammation and insulin resistance.[14]

Alongside the loss of beneficial taxa, the enrichment of dysbiosis-associated and pro-inflammatory organisms including Prevotella spp., Ruminococcus gnavus, Enterobacteriaceae, and other LPS-rich taxa was a defining feature of high IR individuals. These organisms contribute to metabolic dysfunction through multiple pathways. R. gnavus, a mucin-degrading bacterium, weakens the protective mucus layer and increases epithelial vulnerability. Increased abundance of Enterobacteriaceae and other gram-negative taxa contributes to elevated lipopolysaccharide (LPS) burden, a key driver of metabolic endotoxemia.[15] Low-grade systemic inflammation initiated by LPS can disrupt insulin signaling pathways and is recognized as a crucial step in the transition from normoglycemia to insulin resistance. The findings of this study support this mechanistic model, demonstrating that individuals with higher IR exhibit a microbiome skewed toward organisms that compromise barrier function and promote inflammation.[16]

Anthropometric and biochemical correlates showed expected patterns, with BMI, waist circumference, triglycerides, and fasting insulin rising across IR categories. However, the persistence of microbiome associations even after adjusting for these variables underscores the potential role of microbial diversity and composition as independent metabolic regulators. This independence is clinically meaningful, as it suggests that microbiome profiling may offer predictive value beyond conventional markers in identifying individuals at risk for metabolic deterioration.[17,18]

The cross-sectional nature of this study allows identification of associations but not determination of causality. However, the findings align with mechanistic and interventional research indicating that microbiome-directed strategies such as dietary fiber enrichment, prebiotic supplementation, probiotic therapies, and lifestyle modification can improve insulin sensitivity.[19] Moreover, emerging trials on fecal microbiota transplantation (FMT) provide proof-of-concept evidence that manipulation of gut microbial communities can alter metabolic outcomes. Although such interventions were not part of the current study, the results presented here offer a compelling rationale for future randomized trials targeting the microbiome in prediabetes.[20]

Another strength of this study is the use of 16S rRNA PCR sequencing, which provides robust taxonomic profiling and reliable diversity metrics. Population-specific microbiome data remain scarce, particularly in regions undergoing rapid nutritional and lifestyle transitions. Thus, the data generated hold value not only for understanding pathophysiology but also for informing culturally tailored interventions.

Nevertheless, certain limitations merit consideration. The single-time-point sampling does not account for intra-individual microbiome variability. Diet, although recorded as a broad category, was not quantified using detailed food frequency questionnaires. Lifestyle factors such as sleep quality, stress, and physical activity, all of which influence the microbiome–metabolic axis, were not deeply evaluated. Future longitudinal and interventional studies are needed to clarify directionality, establish causality, and test whether microbiome modification can halt or reverse the progression to T2DM.

In conclusion, this study demonstrates that reduced gut microbiome diversity, diminished SCFA-producing capacity, and increased abundance of pro-inflammatory microbial taxa are strongly associated with insulin resistance in prediabetic adults. These findings highlight dysbiosis as a significant metabolic determinant and suggest that microbiome-based assessment may enhance early metabolic risk stratification. Interventions aimed at restoring microbial diversity represent a promising avenue for preventive strategies targeting the rising global burden of T2DM.

CONCLUSION

Gut microbiome diversity demonstrates a clear and clinically meaningful inverse association with insulin resistance in prediabetic adults, with reduced richness, diminished SCFA-producing capacity, and enrichment of pro-inflammatory taxa characterizing individuals with higher HOMA-IR values. These findings position microbial dysbiosis as an independent and biologically relevant contributor to early metabolic dysfunction. Strengthening gut microbial diversity may offer a promising preventive

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strategy to delay or halt progression to type 2 diabetes.

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