

# Gut Microbiota Alterations, Biochemical Markers, and Dietary Patterns in Type 2 Diabetes Mellitus: A Cross-Sectional Study in Sokoto, Nigeria

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## Abstract

**Background:** Type 2 diabetes mellitus (T2DM) is increasingly linked to gut microbiota dysbiosis, yet the relationship between microbial alterations, specific biochemical markers, and dietary patterns remains underexplored in African populations. This study examined associations between gut microbiota composition, circulating pro-inflammatory cytokines, fecal calprotectin, and dietary patterns in T2DM patients in Sokoto, Nigeria. **Methods:** A cross-sectional study enrolled 126 participants (63 T2DM patients and 63 age- and sex-matched healthy controls). We quantified gut microbiota (*Bacteroidetes* and *Firmicutes*) using qPCR. We measured plasma levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), as well as fecal calprotectin, using ELISA. We assessed dietary patterns using a structured questionnaire. We assessed associations between microbial abundance and biomarkers using Spearman's correlation, and analyzed clinical/dietary factors associated with dysbiosis using chi-square tests and logistic regression. **Results:** Among T2DM patients, 51 (81.0%) exhibited gut dysbiosis, characterized by an elevated *Firmicutes/Bacteroidetes* (F/B) ratio. Participants with dysbiosis had significantly higher median concentrations of TNF- $\alpha$  (41.41 ng/L), IL-6 (2.34 pg/mL), and fecal calprotectin (47.05 pg/mL) than controls ( $p < 0.05$  for all). Hyperglycemia showed the strongest association with dysbiosis ( $\chi^2 = 107.47$ ,  $p < 0.001$ ). Dietary analysis showed that rice/swallow consumption at breakfast was associated with 83.3% dysbiosis prevalence ( $\chi^2 = 13.321$ ,  $p = 0.038$ ), while 100% of participants who consumed oats at dinner had dysbiosis ( $\chi^2 = 53.784$ ,  $p < 0.001$ ). In dysbiotic patients, the F/B ratio correlated strongly with TNF- $\alpha$  ( $r = 0.782$ ,  $p = 0.001$ ), moderately with IL-6 ( $r = 0.352$ ,  $p = 0.032$ ) and faecal calprotectin ( $r = -0.530$ ,  $p = 0.001$ ). We observed no significant correlations in the eubiotic or control groups. **Conclusion:** Gut dysbiosis in T2DM is associated with elevated systemic inflammation, altered microbial metabolites, intestinal inflammation, and specific dietary patterns. These findings support the gut–systemic inflammatory axis and highlight the potential for microbiome-targeted dietary interventions in T2DM management, particularly in resource-limited settings.

**Keywords:** Type 2 diabetes mellitus, gut dysbiosis, *Firmicutes/Bacteroidetes* ratio, TNF- $\alpha$ , TMAO, branched-chain amino acids, fecal calprotectin, dietary patterns, Nigeria

## Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent Hyperglycemia due to impaired insulin secretion, insulin resistance, or both [1]. With an estimated 589 million adults affected globally in 2024, and projections rising to 853 million by 2050, T2DM represents a growing public health crisis, particularly in low- and middle-income countries [2]. Nigeria's diabetes prevalence is estimated at 4.3%, affecting approximately 5.5 million individuals [2].

The gut microbiota—a complex community of trillions of microorganisms in the gastrointestinal tract—plays a crucial role in host metabolism, immune modulation, and maintaining intestinal barrier integrity [3,4]. The phyla *Bacteroidetes* and *Firmicutes* account for approximately 90% of the total human gut microbiota, and their relative abundance serves as a key indicator of microbial balance [5]. A clinical laboratory index to assess gut dysbiosis is the F/B ratio, with values exceeding 0.8–1.0 indicating imbalance.

Emerging evidence suggests that gut microbiota dysbiosis contributes to T2DM pathogenesis through multiple mechanisms, including metabolic endotoxemia, chronic low-grade inflammation, and altered microbial metabolite production [6,7]. Dysbiosis compromises intestinal barrier integrity, allowing bacterial lipopolysaccharide (LPS) to translocate into systemic circulation, activate Toll-like receptor 4 (TLR4) on immune cells, and trigger pro-inflammatory cytokine release (TNF- $\alpha$ , IL-6) [8,9]. This "metabolic endotoxemia" perpetuates insulin resistance and  $\beta$ -cell dysfunction [10].

Microbial metabolites have emerged as critical mediators linking gut dysbiosis to metabolic dysfunction. Trimethylamine N-oxide (TMAO), derived from dietary choline and carnitine through gut microbial metabolism, is associated with insulin resistance and cardiovascular risk in T2DM [11,12]. Similarly, elevated circulating branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine—are recognized as strong biomarkers of insulin resistance and future diabetes risk, and gut microbiota composition influences host BCAA metabolism [13,14]. Fecal calprotectin, a marker of intestinal inflammation, is elevated in T2DM patients, suggesting subclinical gut inflammation that may exacerbate systemic insulin resistance [15].

Dietary patterns are increasingly recognized as key modulators of gut microbiota composition and function. Traditional African diets, characterized by high fiber from whole grains, legumes, and fibrous vegetables, promote gut lining integrity and microbial diversity. Conversely, urbanization-driven dietary transitions towards processed foods and refined carbohydrates are associated with reduced microbial diversity and increased risk of non-communicable diseases. In Nigeria, common dietary staples include rice and "swallow" (stiff porridges made from yam, cassava, or grains), which vary considerably in their fiber content and glycaemic impact. Understanding how these dietary patterns relate to gut dysbiosis in Nigerian T2DM patients is critical for developing culturally appropriate interventions.

Despite growing evidence linking gut microbiota alterations to T2DM, significant gaps remain in understanding these relationships in African populations. Most studies have focused on broad taxonomic shifts rather than specific microbial–biomarker associations, and data on gut microbiota changes and their dietary determinants in T2DM patients in Northern Nigeria remain limited. This study aimed to evaluate the association between gut microbiota alterations (Bacteroidetes and Firmicutes), biochemical markers (TNF- $\alpha$ , IL-6, BCAAs, TMAO, and fecal calprotectin), and dietary patterns in T2DM patients in Sokoto metropolis, Nigeria.

## Materials and Methods

### Study Area and Population

The study was conducted at Specialist Hospital, Sokoto (SHS) and Maryam Abacha Women and Children Hospital (MAWCH), Sokoto, Nigeria. The study enrolled 126 participants: 63 T2DM patients and 63 age- and sex-matched apparently healthy controls recruited from staff and students of Usmanu Danfodiyo University, Sokoto.

### Inclusion and Exclusion Criteria

Inclusion criteria: male and female patients diagnosed with T2DM, aged  $\geq 18$  years, who provided informed consent. Exclusion criteria: type 1 diabetes; comorbid conditions known to cause gut dysbiosis (autoimmune diseases, inflammatory bowel disease); antibiotic use within 4 weeks; chemotherapy; gastrointestinal surgery; pregnancy; and lactation.

### Sample Size Determination

We calculated the sample size using the formula for analytical studies involving two groups, yielding 63 participants per group (total  $N = 126$ ). The calculation assumed 80% power and an alpha level of 0.05, based on an expected moderate effect size for microbiome–biomarker associations.

### Ethical Approval

The Ethics and Research Committee of Usmanu Danfodiyo University Sokoto and the Sokoto State Ministry of Health approved the study. The study followed the Declaration of Helsinki.

### Blood Sample Collection and Processing

Venous blood (5.0 mL) was collected into EDTA tubes, centrifuged at 3000 rpm for 5 minutes, and plasma stored at  $-20^{\circ}\text{C}$  until analysis.

## Stool Sample Collection and DNA Extraction

Fresh stool samples were collected in sterile containers and stored at 4°C until analysis. DNA was extracted using a QIAGEN extraction kit following the manufacturer's instructions.

## Quantification of Gut Microbiota

Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix (Vazyme Biotech) with primers specific for Firmicutes (Firm934F/Firm1060R) and Bacteroidetes (Bac960F/Bac1100R) [16]. Cycling conditions: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of 95°C for 3 seconds and 60°C for 40 seconds. Bacterial abundance was expressed as log<sub>10</sub> colony-forming units (CFU) per gram of feces.

## Biochemical Analysis

Plasma TNF- $\alpha$ , IL-6, as well as fecal calprotectin, were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturers' protocols. Absorbance was read at 450 nm using a microplate reader, and concentrations were calculated from standard curves.

## Dietary Assessment

Dietary patterns were assessed using a structured questionnaire administered by trained research assistants. Participants reported their typical food choices for breakfast, lunch, and dinner, with response options including rice/swallow, oats, tea, gruel, and vegetables/fibre/fruits. The questionnaire was designed to capture predominant dietary patterns in the study population and was pilot-tested with 10 participants before the main study.

## Definitions and Classifications

Gut dysbiosis: Bacterial load >75th percentile of the normal control group. This threshold was selected to identify individuals with substantially elevated bacterial loads relative to the healthy reference population, consistent with the concept that dysbiosis involves deviation from the normal range.

Gut eubiosis: Bacterial load <75th percentile of the normal control group.

BMI classification: Normal weight (18.5–24.9 kg/m<sup>2</sup>), Overweight (25.0–29.9 kg/m<sup>2</sup>), Obesity Class I (30.0–34.9 kg/m<sup>2</sup>).

Glycaemic status: Normoglycaemia (fasting blood glucose 3.9–5.8 mmol/L), Hyperglycemia (>5.8 mmol/L).

## Statistical Analysis

Data were analyzed using SPSS version 27. Numerical variables were presented as median and interquartile range (IQR). We used the Kruskal-Wallis H test for group comparisons, with Dunn's post-hoc test for multiple comparisons. Effect sizes for Kruskal-Wallis tests were estimated using eta-squared ( $\eta^2$ ), calculated as  $\eta^2 H = (H - k + 1)/(N - k)$ , where  $k$  is the number of groups and  $N$  is the total sample size. We interpreted values of 0.01, 0.06, and 0.14 as small, medium, and large effect sizes, respectively.

We assessed associations between categorical variables using Pearson's chi-square test. We further analyzed significant associations using binary or multinomial logistic regression, adjusting for age, sex, and BMI. We used Spearman's rho to evaluate relationships between microbial abundance and biochemical markers in the full T2DM group (regardless of dysbiosis status) to avoid selection bias. We set statistical significance at  $p < 0.05$ .

## Result and Discussion

### Results

#### Demographic and Clinical Characteristics

We enrolled 126 participants: 80 females (63.5%) and 46 males (36.5%). Most participants (57.9%) were aged 46–60 years. Over half (54.8%) were overweight, and 46.0% presented with Hyperglycemia. Among T2DM patients, 51 (81.0%) exhibited gut dysbiosis, while only 12 (19.0%) showed eubiosis (Table 1).

**Table 1.** Demographic and Clinical Characteristics of Study Participants

| Characteristic            | Category        | T2DM Patients (n = 63) | Controls (n = 63) | p-value |
|---------------------------|-----------------|------------------------|-------------------|---------|
| <b>Gender</b>             | Male            | 23 (36.5)              | 23 (36.5)         | 1.000   |
|                           | Female          | 40 (63.5)              | 40 (63.5)         |         |
| <b>Age (years)</b>        | 31–45           | 16 (25.4)              | 15 (23.8)         | 0.967   |
|                           | 46–60           | 36 (57.1)              | 37 (58.7)         |         |
|                           | >60             | 11 (17.5)              | 11 (17.5)         |         |
| <b>BMI Classification</b> | Normal Weight   | 20 (31.7)              | 25 (39.7)         | 0.624   |
|                           | Overweight      | 36 (57.1)              | 33 (52.4)         |         |
|                           | Obesity Class I | 7 (11.1)               | 5 (7.9)           |         |
| <b>Glycaemia</b>          | Normoglycaemia  | 5 (7.9)                | 63 (100.0)        | <0.001  |
|                           | Hyperglycaemia  | 58 (92.1)              | 0 (0.0)           |         |
| <b>F/B Ratio Status</b>   | Gut Eubiosis    | 12 (19.0)              | —                 | —       |
|                           | Gut Dysbiosis   | 51 (81.0)              | —                 | —       |

### Quantification of Gut Microbiota

Based on *Bacteroidetes* quantification, we classified 49 participants (38.9%) as having dysbiosis. Based on *Firmicutes* quantification, we classified 47 participants (37.3%) as having dysbiosis. The *Firmicutes/Bacteroidetes* (F/B) ratio was significantly elevated in the dysbiosis group (median 1.496, IQR: 1.339–2.005) compared to both eubiosis (0.928, IQR: 0.858–0.964) and control (0.932, IQR: 0.868–0.990) groups ( $H = 86.657$ ,  $p < 0.001$ ,  $\eta^2H = 0.688$ , large effect).

### Pro-inflammatory Cytokines

Plasma TNF- $\alpha$  concentrations differed significantly across groups ( $H = 6.370$ ,  $p = 0.041$ ,  $\eta^2H = 0.043$ , small-to-moderate effect). The dysbiosis group had the highest median TNF- $\alpha$  concentration (41.406 ng/L, IQR: 38.460–46.002 ng/L), compared with eubiosis (38.934 ng/L, IQR: 37.502–46.683 ng/L) and controls (39.480 ng/L, IQR: 35.760–43.294 ng/L).

Similarly, IL-6 concentrations differed significantly across groups ( $H = 8.854$ ,  $p = 0.012$ ,  $\eta^2H = 0.063$ , moderate effect), with the dysbiosis group showing the highest median value (2.342 pg/mL, IQR: 1.930–2.702 pg/mL) compared to eubiosis (2.107 pg/mL, IQR: 1.859–2.945 pg/mL) and controls (1.950 pg/mL, IQR: 1.580–2.429 pg/mL).

### Microbial Metabolites and Intestinal Inflammation

Plasma BCAAs concentrations showed highly significant differences across groups ( $H = 41.774$ ,  $p < 0.001$ ,  $\eta^2H = 0.323$ , large effect), with the dysbiosis group demonstrating the highest median value (12.538 pg/mL, IQR: 11.690–14.570 pg/mL) compared to eubiosis (11.550 pg/mL, IQR: 9.294–13.844 pg/mL) and controls (9.248 pg/mL, IQR: 7.462–10.640 pg/mL).

Fecal calprotectin levels also differed significantly ( $H = 11.582$ ,  $p = 0.003$ ,  $\eta^2H = 0.078$ , moderate effect), with the dysbiosis group showing the highest median concentration (47.045 pg/mL, IQR: 42.990–60.280 pg/mL) compared to controls (40.462 pg/mL).

### Factors Associated with Gut Dysbiosis

We observed significant associations between gut dysbiosis and glycaemic status ( $\chi^2 = 107.472$ ,  $p < 0.001$ ). Among hyperglycaemic participants, 47 (81.0%) had dysbiosis. Hyperglycemia was associated with increased odds of dysbiosis (adjusted odds ratio [AOR] = 2.30, 95% CI: 1.33–3.98, adjusted for age, sex, and BMI).

BMI classification was significantly associated with dysbiosis ( $\chi^2 = 12.297$ ,  $p = 0.015$ ). Normal-weight participants had the highest proportion of dysbiosis (55.6%), while only 27.5% of overweight participants had dysbiosis. Oral hypoglycaemic agent (OHA) use was strongly associated with dysbiosis ( $\chi^2 = 107.471$ ,  $p < 0.001$ ; AOR = 2.41, 95% CI: 1.92–3.81). All 63 OHA users had T2DM; among them, 51 (82.4%) had

dysbiosis and 12 (17.6%) had eubiosis.

### Dietary Patterns and Gut Dysbiosis

Dietary analysis revealed significant associations between meal-specific food choices and gut dysbiosis status (Table 2).

**Table 2.** Association between Dietary Patterns and Gut Dysbiosis

| Meal             | Food Category           | Frequency n (%) | Eubiosis n (%) | Dysbiosis n (%) | Control n (%) | $\chi^2$ | p-value | AOR (95% CI)       |
|------------------|-------------------------|-----------------|----------------|-----------------|---------------|----------|---------|--------------------|
| <b>Breakfast</b> | Rice/swallow            | 6 (4.8)         | 1 (16.7)       | 5 (83.3)        | 0 (0.0)       | 13.321   | 0.038   | 0.31 (0.21 – 0.91) |
|                  | Oats                    | 21 (16.7)       | 3 (14.3)       | 4 (19.0)        | 14 (66.7)     |          |         | 0.45 (0.31 – 0.98) |
|                  | Tea                     | 46 (36.5)       | 3 (6.5)        | 16 (34.8)       | 27 (58.7)     |          |         | 1.21 (1.25 – 3.32) |
|                  | Gruel                   | 53 (42.1)       | 5 (9.4)        | 26 (49.1)       | 22 (41.5)     |          |         | 2.05 (2.56 – 3.78) |
| <b>Lunch</b>     | Rice/Swallow            | 94 (74.6)       | 7 (7.5)        | 24 (25.5)       | 63 (67.0)     | 44.594   | <0.001  | 2.45 (3.67 – 4.76) |
|                  | Oats                    | 3 (2.4)         | 0 (0.0)        | 3 (100.0)       | 0 (0.0)       |          |         | 1.29 (0.98 – 3.04) |
|                  | Vegetables/Fibre/Fruits | 29 (23.0)       | 5 (17.2)       | 24 (82.8)       | 0 (0.0)       |          |         | 1.98 (0.99 – 4.67) |
| <b>Dinner</b>    | Rice/Swallow            | 80 (63.5)       | 9 (11.3)       | 39 (48.7)       | 32 (40.0)     | 53.784   | <0.001  | 1.67 (1.57 – 1.77) |

|                |                         |           |          |           |         |        |       |       |
|----------------|-------------------------|-----------|----------|-----------|---------|--------|-------|-------|
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 3.67) |
|                | Oats                    | 5 (4.0)   | 0 (0.0)  | 5 (100.0) | 0 (0.0) |        |       | 0.98  |
|                |                         |           |          |           |         |        |       | (0.87 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 2.65) |
|                | Vegetables/Fibre/Fruits | 32 (25.4) | 0 (0.0)  | 1 (3.1)   | 31      |        |       | 0.67  |
|                |                         |           |          |           | (96.9)  |        |       | (0.37 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 2.09) |
|                | Tea                     | 2 (1.6)   | 0 (0.0)  | 2 (100.0) | 0 (0.0) |        |       | 2.07  |
|                |                         |           |          |           |         |        |       | (1.05 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 4.45) |
|                | Gruel                   | 7 (5.6)   | 3 (42.9) | 4 (57.1)  | 0 (0.0) |        |       | 1.08  |
|                |                         |           |          |           |         |        |       | (0.97 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 2.89) |
| <b>OHA Use</b> | No                      | 63 (50.0) | 0 (0.0)  | 0 (0.0)   | 63      | 107.47 | <0.00 | 2.41  |
|                |                         |           |          |           | (100.0) | 1      | 1     | (1.92 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 3.81) |
|                | Yes                     | 63 (50.0) | 12       | 51 (82.4) | 0 (0.0) |        |       | 1.63  |
|                |                         |           | (17.6)   |           |         |        |       | (0.87 |
|                |                         |           |          |           |         |        |       | —     |
|                |                         |           |          |           |         |        |       | 1.77) |

Association determined using Pearson's chi-square test. We further analyzed significant associations using binary or multinomial logistic regression, adjusted for age, sex, and BMI. AOR = Adjusted Odds Ratio; CI = Confidence Interval.

Participants who consumed rice/swallow as breakfast had the highest prevalence of dysbiosis (83.3%), and this association was statistically significant ( $\chi^2 = 13.321$ ,  $p = 0.038$ ). Those who consumed vegetables/fruits at lunch had an 82.8% prevalence of dysbiosis ( $\chi^2 = 44.594$ ,  $p < 0.001$ ). Notably, 100% of participants who consumed oats for dinner had dysbiosis ( $\chi^2 = 53.784$ ,  $p < 0.001$ ), and 100% of those who consumed oats for lunch also had dysbiosis. Gruel for breakfast was associated with a 49.1% prevalence of dysbiosis. By BMI, 55.6% of dysbiosis cases occurred among normal-weight individuals, while only 27.5% of overweight participants had dysbiosis (63.8% were healthy controls). Gender ( $p = 0.250$ ) and age ( $p = 0.168$ ) were not significantly associated.

### Correlations between Microbial Abundance and Biomarkers

Among T2DM patients (regardless of dysbiosis status), the F/B ratio showed significant correlations with multiple biomarkers (Table 3). The F/B ratio showed a strong positive correlation with TNF- $\alpha$  ( $r = 0.782$ ,  $p = 0.001$ ), moderate positive correlations with IL-6 ( $r = 0.352$ ,  $p = 0.032$ ) and TMAO ( $r = 0.407$ ,  $p = 0.002$ ), and negative correlations with BCAAs ( $r = -0.452$ ,  $p = 0.008$ ) and fecal calprotectin ( $r = -0.53$ ,  $p = 0.001$ ). We observed negative correlations with BCAAs ( $r = -0.452$ ,  $p = 0.008$ ) and fecal calprotectin ( $r =$

-0.530,  $p = 0.001$ ). No significant correlations were observed in the control group.

**Table 3.** Correlations between Firmicutes/Bacteroidetes Ratio and Biomarkers in T2DM Patients

| Biomarker           | r (Spearman's rho) | p-value |
|---------------------|--------------------|---------|
| TNF- $\alpha$       | 0.782*             | 0.001   |
| IL-6                | 0.352*             | 0.032   |
| Faecal calprotectin | -0.530*            | 0.001   |
| BMI                 | 0.153              | 0.092   |
| Fasting blood sugar | -0.340*            | 0.040   |

*Spearman's correlation is significant at  $p < 0.05$ . FCALPRO = fecal calprotectin; FBS = fasting blood sugar.*

## Discussion

This study provides novel insights into the association between gut microbiota alterations, biochemical markers, and dietary patterns in T2DM patients in Sokoto, Nigeria. The findings show that gut dysbiosis characterized by an elevated Firmicutes/Bacteroidetes ratio is prevalent among T2DM patients and associated with increased systemic inflammation, altered microbial metabolites, intestinal inflammation, and specific dietary patterns.

The high prevalence of dysbiosis among T2DM patients (81.0%) aligns with international studies reporting gut microbial alterations in metabolic diseases [17,18]. Our observation that dysbiosis was more frequently identified through Bacteroidetes quantification than Firmicutes suggests that dysbiosis in this population may involve expansion of specific Bacteroidetes taxa rather than simple phylum-level shifts. This finding supports emerging research indicating that the Firmicutes/Bacteroidetes ratio alone may be insufficient to capture the complexity of dysbiosis, and absolute quantification of specific taxa is necessary [19].

The elevated TNF- $\alpha$  and IL-6 concentrations in dysbiotic patients support the gut–systemic inflammatory axis hypothesis. This finding is consistent with mechanistic studies demonstrating that dysbiosis compromises intestinal barrier integrity, enabling translocation of bacterial LPS, which activates TLR4 on immune cells and triggers pro-inflammatory cytokine production [8,9]. Our results directly translate this mechanistic model to a clinical T2DM population in Nigeria, corroborating previous Nigerian studies that reported elevated inflammatory markers in T2DM patients [20].

The dietary findings provide important insights into modifiable risk factors for gut dysbiosis in T2DM. The observation that rice/swallow consumption at breakfast was associated with 83.3% dysbiosis prevalence is concerning, as these foods are staple components of Nigerian diets. Rice and swallow foods (such as pounded yam, eba, and fufu) are high in rapidly digestible carbohydrates and low in fiber, which may promote the growth of pro-inflammatory microbial taxa at the expense of beneficial fiber-fermenting bacteria. Conversely, the finding that 100% of participants consuming oats at dinner or lunch exhibited dysbiosis was unexpected and warrants cautious interpretation. Oats are generally considered beneficial for gut health because gut bacteria ferment their high  $\beta$ -glucan content to produce short-chain fatty acids. However, in this population, oats consumption may reflect dietary modification after T2DM diagnosis, with individuals with more severe disease adopting oats as a "healthy" alternative while already harboring established dysbiosis. Alternatively, the very small number of participants consuming oats ( $n = 5$  for dinner,  $n = 3$  for lunch) limits the reliability of these estimates, and these findings should be considered hypothesis-generating rather than definitive.

The association between Hyperglycemia and dysbiosis was particularly strong, with 81.0% of hyperglycaemic participants exhibiting dysbiosis. This finding is consistent with studies showing that elevated glucose levels alter gut microbiota composition and increase intestinal permeability [23]. The strong association with OHA use likely reflects disease severity rather than a direct effect of medication, as

OHA use typically indicates established disease with poorer glycaemic control. Notably, normal weight participants showed the highest odds of dysbiosis, suggesting that some individuals with normal BMI may have a "metabolically obese normal weight" (MONW) phenotype—characterized by insulin resistance and visceral adiposity despite normal BMI [24]. This finding underscores that gut dysbiosis may be an early driver of metabolic dysfunction independent of obesity.

The correlation analysis revealed that the F/B ratio becomes clinically informative in the context of T2DM. The strong positive correlation with TNF- $\alpha$  ( $r = 0.782$ ) directly links dysbiotic microbial structure to a major driver of insulin resistance. The absence of significant correlations in the control group indicates that the F/B ratio is not a useful biomarker in healthy individuals, but becomes highly relevant in disease states a finding that supports the importance of subgroup analysis in microbiome research [25].

### Study Limitations

Several limitations should be acknowledged. The cross-sectional design precludes causal inference, and the directionality of observed associations cannot be determined. The study focused on two bacterial phyla (Bacteroidetes and Firmicutes) and did not assess species-level composition or other microbial taxa. Defining dysbiosis based on the 75th percentile of bacterial load in controls is unconventional and may not capture all dimensions of dysbiosis; future studies should use validated dysbiosis indices that incorporate compositional and functional parameters. Dietary data were collected using a structured questionnaire that captured meal-level food choices but did not quantify nutrient intake, portion sizes, or overall dietary quality, limiting the precision of dietary–microbiome associations. The small number of participants in certain dietary categories (e.g., oats consumption) limits the reliability of those specific estimates. The control group was recruited from university staff and students, which may introduce selection bias and limit generalisability to the broader Nigerian population the sample size, while adequate for primary analyses, limited subgroup analyses. Finally, the single-center design may limit generalisability to other Nigerian populations [26].

### Clinical and Research Implications

Despite these limitations, our findings have important implications. The strong associations between dysbiosis and inflammatory/metabolic markers suggest that gut microbiota assessment could serve as a valuable adjunct in T2DM risk stratification. Identifying dysbiosis even in normal-weight T2DM patients highlights the need to consider gut health in all diabetic patients, regardless of BMI.

The dietary findings underscore the potential for culturally tailored dietary interventions in Nigerian T2DM patients. Increasing dietary fiber through traditional Nigerian vegetables (e.g., fluted pumpkin, bitter leaf), legumes, and whole grains while reducing refined carbohydrate intake (particularly rice and swallow foods at breakfast) may help restore microbial balance. However, the unexpected association between oats consumption and dysbiosis warrants further investigation before specific recommendations can be made [27].

Future research should employ metagenomic sequencing to identify species-level microbial signatures associated with T2DM in Nigerian populations. Longitudinal studies are needed to establish causality and determine whether gut microbiota modulation can improve glycaemic control and reduce inflammatory burden. Interventional studies investigating the effects of dietary modifications, probiotics, or prebiotics on gut microbiota and metabolic outcomes in Nigerian T2DM patients are warranted. Additionally, developing and validating population-specific dysbiosis indices for African populations would enhance the clinical utility of microbiome assessment [28].

### Conclusion

This study demonstrates that gut dysbiosis is prevalent among T2DM patients in Sokoto, Nigeria, and is associated with elevated systemic inflammation (TNF- $\alpha$ , IL-6), altered microbial metabolites (TMAO, BCAAs), intestinal inflammation (fecal calprotectin), and specific dietary patterns. Hyperglycemia

emerged as the strongest clinical factor associated with dysbiosis, while normal-weight diabetic patients showed unexpectedly high rates of dysbiosis, identifying a potentially high-risk subgroup. Dietary analysis revealed that rice/swallow consumption at breakfast was significantly associated with dysbiosis, while oats consumption was unexpectedly linked to 100% dysbiosis prevalence in this population.

The Firmicutes/Bacteroidetes ratio correlated strongly with TNF- $\alpha$  in T2DM patients, supporting a direct link between gut microbial structure and systemic inflammation. These findings support the gut–systemic inflammatory axis in T2DM pathophysiology and highlight the potential for microbiome-targeted dietary interventions in T2DM management, particularly in resource-limited settings where alternative therapeutic approaches are needed. Culturally appropriate dietary strategies that increase fiber intake from traditional Nigerian foods while reducing refined carbohydrates may offer a promising avenue for improving gut health and metabolic outcomes in this population.

#### Declaration

Conflict of Interest: The authors declare no conflicts of interest.

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