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Gut Microbiota Composition and Inflammatory Markers in Type 2 Diabetes: A qPCR- Based Study from Sokoto, Nigeria

Umar Imam Asiya[1]
Usmanu Danfodiyo University
Nura Muhammad Bunza[1]
Usmanu Danfodiyo University
Jafaru Muhammad Bunza
Usmanu Danfodiyo University
Bilkisu Mikàilu Mainasara[1]
Usmanu Danfodiyo University Teaching Hospital, Sokoto
Ogunwale Kolawole Ayobami
university of illorin teaching hospital Kwara state Nigeria
Blessing Shina Oladele
University of Salford
Aliyu Umar Maimuna
Usmanu Danfodiyo University
Jidda Muhammad Lawal
muhammad.lawal@udusok.edu.ng

Usmanu Danfodiyo University

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Abstract

Background

Type 2 diabetes mellitus (T2DM) is associated with gut microbiota dysbiosis, but data from African populations remain limited. This study investigated the composition of gut *Firmicutes* and *Bacteroidetes* and their association with inflammatory markers in T2DM patients from Sokoto, Northwestern Nigeria.

Methods

A cross-sectional study enrolled 126 participants (63 T2DM patients and 63 age- and sex-matched non-diabetic controls). Gut microbiota was assessed using quantitative polymerase chain reaction (qPCR) targeting 16S rRNA genes of *Firmicutes* and *Bacteroidetes*. Plasma tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA). Fecal calprotectin was determined as a marker of intestinal inflammation. Participants were classified into gut eubiosis (n = 14), gut dysbiosis (n = 49), and non-diabetic control (n = 63) groups based on bacterial load thresholds.

Results

The dysbiosis group demonstrated significantly lower *Bacteroidetes* abundance (median: 5.571 log10 CFU; IQR: 4.835–6.520) compared to controls (median: 6.576 log10 CFU; IQR: 5.826–7.826; $p < 0.001$) and higher *Firmicutes* abundance (median: 7.018 log10 CFU; IQR: 6.850–7.526) compared to controls (median: 6.690 log10 CFU; IQR: 5.611–7.110). The *Firmicutes/Bacteroidetes* (F/B) ratio was significantly elevated in dysbiosis (median: 1.496; IQR: 1.339–2.005) compared to eubiosis (0.928) and controls (0.932; $H = 86.657$, $p < 0.001$). Plasma TNF-α concentrations differed significantly across groups ($H = 6.370$, $p = 0.041$), with the dysbiosis group showing the highest levels (41.406 ng/L). Fecal calprotectin was also elevated in dysbiosis (47.045 pg/mL) compared to controls (40.462 pg/mL; $H = 11.582$, $p = 0.003$). In the dysbiosis group, the F/B ratio correlated positively with TNF-α ($r = 0.782$, $p = 0.001$) and IL-6 ($r = 0.352$, $p = 0.032$), and negatively with fecal calprotectin ($r=-0.530$, $p = 0.001$) and fasting blood glucose ($r=-0.340$, $p = 0.040$).

Conclusions

T2DM patients in Sokoto exhibit significant gut dysbiosis characterized by elevated F/B ratio, reduced *Bacteroidetes* abundance, and increased systemic and intestinal inflammation. The strong correlation between F/B ratio and TNF-α in dysbiosis suggests a mechanistic link between gut microbial alteration and inflammatory pathogenesis in T2DM. These findings establish baseline data for gut microbiota research in Northwestern Nigeria and support the potential for microbiome-targeted interventions.

Clinical Trail Number

NOT APPLICABLE

1. INTRODUCTION

The human gut microbiota comprises approximately 100 trillion microorganisms that coexist in a symbiotic relationship with the host, performing essential functions in nutrition, metabolism, and immune regulation (Lynch & Pedersen, 2016; Thursby & Juge, 2017). Among the diverse microbial taxa inhabiting the gastrointestinal tract, the phyla *Firmicutes* and *Bacteroidetes* dominate, together constituting 70–90% of the total bacterial community in healthy individuals (Arumugam et al., 2011). The ratio between these two phyla has emerged as a□□ but informative biomarker of gut ecological status, with deviations from typical ranges associated with various metabolic disorders (Magne et al., 2020).

Type 2 diabetes mellitus (T2DM) represents a global health crisis affecting over 537 million adults worldwide, with prevalence rising most rapidly in low- and middle-income countries (International Diabetes Federation, 2021). Nigeria, the most populous country in Africa, has witnessed a dramatic increase in T2DM prevalence, with current estimates ranging from 3.6% to 5.8% depending on geographic region and urbanicity (Uloko et al., 2018). The pathophysiology of T2DM involves chronic hyperglycemia resulting from pancreatic β-cell dysfunction and peripheral insulin resistance, processes increasingly recognized to be modulated by the gut microbiota (Arora et al., 2021; Gurung et al., 2020).

The landmark metagenome-wide association study by Qin et al. (2012) first demonstrated that T2DM patients exhibit gut dysbiosis characterized by decreased abundance of butyrate-producing bacteria (*Roseburia intestinalis*, *Faecalibacterium prausnitzii*), increased opportunistic pathogens (*Clostridium hathewayi*, *Escherichia coli*), and enrichment of microbial genes involved in sulfate reduction and oxidative stress resistance. Subsequent studies across European and Asian populations have largely confirmed these findings, revealing a consistent signature of reduced *Bacteroidetes* abundance and elevated *Firmicutes/Bacteroidetes* ratio in T2DM (Karlsson et al., 2013; Forslund et al., 2015).

The mechanistic links between gut dysbiosis and T2DM involve multiple interconnected pathways. Dysbiosis compromises intestinal barrier integrity through reduced production of short-chain fatty acids (SCFAs), particularly butyrate, which serves as the primary energy source for colonocytes and regulates tight junction protein expression (Koh et al., 2016; Parada Venegas et al., 2019). Increased intestinal permeability facilitates translocation of bacterial lipopolysaccharide (LPS) into the systemic circulation, a condition termed "metabolic endotoxemia" (Cani et al., 2007). Circulating LPS activates Toll-like receptor 4 (TLR4) on immune cells and adipocytes, triggering NF-κB-mediated production of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which impair insulin signaling in peripheral tissues (Cani et al., 2019; Hersoug et al., 2018).

Despite growing global evidence, research on gut microbiota in African populations remains limited, with most studies conducted in rural, traditional-living communities rather than urban or peri-urban settings where dietary transitions are occurring rapidly (Smits et al., 2017). In Nigeria, only a handful of studies have examined gut microbiota composition, primarily in southern regions and healthy populations (Adeniji et al., 2021; Ayeni et al., 2018). A study by Akinyemi et al. (2020) in Lagos reported decreased *Bacteroidetes* and increased *Firmicutes/Bacteroidetes* ratio in T2DM patients compared to controls, but

no study has investigated gut microbiota in Northern Nigeria, where distinctive dietary patterns, cultural practices, and environmental exposures may shape unique microbial ecologies.

Sokoto State in Northwestern Nigeria presents a particularly understudied context. The predominant Hausa-Fulani population maintains dietary patterns characterized by high consumption of cereals (millet, sorghum), legumes, dairy products, and limited intake of fruits and vegetables (Yahaya et al., 2020). The region also experiences extreme climatic conditions, including temperatures reaching 45°C and seasonal harmattan dust storms, which may influence microbial composition. Understanding gut microbiota alterations in this context is essential for developing regionally appropriate prevention and treatment strategies.

This study therefore aimed to: (1) quantify *Firmicutes* and *Bacteroidetes* abundance using qPCR in T2DM patients and non-diabetic controls in Sokoto Metropolis; (2) determine the association between gut microbiota composition and systemic inflammatory markers (TNF-α, IL-6); and (3) assess the relationship between gut microbiota and intestinal inflammation measured by fecal calprotectin.

2. MATERIALS AND METHODS

2.1 Study Area and Design

This cross-sectional comparative study was conducted in Sokoto Metropolis, Sokoto State, Northwestern Nigeria (latitudes 13°N-15°N, longitudes 4°E-6°E). Participants were recruited from three government hospitals: Specialist Hospital Sokoto (SHS), Maryam Abacha Women and Children Hospital Sokoto (MAWCH), and Women and Children Welfare Clinic Sokoto (WCWC). Molecular analyses were performed at the Centre for Advance Medical Research and Training (CAMRET), Usmanu Danfodiyo University Sokoto. The study was conducted between June and December 2025.

2.2 Study Population

A total of 126 participants were enrolled, comprising 63 T2DM patients and 63 age- and sex-matched non-diabetic controls. T2DM patients were recruited consecutively from medical outpatient clinics of the participating hospitals. Controls were recruited from staff and students of Usmanu Danfodiyo University Sokoto with no history of diabetes or other metabolic disorders.

Inclusion Criteria

(1) Confirmed T2DM diagnosis based on fasting blood glucose ≥ 7.0 mmol/L or random blood glucose ≥ 11.1 mmol/L (for cases); (2) age ≥ 18 years; (3) willingness to provide written informed consent; (4) residence in Sokoto Metropolis for at least one year prior to enrollment.

Exclusion Criteria

(1) Type 1 diabetes mellitus or gestational diabetes; (2) autoimmune diseases; (3) inflammatory bowel disease (Crohn's disease, ulcerative colitis); (4) antibiotic use within four weeks prior to enrollment; (5) current or recent (within three months) chemotherapy; (6) history of gastrointestinal surgery (excluding appendectomy); (7) pregnancy or lactation; (8) refusal to provide informed consent.

2.3 Sample Size Determination

Sample size was calculated using Cohen's (1988) formula for analytical studies comparing two independent groups:

$n = 2 \times (Z\alpha/2 + Z\beta)^2 \times \delta^2 / d^2$

Where $Z\alpha/2 = 1.96$ (95% confidence level), $Z\beta = 1.28$ (90% statistical power), $\delta = 0.5$ (estimated effect size based on previous studies), and $d = 0.5$ (margin of error). This calculation yielded 63 participants per group, for a total sample size of 126 participants.

2.4 Ethical Considerations

Ethical approval

was obtained from the Ethics and Research Committee of Sokoto State Ministry of Health (Reference Number: SMH/1580/V.IV). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants after providing detailed information about study procedures, risks, and benefits. Participants were informed of their right to withdraw from the study at any time without affecting their medical care.

2.5 Data Collection

Sociodemographic and Clinical Data

A structured interviewer-administered questionnaire was used to collect information on age, sex, occupation, educational level, medication history, and duration of diabetes diagnosis. Weight was measured to the nearest 0.1 kg using a calibrated digital scale, and height was measured to the nearest 0.1 cm using a stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Glycemic Assessment

Fasting blood glucose was measured using the glucose oxidase method from venous blood samples collected after an overnight fast of at least eight hours. Hyperglycemia was defined as fasting blood glucose > 5.8 mmol/L.

2.6 Sample Collection and Processing

Stool Samples

Fecal samples (approximately 5 g) were collected by participants in sterile, screw-cap, wide-mouth plastic containers. Samples were transported to the laboratory on dry ice within four hours of collection and stored at -80°C until analysis. All samples were processed within three months of collection to minimize degradation.

Blood Samples

Venous blood (5 mL) was collected from each participant into EDTA-containing vacutainer tubes. Samples were centrifuged at 3,000 rpm for 5 minutes within 30 minutes of collection to obtain plasma. Plasma was aliquoted into sterile cryovials and stored at -20°C until analysis for TNF-α and IL-6.

2.7 DNA Extraction

DNA was extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol with minor modifications. Briefly, 200 mg of stool was added to a 2 mL tube containing 1.4 mL Buffer ASL and vortexed continuously for 1 minute. The sample was heated at 95°C for 5 minutes, vortexed, and centrifuged at 14,000 rpm for 1 minute. A 1.2 mL aliquot of supernatant was transferred to a new tube containing one InhibitEX tablet, vortexed until dissolved, and incubated at room temperature for 1 minute. After centrifugation at 14,000 rpm for 3 minutes, 200 µL of supernatant was transferred to a new tube containing 15 µL proteinase K and 200 µL Buffer AL. The mixture was incubated at 70°C for 10 minutes. Absolute ethanol (200 µL) was added, and the mixture was transferred to a QIAamp spin column. The column was washed with 500 µL Buffer AW1 and 500 µL Buffer AW2, and DNA was eluted in 50 µL Buffer ATE. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), with acceptable A260/A280 ratios between 1.8 and 2.0.

2.8 Quantitative PCR Amplification

Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using SYBR Green chemistry. Primer sequences targeting the 16S rRNA gene of *Firmicutes* and *Bacteroidetes* were used as described by Guo et al. (2008):

- *Firmicutes* forward (Firm934F): 5'-GGAGYATGTGGTTTAATTCGAAGCA-3'
- *Firmicutes* reverse (Firm1060R): 5'-AGCTGACGACAACCATGCAC-3'
- *Bacteroidetes* forward (Bac960F): 5'-GTTTAATTCGATGATACGCGAG-3'
- *Bacteroidetes* reverse (Bac1100R): 5'-TTAASCCGACACCTCACGG-3'

Each 10 µL reaction mixture contained: 5 µL AceQ qPCR SYBR Green Master Mix (Qiagen, Hilden, Germany), 0.25 µL of each primer (10 µM concentration), 1 µL template DNA, and 3.5 µL nuclease-free water. Thermal cycling conditions were: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 3 seconds and annealing/elongation at 60°C for 40 seconds. A

melting curve analysis was performed from 60°C to 95°C to confirm amplification specificity. Each sample was analyzed in duplicate, and the mean cycle threshold (Ct) value was used for calculations. Standard curves were generated using 10-fold serial dilutions of plasmid DNA containing the target amplicons, and bacterial loads were expressed as log10 colony-forming units (CFU) per gram of feces.

2.9 Determination of Inflammatory Markers

Plasma TNF-α and IL-6: Concentrations of TNF-α and IL-6 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols. Briefly, 96-well microplates pre-coated with capture antibodies were incubated with 100 µL of plasma samples or standards at room temperature for 2 hours. After washing, detection antibodies were added and incubated for 2 hours. Streptavidin-horseradish peroxidase conjugate was added, followed by substrate solution. The reaction was stopped with 2N sulfuric acid, and absorbance was read at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Sample concentrations were calculated from standard curves generated using recombinant human TNF-α (range: 15.6–1000 pg/mL) and IL-6 (range: 3.1–300 pg/mL). The lower limits of detection were 4.5 pg/mL for TNF-α and 0.7 pg/mL for IL-6. See questionnaire.

Fecal Calprotectin: Fecal calprotectin was measured using a commercial ELISA kit (Bühlmann Laboratories, Schönenbuch, Switzerland) according to the manufacturer's instructions. Fecal samples were extracted using extraction buffer, vortexed, and centrifuged at 3,000 rpm for 5 minutes. The supernatant was diluted and added to anti-calprotectin antibody-coated microplate wells. After incubation for 30 minutes at room temperature, wells were washed, and peroxidase-conjugated detection antibody was added. After a second incubation and wash, substrate solution was added, and absorbance was read at 450 nm. Calprotectin concentrations were calculated from a standard curve (range: 30-3000 ng/g).

2.10 Classification of Gut Microbiome Status

Dysbiosis was defined as bacterial load exceeding the 75th percentile of the non-diabetic control group for either *Firmicutes* or *Bacteroidetes*. Eubiosis was defined as bacterial load below the 75th percentile of the non-diabetic control group but with a confirmed T2DM diagnosis. Non-diabetic control participants were all normoglycemic with no history of diabetes.

2.11 Statistical Analysis

Statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to assess normality of continuous variables. Since most variables deviated from normal distribution, non-parametric tests were employed throughout. Continuous variables were presented as median and interquartile range (IQR). Categorical variables were presented as frequencies and percentages.

The Kruskal-Wallis H test was used to compare continuous variables across the three groups (gut eubiosis, gut dysbiosis, non-diabetic control). Effect sizes were calculated using eta squared (η^2H).

When significant differences were detected, post-hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction for multiple comparisons.

Spearman's rank correlation coefficient (r) was used to assess relationships between the *Firmicutes/Bacteroidetes* ratio and inflammatory markers (TNF-α, IL-6, fecal calprotectin), as well as clinical parameters (BMI, fasting blood glucose). Correlation strength was interpreted as: weak ($r < 0.3$), moderate ($r = 0.3–0.7$), and strong ($r > 0.7$).

All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant. The Benjamini-Hochberg procedure was applied to control the false discovery rate for multiple comparisons where appropriate.

3. RESULTS

3.1 Participant Characteristics

A total of 126 participants were enrolled, including 80 females (63.5%) and 46 males (36.5%). The median age was 52 years (IQR: 46–61). Among T2DM patients, the median duration of diabetes diagnosis was 4 years (IQR: 2–7). Baseline characteristics of the study population are presented in Table 1. The three groups (gut eubiosis, n = 14; gut dysbiosis, n = 49; non-diabetic control, n = 63) were comparable with respect to age and sex distribution ($p > 0.05$ for both). As expected, fasting blood glucose was significantly higher in both T2DM groups compared to controls ($p < 0.001$).

Table 1
Baseline Characteristics of Study Participants by Group

Characteristic	Gut Eubiosis (n = 14)	Gut Dysbiosis (n = 49)	Non-diabetic Control (n = 63)	p-value
Age, years	53 (47–60)	54 (48–62)	51 (45–58)	0.168
Sex, n (%) female	9 (64.3)	31 (63.3)	40 (63.5)	0.998
BMI, kg/m²	27.4 (24.2–30.1)	26.8 (24.5–29.6)	25.9 (23.8–28.4)	0.087
Fasting blood glucose, mmol/L	7.4 (6.8–8.9)*	8.2 (7.1–10.5)*	4.8 (4.3–5.2)	< 0.001
Diabetes duration, years	3.5 (2.0–6.0)	4.5 (2.0–8.0)	NA	0.342

**Values presented as median (IQR) unless otherwise indicated. p < 0.05 compared to control group. BMI: Body mass index; NA: Not applicable.*

3.2 Quantification of *Bacteroidetes* and *Firmicutes*

qPCR analysis revealed significant differences in bacterial loads across the three groups (Table 2; Fig. 1). For *Bacteroidetes*, the non-diabetic control group exhibited the highest median load (6.576 log10 CFU;

IQR: 5.826–7.826), followed by the gut dysbiosis group (5.571 log10 CFU; IQR: 4.835–6.520), and the gut eubiosis group showed the lowest load (4.830 log10 CFU; IQR: 3.830–5.244). The Kruskal-Wallis test confirmed a statistically significant difference across groups ($H = 18.342$, $p < 0.001$, $\eta^2H = 0.146$). Post-hoc analysis revealed that both T2DM groups (eubiosis and dysbiosis) had significantly lower *Bacteroidetes* loads compared to controls ($p < 0.001$ for both comparisons), while the difference between eubiosis and dysbiosis was not statistically significant ($p = 0.087$).

For *Firmicutes*, a different pattern emerged. The gut dysbiosis group demonstrated the highest median load (7.018 log10 CFU; IQR: 6.850–7.526), followed by the non-diabetic control group (6.690 log10 CFU; IQR: 5.611–7.110), and the gut eubiosis group showed the lowest load (5.825 log10 CFU; IQR: 5.065–6.541). These differences were statistically significant ($H = 21.765$, $p < 0.001$, $\eta^2H = 0.173$). Post-hoc analysis indicated that the dysbiosis group had significantly higher *Firmicutes* loads compared to both eubiosis ($p < 0.001$) and control ($p = 0.003$) groups, while eubiosis and control groups did not differ significantly ($p = 0.156$).

Table 2
Bacterial Loads of *Bacteroidetes* and *Firmicutes* by Group

Group	**n**	***Bacteroidetes* (log10 CFU)**	***Firmicutes* (log10 CFU)**
Gut eubiosis	14	4.830 (3.830–5.244)*	5.825 (5.065–6.541)
Gut dysbiosis	49	5.571 (4.835–6.520)*	7.018 (6.850–7.526)*†
Non-diabetic control	63	6.576 (5.826–7.826)	6.690 (5.611–7.110)
Kruskal-Wallis H		18.342	21.765
p-value		< 0.001	< 0.001
η^2H		0.146	0.173

**Values presented as median (IQR). $p < 0.05$ compared to control group; †$p < 0.05$ compared to eubiosis group.*

3.3 *Firmicutes/Bacteroidetes* Ratio

The *Firmicutes/Bacteroidetes* (F/B) ratio showed a striking gradient across the three groups. The gut dysbiosis group exhibited a markedly elevated median F/B ratio of 1.496 (IQR: 1.339–2.005), while both the gut eubiosis group (median: 0.928; IQR: 0.858–0.964) and the non-diabetic control group (median: 0.932; IQR: 0.868–0.990) showed ratios below 1.0. The Kruskal-Wallis test revealed a highly significant difference across groups ($H = 86.657$, $p < 0.001$, $\eta^2H = 0.688$). Post-hoc analysis confirmed that the dysbiosis group had significantly higher F/B ratios compared to both eubiosis ($p < 0.001$) and control ($p < 0.001$) groups, while the eubiosis and control groups did not differ significantly ($p = 0.892$).

3.4 Inflammatory Markers

Plasma TNF-α and IL-6: Concentrations of pro-inflammatory cytokines differed significantly across the three groups (Table 3; Fig. 2). Plasma TNF-α was highest in the gut dysbiosis group (median: 41.406 ng/L; IQR: 38.460-46.002), followed by the non-diabetic control group (median: 39.480 ng/L; IQR: 35.760-43.294) and the gut eubiosis group (median: 38.934 ng/L; IQR: 37.502–46.683). The Kruskal-Wallis test indicated a statistically significant difference ($H = 6.370$, $p = 0.041$, $\eta^2H = 0.036$). Post-hoc analysis revealed that the dysbiosis group had significantly higher TNF-α levels compared to controls ($p = 0.038$), while differences between eubiosis and controls ($p = 0.412$) and between eubiosis and dysbiosis ($p = 0.206$) did not reach statistical significance.

Plasma IL-6 concentrations followed a similar pattern, with the gut dysbiosis group showing the highest median level (2.342 pg/mL; IQR: 1.930–2.702), followed by the gut eubiosis group (2.107 pg/mL; IQR: 1.859–2.945) and the control group (1.950 pg/mL; IQR: 1.580–2.429). The difference across groups was statistically significant ($H = 8.854$, $p = 0.012$, $\eta^2H = 0.056$). Post-hoc analysis indicated that the dysbiosis group had significantly higher IL-6 levels compared to controls ($p = 0.009$), while other pairwise comparisons were not significant.

Fecal Calprotectin: Fecal calprotectin, a marker of intestinal inflammation, showed significant differences across groups ($H = 11.582$, $p = 0.003$, $\eta^2H = 0.078$). The gut dysbiosis group exhibited the highest median concentration (47.045 pg/mL; IQR: 42.990–60.280), followed by the gut eubiosis group (45.553 pg/mL; IQR: 40.089–51.080) and the control group (40.462 pg/mL; IQR: 37.993–48.292). Post-hoc analysis revealed that the dysbiosis group had significantly higher calprotectin levels compared to controls ($p = 0.002$), while the eubiosis group did not differ significantly from either dysbiosis ($p = 0.187$) or control ($p = 0.098$) groups.

Table 3
Inflammatory Markers by Group

Marker	Gut Eubiosis (n = 14)	Gut Dysbiosis (n = 49)	Non-diabetic Control (n = 63)	H	p	η^2H
TNF-α (ng/L)	38.934 (37.502–46.683)	41.406 (38.460-46.002)*	39.480 (35.760-43.294)	6.370	0.041	0.036
IL-6 (pg/mL)	2.107 (1.859–2.945)	2.342 (1.930–2.702)*	1.950 (1.580–2.429)	8.854	0.012	0.056
Fecal calprotectin (pg/mL)	45.553 (40.089–51.080)	47.045 (42.990–60.280)*	40.462 (37.993–48.292)	11.582	0.003	0.078
F/B ratio	0.928 (0.858–0.964)	1.496 (1.339–2.005)*†	0.932 (0.868–0.990)	86.657	< 0.001	0.688

Values presented as median (IQR). *p < 0.05 compared to control group; †p < 0.05 compared to eubiosis group. TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; F/B: Firmicutes/Bacteroidetes.

3.5 Correlations Between F/B Ratio and Clinical Parameters

Spearman's correlation analysis revealed striking differences in association patterns depending on gut microbiome status (Table 4; Fig. 3). In the gut dysbiosis group, the F/B ratio demonstrated a strong positive correlation with plasma TNF-α (r = 0.782, p = 0.001), a moderate positive correlation with IL-6 (r = 0.352, p = 0.032), a moderate negative correlation with fecal calprotectin (r = -0.530, p = 0.001), and a weak negative correlation with fasting blood glucose (r = -0.340, p = 0.040). No significant correlation was observed between F/B ratio and BMI in this group (r = 0.153, p = 0.092).

In contrast, the gut eubiosis group showed no significant correlations between F/B ratio and any of the measured parameters. The strongest correlation in this group was with TNF-α (r = 0.235, p = 0.514), which did not reach statistical significance, likely due to the small sample size (n = 14).

In the non-diabetic control group, the only significant correlation was between F/B ratio and BMI (r = 0.353, p = 0.005), representing a weak to moderate positive association. No significant correlations were observed with TNF-α (r = -0.096, p = 0.453), IL-6 (r = 0.176, p = 0.168), fecal calprotectin (r = -0.145, p = 0.256), or fasting blood glucose (r = 0.107, p = 0.403).

Table 4
Spearman's Correlations Between F/B Ratio and Clinical Parameters

Parameter	**Gut Eubiosis (n = 14)**	**Gut Dysbiosis (n = 49)**	**Non-diabetic Control (n = 63)**			
	r	**p**	**r**	**P**	**r**	**p**
TNF-α	0.235	0.514	0.782	**0.001**	-0.096	0.453
IL-6	-0.084	0.795	0.352	**0.032**	0.176	0.168
Fecal calprotectin	0.210	0.513	-0.530	**0.001**	-0.145	0.256
BMI	-0.142	0.189	0.153	0.092	0.353	**0.005**
Fasting blood glucose	-0.194	0.546	-0.340	**0.040**	0.107	0.403

Bolded p-values indicate statistical significance (p < 0.05). F/B: Firmicutes/Bacteroidetes; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; BMI: Body mass index.

4. DISCUSSION

This study provides the first comprehensive quantification of gut microbiota composition and its association with inflammatory markers in T2DM patients from Northwestern Nigeria. Our findings demonstrate that T2DM patients in Sokoto Metropolis exhibit significant gut dysbiosis characterized by

elevated *Firmicutes/Bacteroidetes* ratio, reduced *Bacteroidetes* abundance, and increased *Firmicutes* abundance. These microbial alterations are associated with elevated systemic (TNF-α, IL-6) and intestinal (fecal calprotectin) inflammation, with the strongest correlations observed specifically in the dysbiosis group.

4.1 Gut Microbiota Composition in T2DM

The pattern of gut dysbiosis observed in this study—elevated F/B ratio with *Bacteroidetes* depletion—is consistent with findings from global T2DM research. The landmark metagenome-wide association study by Qin et al. (2012) in Chinese populations first reported decreased *Bacteroidetes* and increased *Firmicutes* in T2DM patients, a finding subsequently replicated in European cohorts (Karlsson et al., 2013) and meta-analyses (Gurung et al., 2020). Our study extends these findings to an understudied African population, suggesting that the association between T2DM and gut dysbiosis transcends geographic and dietary boundaries.

The median F/B ratio of 1.496 in our dysbiosis group is notably higher than the 0.932 observed in controls, representing a 60% relative increase. This magnitude of change is comparable to that reported by Akinyemi et al. (2020) in Lagos, Nigeria, who found elevated F/B ratios in T2DM patients using similar qPCR methodology. However, direct comparison is complicated by methodological differences in primer design and quantification approaches. Importantly, our classification of eubiosis and dysbiosis based on control group percentiles provides a population-specific reference that may be more relevant than absolute thresholds derived from Western populations.

The observation that some T2DM patients (n = 14, 22.2% of diabetic participants) maintained F/B ratios comparable to healthy controls (eubiosis group) is noteworthy. This suggests that not all individuals with T2DM develop significant gut dysbiosis, and that microbial resilience or protective factors may exist. Factors that distinguished eubiosis from dysbiosis in our cohort included lower fasting blood glucose (median 7.4 vs. 8.2 mmol/L) and shorter diabetes duration (3.5 vs. 4.5 years), though neither difference reached statistical significance, likely due to limited sample size in the eubiosis group.

4.2 Inflammatory Profile and Gut Microbiota

The elevated concentrations of TNF-α and IL-6 observed in the dysbiosis group compared to controls are consistent with the established paradigm of "metabolic endotoxemia" (Cani et al., 2007). This paradigm proposes that gut dysbiosis compromises intestinal barrier integrity, facilitating translocation of bacterial lipopolysaccharide into the systemic circulation, where it activates TLR4 on immune cells and triggers pro-inflammatory cytokine production (Cani et al., 2019). Our finding of elevated fecal calprotectin—a sensitive marker of neutrophil-driven intestinal inflammation—in the dysbiosis group provides direct evidence of mucosal inflammation that may underlie increased intestinal permeability.

The strong positive correlation between F/B ratio and TNF-α specifically in the dysbiosis group ($r = 0.782$, $p = 0.001$) is a key finding of this study. This correlation suggests that once a threshold of dysbiosis is

crossed, the degree of microbial imbalance directly predicts the magnitude of systemic inflammation. The strength of this association ($r > 0.7$) is remarkable given the inherent variability in both microbiome and cytokine measurements, indicating a robust biological relationship. This finding aligns with Vieira-Silva et al. (2020), who demonstrated that microbiome-host correlations are context-dependent and most pronounced in disease states.

The moderate positive correlation between F/B ratio and IL-6 ($r = 0.352$, $p = 0.032$) further supports the link between gut dysbiosis and systemic inflammation, though the weaker association compared to TNF-α may reflect differences in the cellular sources and regulation of these cytokines. TNF-α is primarily produced by macrophages in response to LPS stimulation, while IL-6 has multiple cellular sources and is regulated by additional factors including adipocyte-derived signals (McNelis & Olefsky, 2014).

4.3 Unexpected Findings: Negative Correlation with Fecal Calprotectin

The negative correlation between F/B ratio and fecal calprotectin in the dysbiosis group ($r = -0.530$, $p = 0.001$) was unexpected and warrants careful interpretation. This finding suggests that within the dysbiosis group, a lower F/B ratio (i.e., relatively more *Bacteroidetes* compared to *Firmicutes*) is associated with higher intestinal inflammation. Several explanations are possible.

First, the F/B ratio is a coarse metric that may obscure important species-level variations. Certain *Bacteroidetes* species, such as *Bacteroides fragilis* producing enterotoxin, can be pro-inflammatory (Round & Mazmanian, 2009). Thus, elevated *Bacteroidetes* abundance does not necessarily indicate a beneficial microbial community. Second, intestinal inflammation measured by fecal calprotectin may be driven by specific microbial metabolites or structural components rather than phylum-level ratios. Third, the negative correlation may reflect a compensatory response whereby intestinal inflammation alters the luminal environment (e.g., increased oxygenation, altered nutrient availability) that selectively inhibits *Firmicutes* while favoring *Bacteroidetes*.

This finding highlights the limitations of relying solely on the F/B ratio as a marker of gut health and underscores the need for higher-resolution taxonomic and functional analyses. As noted by Magne et al. (2020) and Wilkins et al. (2021), the F/B ratio is an oversimplification of complex microbial ecology, and its utility as a biomarker is context-dependent.

4.4 Comparison with Previous Studies

Our findings are broadly consistent with the limited existing literature on gut microbiota in Nigerian T2DM patients. Akinyemi et al. (2020) studied 50 T2DM patients and 50 controls in Lagos, reporting decreased *Bacteroidetes* and increased F/B ratio in diabetics, along with specific reductions in *Bacteroides vulgatus* and *Faecalibacterium prausnitzii*. Our study extends these findings by including fecal calprotectin and multiple cytokine measurements, providing a more comprehensive assessment of the gut-systemic inflammatory axis.

International comparisons reveal both similarities and differences. The elevated F/B ratio observed in our dysbiosis group (median 1.496) is comparable to values reported in European T2DM cohorts (Karlsson et al., 2013) but lower than some Asian studies (Qin et al., 2012). These differences may reflect population-specific dietary patterns, genetic backgrounds, or environmental exposures. Notably, the non-diabetic controls in our study had median F/B ratio of 0.932, which is within the range reported for healthy individuals in other African populations (De Filippo et al., 2010; Schnorr et al., 2014).

The correlation between F/B ratio and TNF-α in our dysbiosis group (r = 0.782) is stronger than that reported in most previous studies. For example, Pedersen et al. (2016) found correlations between specific microbial genera and inflammatory markers but did not report such strong associations with phylum-level ratios. This discrepancy may reflect differences in study populations (treatment-naïve vs. treated patients), analytical methods, or the relatively advanced disease stage in our cohort.

4.5 Mechanistic Implications

Our findings support a mechanistic model in which gut dysbiosis drives systemic inflammation through multiple interconnected pathways. The elevated F/B ratio in dysbiosis likely reflects reduced abundance of butyrate-producing *Firmicutes* (e.g., *Faecalibacterium prausnitzii*, *Roseburia intestinalis*) and relative expansion of other taxa. Butyrate deficiency impairs colonic epithelial energy metabolism, reduces tight junction protein expression, and compromises barrier integrity (Parada Venegas et al., 2019). Increased intestinal permeability facilitates LPS translocation, activating TLR4 on intestinal macrophages and dendritic cells.

Activated immune cells migrate to mesenteric lymph nodes and enter the systemic circulation, where they infiltrate adipose tissue and differentiate into pro-inflammatory M1 macrophages (McNelis & Olefsky, 2014). These adipose tissue macrophages secrete TNF-α, IL-6, and other cytokines that impair insulin signaling in adipocytes, hepatocytes, and myocytes through serine phosphorylation of insulin receptor substrate-1 (IRS-1) (Donath & Shoelson, 2011). The strong correlation between F/B ratio and TNF-α observed in our study is consistent with this pathway.

The elevated fecal calprotectin in dysbiosis indicates that intestinal inflammation is present and may serve as the initiating event in this cascade. Calprotectin, a heterodimer of S100A8 and S100A9 proteins, is released by activated neutrophils and is a sensitive marker of mucosal inflammation (Heida et al., 2020). The fact that calprotectin was elevated even in the eubiosis group, though not significantly, suggests that some degree of intestinal inflammation may precede or occur independently of phylum-level dysbiosis.

4.6 Clinical Significance

These findings have several clinical implications. First, the strong association between gut dysbiosis and systemic inflammation suggests that targeting the gut microbiota could be a therapeutic strategy for reducing inflammation and improving glycemic control in T2DM. Interventions such as prebiotics,

probiotics, and dietary fiber supplementation have shown modest benefits in meta-analyses (Kocsis et al., 2020), and our findings provide mechanistic support for such approaches.

Second, the correlation between F/B ratio and TNF-α suggests that the F/B ratio, despite its limitations, may serve as a useful clinical biomarker for identifying T2DM patients with heightened inflammatory status who might benefit from microbiome-targeted therapies. The 75th percentile cutoff used in this study provides a population-specific reference that could be refined in future research.

Third, the finding that not all T2DM patients exhibit dysbiosis suggests that gut microbiota assessment could help stratify patients for personalized interventions. Those with eubiosis may require different management approaches compared to those with severe dysbiosis.

4.7 Limitations

Several limitations of this study should be acknowledged. First, the cross-sectional design precludes determination of causality. While our data demonstrate associations between dysbiosis and inflammation, we cannot determine whether dysbiosis causes inflammation, inflammation causes dysbiosis, or both are driven by a common factor such as hyperglycemia or medication use.

Second, the qPCR method targets only two bacterial phyla and does not provide information about species-level composition, functional capacity, or other microbial kingdoms (archaea, viruses, fungi). Shotgun metagenomic sequencing would provide more comprehensive insights but was beyond the scope of this study.

Third, the sample size, while adequate for the primary analyses, limited our ability to perform subgroup analyses and resulted in wide confidence intervals for some estimates. The eubiosis group was particularly small (n = 14), which may have contributed to the lack of significant correlations in this group.

Fourth, we did not control for all potential confounders, including detailed dietary intake (beyond broad food categories), physical activity, sleep patterns, and medication adherence. Metformin, the first-line medication for T2DM, is known to alter gut microbiota composition (Wu et al., 2017), and its use may have influenced our findings. However, our objective was to describe microbiota composition in real-world T2DM patients, and excluding metformin users would have limited generalizability.

Fifth, the study was conducted in a single geographic region (Sokoto Metropolis), and findings may not be generalizable to other Nigerian populations or to rural settings with different dietary patterns and lifestyles.

4.8 Future Directions

Future research should employ metagenomic sequencing to identify species-level and strain-level microbial signatures associated with T2DM in Nigerian populations. Longitudinal studies are needed to establish temporal relationships between dysbiosis development, inflammatory marker elevation, and

glycemic progression. Interventional trials evaluating prebiotic, probiotic, or dietary interventions for restoring eubiosis and improving metabolic outcomes are warranted.

Additionally, mechanistic studies using animal models or in vitro systems could help establish causality and identify specific microbial metabolites or structural components that drive inflammation. The role of the gut barrier, measured by markers such as zonulin or lactulose/mannitol ratio, should be assessed in future studies.

5. CONCLUSION

This study demonstrates that T2DM patients in Sokoto, Northwestern Nigeria, exhibit significant gut dysbiosis characterized by elevated *Firmicutes/Bacteroidetes* ratio, reduced *Bacteroidetes* abundance, and increased *Firmicutes* abundance. This dysbiosis is associated with elevated systemic (TNF-α, IL-6) and intestinal (fecal calprotectin) inflammation, with the strongest correlations observed specifically in the dysbiosis group. The strong positive correlation between F/B ratio and TNF-α in dysbiosis ($r = 0.782$, $p = 0.001$) suggests a mechanistic link between gut microbial alteration and inflammatory pathogenesis. These findings establish baseline data for gut microbiota research in Northwestern Nigeria and support the potential for microbiome-targeted interventions in T2DM management. Future research should employ higher-resolution metagenomic approaches and longitudinal designs to further elucidate these relationships and inform clinical practice.

Declarations

Ethics approval and consent to participate

This study was approved by the Sokoto State Ministry of Health Health Research and Ethics Committee (SKHREC), with the following approval number SKHREC/040//2025. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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Author Contributions: UI Asiya, ML Jidda, MM Bilkisu conceptualized the study, collected data, performed laboratory analyses, and drafted the manuscript. UI Asiya, J. M. Bunza supervised the research, and contributed to study design, O.A. Kolawale revised the manuscript. NM Bunza, Blessing O.S. O.A. Kolawale and JM Bunza provided technical supervision, assisted with qPCR optimization, and reviewed the manuscript for critical important content. All authors read and approved the final manuscript.

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Figures

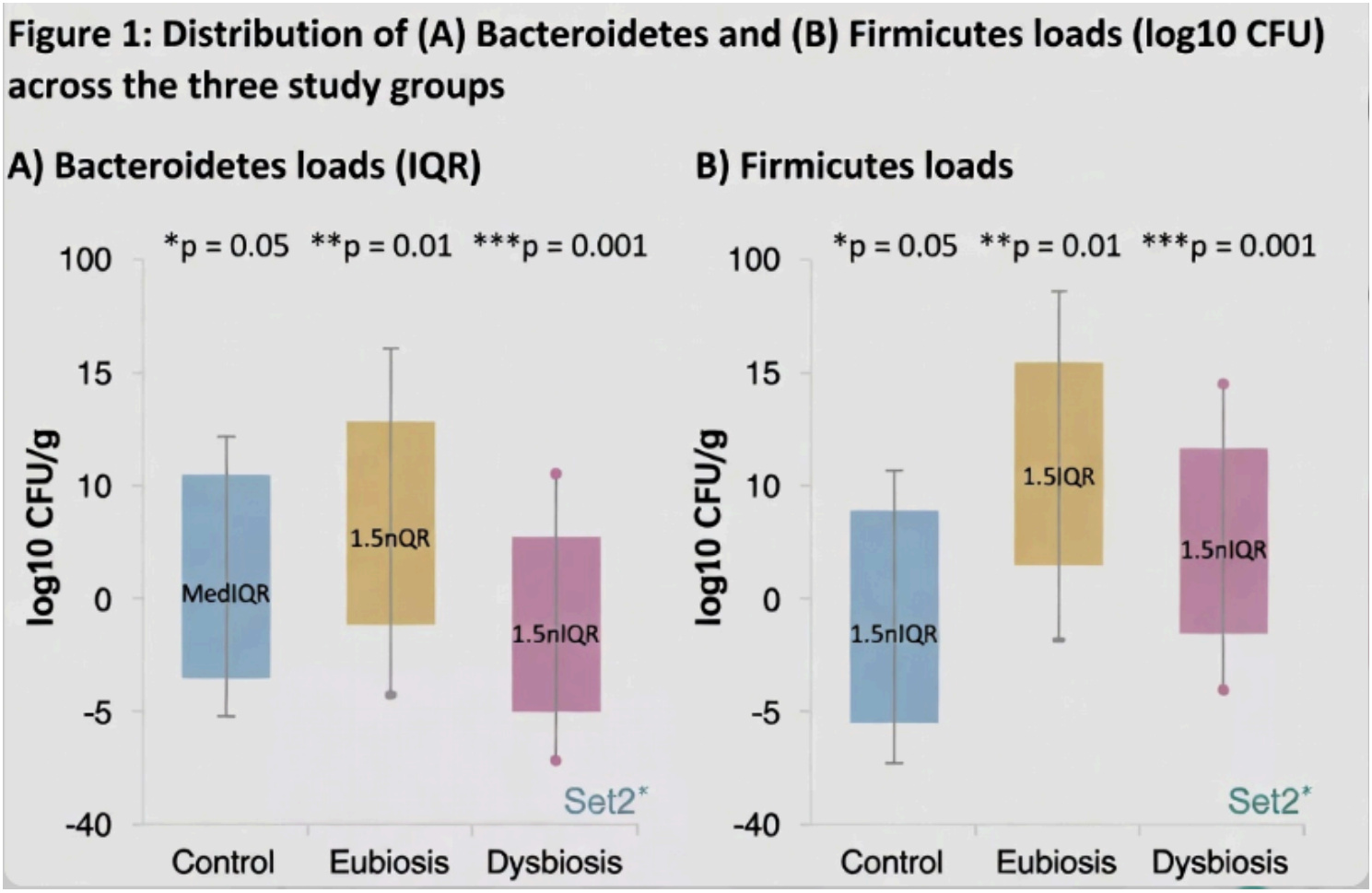


Figure 1

*Box plots showing the distribution of (A) Bacteroidetes and (B) Firmicutes loads (log10 CFU) across the three study groups. Boxes represent interquartile ranges, horizontal lines within boxes represent medians, whiskers represent 1.5× IQR, and circles represent outliers. *p < 0.05; **p < 0.01; ***p < 0.001.*

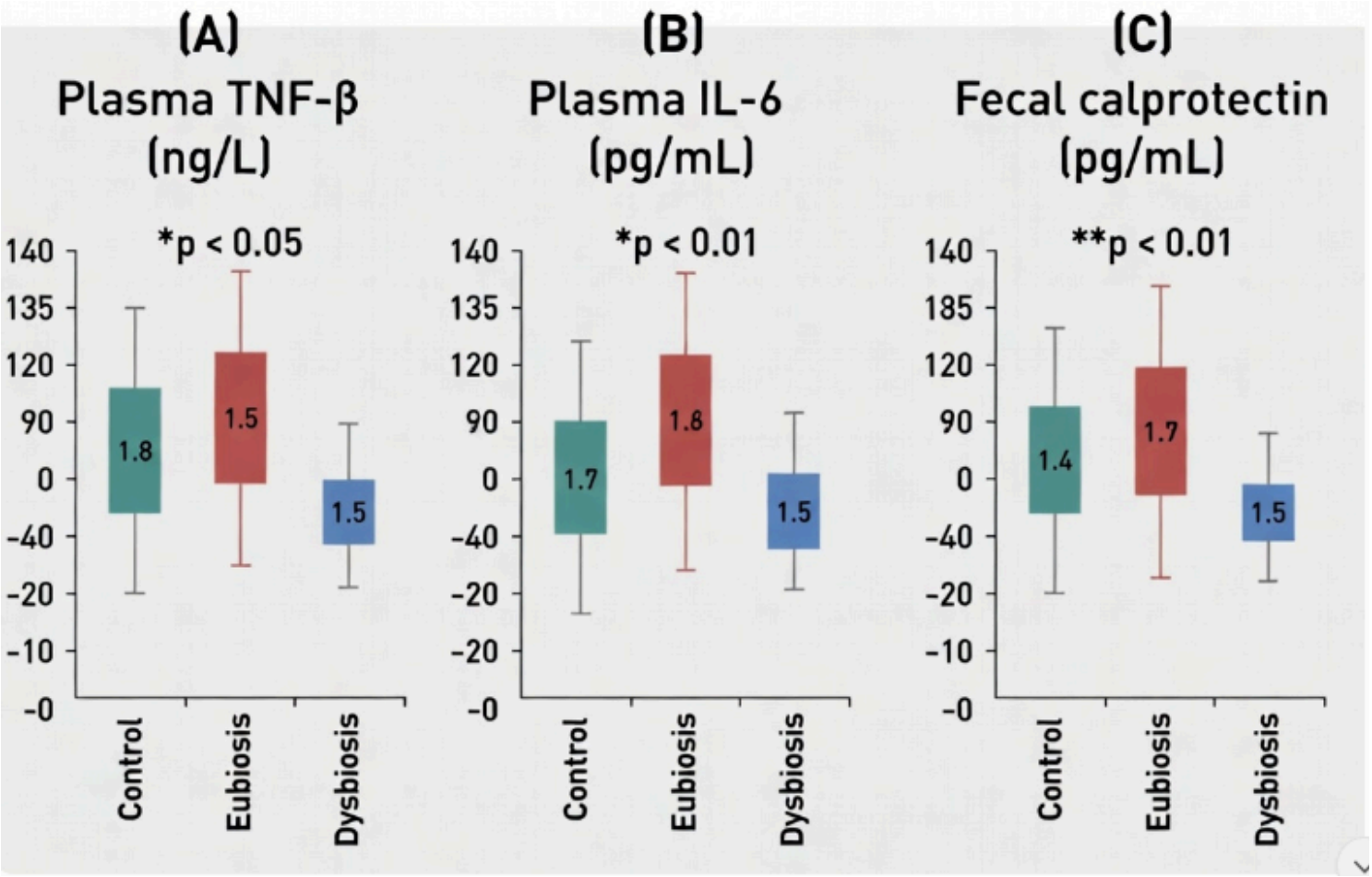


Figure 2

*Box plots showing (A) plasma TNF-α (ng/L), (B) plasma IL-6 (pg/mL), and (C) fecal calprotectin (pg/mL) across the three study groups. *p < 0.05; **p < 0.01.*

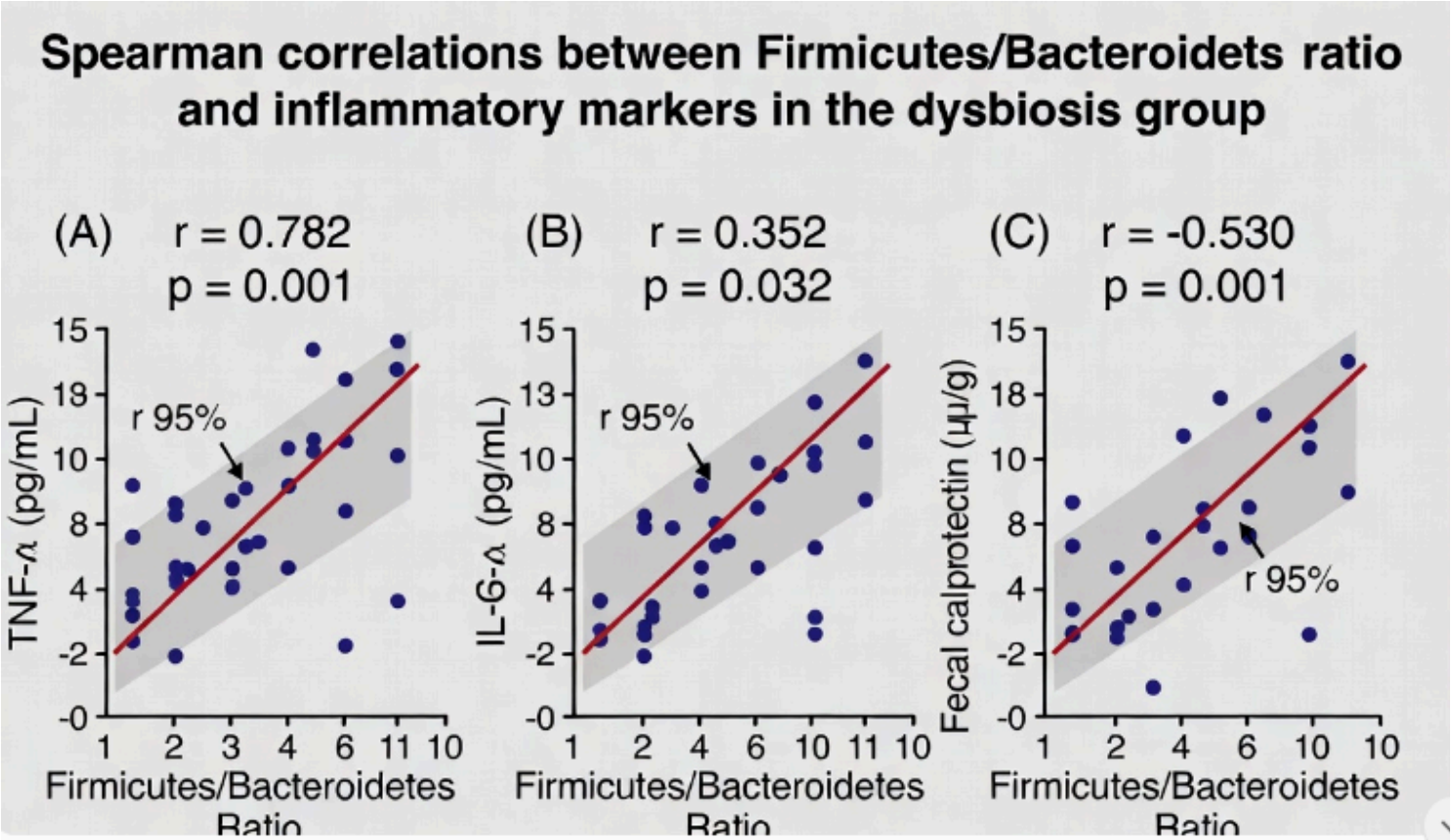


Figure 3

Scatter plots with Spearman correlation lines showing the relationship between Firmicutes/Bacteroidetes ratio and (A) TNF-α in the dysbiosis group (r = 0.782, p = 0.001), (B) IL-6 in the dysbiosis group (r = 0.352, p = 0.032), and (C) fecal calprotectin in the dysbiosis group (r = -0.530, p = 0.001). Shaded areas represent 95% confidence intervals.