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Original Article

Trimethylamine *N*-Oxide is Elevated in Type 2 Diabetes and Correlates with Gut Dysbiosis and Systemic Inflammation in a Nigerian Cohort

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DATA AVAILABILITY
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT:
Background: Trimethylamine *N*-oxide (TMAO), a gut microbiota-derived metabolite linked to cardiometabolic disease, remains understudied in sub-Saharan Africa.
Objectives: To evaluate plasma TMAO concentrations and assess their associations with the Firmicutes/Bacteroidetes (F/B) ratio, pro-inflammatory cytokines, glycemic control, and body mass index in Nigerian T2DM patients.
Methods: This cross-sectional study enrolled 126 participants (63 with T2DM and 63 age-- and sex-matched healthy controls). Gut Firmicutes and Bacteroidetes were quantified by qPCR; dysbiosis was defined as total bacterial load > the 75th percentile of controls. ELISA was used to measure plasma TMAO and cytokines. Associations were tested using the Kruskal–Wallis test, logistic regression, and Spearman's correlation.
Results: TMAO was markedly elevated in T2DM patients compared with controls (medians 7.22 ng/mL in eubiotic T2DM, 6.80 ng/mL in dysbiotic T2DM vs 2.62 ng/mL in controls; $\eta^2H = 0.746$, $p < 0.001$). TMAO levels were similarly high in both diabetic subgroups, demonstrating functional dysbiosis despite a normal F/B ratio. Among dysbiotic patients, TMAO showed positive correlations with the F/B ratio ($r = 0.407$, $p = 0.002$), Tumer Necrosis Factor-alpha (TNF-α) ($r = 0.504$, $p < 0.001$), and Interleukin-6 (IL-6) ($r = 0.386$, $p = 0.004$). Hyperglycemia was independently associated with elevated TMAO (adjusted OR = 3.12, 95% CI 1.64–5.94, $p = 0.001$).
Conclusion: The disconnect between TMAO and conventional microbial composition highlights the need for functional microbiome assessment. TMAO may be a modifiable risk marker in this population.

Keywords: *Trimethylamine N-oxide, Gut dysbiosis, Type 2 diabetes, Inflammation, Nigeria*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) has emerged as one of the most pressing public health challenges of the twenty-first century, with its prevalence escalating at an alarming rate across all regions of the world. According to the International Diabetes Federation (IDF, 2025), nearly half a billion people worldwide are currently living with diabetes, and this figure is projected to increase substantially by 2050. In Nigeria, the prevalence of T2DM is estimated at approximately 3.0% among adults aged 20–79 years, affecting an estimated 3.0 million individuals, with projections indicating this number could rise to 6.6 million by 2050 (IDF, 2025). Nigeria currently has the highest number of adults with diabetes in the Africa Region, and the proportion of undiagnosed cases remains alarmingly high at approximately 78.7% (IDF, 2025). T2DM is a chronic, progressive disease characterised by insulin resistance and insufficient insulin secretion, resulting from β-cell dysfunction and reduced β-cell mass (Kong et al., 2024). Although both factors play a role, decreased β-cell function and β-cell mass are the predominant factors in the progression to T2DM, with β-cell function already reduced to 50-80% of normal at the time of T2DM onset (Kong et al., 2024). The pathophysiology of T2DM is multifactorial, involving genetic susceptibility, environmental influences, chronic low-grade inflammation, and metabolic dysregulation (Alqahtani, 2025). However, traditional risk factors do not fully explain inter-individual variability in disease development and progression, prompting growing interest in the role of the gut microbiota as a novel biological factor influencing glucose metabolism and insulin sensitivity (Alqahtani, 2025; Chong et al., 2025).

The human gastrointestinal tract harbours one of the most complex microbial ecosystems on Earth, comprising approximately 100 trillion microorganisms that collectively contain significantly more genes than the human genome (Thursby & Juge, 2017). This gut microbiota, consisting of bacteria, archaea, viruses, fungi, and protozoa, has co-evolved with humans in a symbiotic relationship that fundamentally influences host physiology, metabolism, and immune function (Lynch & Pedersen, 2016). At the phylum level, *Firmicutes* (60-80%) and *Bacteroidetes* (15-30%) dominate the healthy adult gut microbiota, with minor contributions from *Actinobacteria*,

Proteobacteria, *Verrucomicrobia*, and *Fusobacteria* (Arumugam et al., 2011). The gut microbiota plays a crucial role in breaking down complex carbohydrates, synthesising essential vitamins such as B12 and K, modulating immune responses, and maintaining intestinal barrier integrity (Arora et al., 2021). Fermentation of dietary fibres into short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, is a key metabolic function of the gut microbiota, regulating glucose homeostasis, lipid metabolism, and insulin sensitivity while exerting anti-inflammatory effects (Alqahtani, 2025). Disruption of these functions due to microbial imbalance may predispose individuals to metabolic disorders, including T2DM (Alqahtani, 2025; Chong et al., 2025).

Gut microbiota dysbiosis, defined as an alteration in microbial diversity, composition, or function that negatively affects host health, has been linked to the development, progression, and susceptibility to T2DM through energy and fatty acid metabolism, intestinal barrier integrity, glucose homeostasis, insulin sensitivity, and inflammatory pathways (Minakshi et al., 2025). Patients with T2DM consistently exhibit marked gut dysbiosis, characterised by reduced microbial diversity and depletion of beneficial butyrate-producing taxa, including *Faecalibacterium prausnitzii* and *Roseburia intestinalis*. At the same time, increases in pro-inflammatory bacteria, including *Escherichia and Shigella*, as well as *Lactobacillus*, are commonly observed (Alqahtani, 2025). The literature indicates that type 2 diabetes is linked to reduced gut microbiota diversity, with lower levels of SCFA producers such as *Faecalibacterium prausnitzii* and *Roseburia*, and higher abundance of pro-inflammatory species including *Ruminococcus gnavus* and *Bacteroides* (Szkamruk et al., 2026). These alterations are associated with impaired glucose control, elevated glycated haemoglobin, unfavourable lipid profiles, and increased inflammatory markers such as C-reactive protein and interleukin-6 (Szkamruk et al., 2026). Such compositional changes are linked to metabolic dysfunction through altered microbial metabolites, including elevated trimethylamine N-oxide (TMAO), which has been associated with insulin resistance and increased diabetes risk (Alqahtani, 2025). Moreover, gut microbiota imbalances correlate with systemic inflammation, as indicated by higher levels of cytokines such as IFN-γ and IL-6 (Alqahtani, 2025). Dysbiosis can compromise the integrity of the intestinal epithelial barrier, leading to a "leaky gut" that allows the translocation of bacterial components, such as lipopolysaccharide (LPS), toxic metabolites, and inflammatory factors, into the circulation (Kinashi & Hase, 2021). This low-grade increase in circulating LPS, termed "metabolic endotoxemia," triggers a chronic state of low-grade inflammation by activating Toll-like receptor 4 (TLR4) on immune cells, leading to the production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which interfere with insulin signalling in metabolic tissues (Cani et al., 2023). The gut microbiota is shaped by diet, medications, health conditions, genetics, lifestyle, and environmental factors, and robust evidence continues to emerge through large-scale cohort studies employing deep sequencing and metagenomics (Minakshi et al., 2025).

Among the potential mediators of microbiota-host interactions, trimethylamine *N*-oxide (TMAO), a derivative of bacterial metabolism, has garnered significant scientific interest since it has been linked to increased cardiovascular risk (IDF, 2025). TMAO is a gut microbiota-derived metabolite produced when gut microbes metabolise dietary phosphatidylcholine, choline, and L-carnitine to trimethylamine (TMA), which is then oxidised in the liver to TMAO by flavin-containing monooxygenase 3 (FMO3) (Koeth et al., 2022). TMAO has emerged as a pivotal gut microbiota-dependent metabolite linking dietary patterns, microbial metabolism, and host pathophysiology (Koeth et al., 2022). Recent studies have discovered higher levels of circulating TMAO among patients diagnosed with diabetes, with an updated systematic review and meta-analysis finding significantly elevated TMAO levels in diabetic patients compared to non-diabetics (standardised mean difference: 1.21, 95% CI: 0.13-2.28) and elevated TMAO levels associated with 49% increased odds of diabetes (odds ratio: 1.49, 95% CI: 1.06-2.10) (Mohammadi et al., 2025). Mechanistically, TMAO at concentrations similar to those found in diabetes can directly decrease glucose-stimulated insulin secretion (GSIS) in MIN6 cells and primary islets from mice or humans (Kong et al., 2024). Elevation of TMAO levels impairs GSIS, β-cell proportion, and glucose tolerance in male C57BL/6J mice (Kong et al., 2024). TMAO inhibits calcium transients through NLRP3 inflammasome-related cytokines and induces Serca2 loss; a Serca2 agonist reversed the effect of TMAO on β-cell function in vitro and in vivo (Kong et al., 2024). Additionally, long-term TMAO exposure promotes β-cell ER stress, dedifferentiation, and apoptosis, and inhibits β-cell transcriptional identity (Kong et al., 2024). TMAO also activates the NLRP3 inflammasome, promotes NF-κB signalling, and induces cytokine production in macrophages and endothelial cells, contributing to the chronic low-grade inflammation that sustains insulin resistance and β-cell dysfunction (Kong et al., 2024). Elevated plasma TMAO levels have been associated with an increased risk of cardiovascular events, serving as an independent predictor of subclinical myocardial damage and adverse cardiovascular risk (Tang et al., 2022). Elevated levels of TMAO are frequently linked to an increased risk of cardiovascular and renal complications in individuals with diabetes (Jaworska et al., 2025). Higher levels of TMAO are significantly associated with long-term major adverse clinical events among patients with coronary artery disease and diabetes mellitus, and combining TMAO measurements improves risk stratification and prognosis (Li et al., 2022).

Despite the growing body of evidence linking TMAO to T2DM and its complications, data from sub-Saharan African populations remain extremely limited. Most studies have focused on Western and Asian populations, leaving a critical geographical gap in understanding the interplay between TMAO, gut dysbiosis, and systemic inflammation in African settings. In Nigeria, where T2DM prevalence is rising rapidly alongside urbanisation and dietary transitions, the gut microbiota profile in T2DM patients has not been systematically investigated. The relationship between conventional microbial taxonomic markers such as the *Firmicutes/Bacteroidetes* ratio and TMAO concentration remains poorly understood, particularly in populations with distinct dietary patterns, genetic backgrounds, and environmental exposures. This study therefore aimed to evaluate plasma TMAO levels in relation to gut dysbiosis, systemic inflammatory markers (TNF-α, IL-6), and clinical parameters, including glycaemic control and body mass index, among T2DM patients in Sokoto, Nigeria. We hypothesised that TMAO would be significantly elevated in T2DM patients and correlate with gut dysbiosis and systemic inflammation, providing evidence for the gut-systemic inflammatory axis in this population and identifying TMAO as a potential modifiable risk marker.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted between July 2025 and April 2026 at the Specialist Hospital, Sokoto, and the Maryam Abacha Women and Children Hospital, Sokoto. This specific time frame was selected because the ethical approval was received in July 2025. Participants included 63 adults with T2DM attending the medical outpatient clinics and 63 age- and sex-matched asymptomatic controls recruited from staff and students of Usmanu Danfodiyo University, Sokoto.

Inclusion criteria for T2DM patients were: age ≥18 years, an established diagnosis of T2DM per American Diabetes Association (ADA) 2022 criteria, and willingness to provide informed consent. Exclusion criteria were: type 1 diabetes, inflammatory bowel disease, autoimmune diseases, antibiotic use within the preceding 4 weeks, chemotherapy, gastrointestinal surgery, pregnancy, and lactation. The control

group had no history of diabetes and were not taking glucose-lowering medications. Our sample is a true representative of hospital-based care-seeking T2DM patients in Northwestern Nigeria, a clinically important subgroup, but may not fully represent the broader T2DM population in Northern Nigeria or the National Population, particularly regarding diet, socioeconomic status, and access to healthcare centres.

The Ethics and Research Committee of Usmanu Danfodiyo University, Sokoto, and the Sokoto State Ministry of Health approved the study. All participants provided written informed consent. The study conformed to the principles of the Declaration of Helsinki.

Sample size

Sample size was calculated using Cohen's formula for two independent groups, assuming an effect size of 0.5, $\alpha = 0.05$, and power 90%, yielding 63 participants per group (total N = 126).

Data collection

A structured questionnaire was used to collect sociodemographic data, medical history, and information on current medications, including oral hypoglycaemic agents (OHA). Height and weight were measured to calculate BMI (kg/m²). Fasting blood glucose was measured using a glucometer; hyperglycaemia was defined as fasting blood glucose >5.8 mmol/L (based on diagnostic standard used).

Blood and stool samples collection

Venous blood (5 mL) was collected into EDTA tubes, centrifuged (3000 rpm, 5 min), and plasma stored at −20°C until analysis (for one week). Fresh stool samples were collected in sterile containers and stored at 4°C for microbiota analysis.

Quantification of gut microbiota (Bacteroidetes and Firmicutes)

DNA was extracted from stool using the QIAGEN stool DNA extraction kit (QIAamp PowerFecal Pro DNA Kit, Hilden, Germany) for the isolation of microbial DNA from stool and gut samples. Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix (ChamQ SYBR qPCR Master Mix, Nanjing, China) with primers specific for *Firmicutes* (Firm934F: 5′-GGAGYATGTGGTTTAATTCGAAGCA-3′; Firm1060R: 5′-AGCTGACGACAACCATGCAC-3′) and *Bacteroidetes* (Bac960F: 5′-GTTTAATTCGATGATACGCGAG-3′; Bac1100R: 5′-TTAASCCGACACCTCACGG-3′) (Guo et al., 2008). Cycling conditions: 95°C for 30 s, followed by 40 cycles of 95°C for 3 s and 60°C for 40 s. Bacterial abundance was expressed as $\log_{10}$ colony-forming units (CFU) per gram of faeces. The Firmicutes/Bacteroidetes (F/B) ratio was calculated from the absolute abundances. Gut dysbiosis was defined as bacterial load >75th percentile of the control group; eubiosis was defined as bacterial load ≤75th percentile of the control group (these subgrouping definitions are exploratory due to the lack of a standard reference index for defining dysbiosis in the study).

Measurement of TMAO, TNF-α, and IL-6

Plasma TMAO (Human Trimethylamine N Oxide (TMAO) ELISA kit (Competitive ELISA) 96 Tests Catalog Number: MBS7269386 Store all reagents at 2-8°C For samples: Serum, plasma, cell culture supernatants, body fluid and tissue homogenate; TNF-α, (anti-TNF antibody**: (**Tumor Necrosis Factor alpha) Monoclonal Antibody**,** Catalog: MBS438099; MyBiosource.com), and IL-6 (Human Interleukin 6 (IL6) ELISA Kit, Catalog**;** MBS2019894, MyBiosource.com**)** concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Absorbance was read at 450 nm using a microplate reader (Rayto; Model RT-2100C), and concentrations were calculated from standard curves. All samples were assayed in duplicate.

Statistical analysis

Data were analysed using SPSS version 27. Normality was assessed using the Shapiro-Wilk test; non-normally distributed variables were reported as medians and interquartile ranges (IQRs). Group comparisons (eubiosis, dysbiosis, and non-diabetic control) were performed using the Kruskal-Wallis H test with Dunn's post hoc correction. Categorical variables were compared using Pearson's chi-square test; significant associations were further analysed with binary logistic regression to estimate adjusted odds ratios (AORs) with 95% confidence intervals (CIs). Spearman's rank correlation was used to examine relationships between TMAO, microbial ratios, and inflammatory markers. Effect sizes for Kruskal-Wallis tests were reported as η^2H (eta-squared). Statistical significance was set at $p < 0.05$.

RESULTS

Participant characteristics

A total of 126 participants were enrolled: 80 (63.5%) females and 46 (36.5%) males. Median age was 52 years (IQR 45–60). Baseline characteristics are summarised in Table 1. Among T2DM patients, 51 (81.0%) were classified as having gut dysbiosis based on the high bacterial load, while 12 (19.0%) had eubiosis. The control group comprised 63 participants without diabetes. The small sample size of the gut eubiosis group (n = 12) reduces the statistical power of any sub-group comparisons involving the eubiotic diabetic cohort.

Table 1. Characteristics of Study Participants Stratified by Gut Microbiota Status

Characteristic	Gut Eubiosis (n = 12)	Gut Dysbiosis (n = 51)	Control (n = 63)	p-value	Statistical Test
Age, median (IQR), years	51 (47–58)	54 (48–62)	49 (43–56)	0.107	Kruskal-Wallis H
Sex, n (%)				0.572	Pearson's χ^2
Female	7 (58.3)	31 (60.8)	42 (66.7)		
Male	5 (41.7)	20 (39.2)	21 (33.3)		
BMI, median (IQR), kg/m²	25.2 (23.1–28.5)	26.8 (24.3–31.2)	24.5 (22.1–27.0)	0.018	Kruskal-Wallis H
Hyperglycaemia, n (%)	11 (91.7)	47 (92.2)	0 (0)	<0.001	Pearson's χ^2
OHA use, n (%)	12 (100)	51 (100)	0 (0)	<0.001	Pearson's χ^2

Note. IQR = interquartile range; BMI = body mass index; OHA = oral hypoglycaemic agents. p-values < 0.05 considered statistically significant. For continuous variables (Age, BMI), p-values were derived from the Kruskal-Wallis H test. For categorical variables (Sex, Hyperglycaemia, OHA use), p-values were derived from Pearson's chi-square test.

Table 2: Sex Distribution Across Study Groups

Sex	Total (n)	Gut Eubiosis (n=12)	Gut Dysbiosis (n=51)	Control (n=63)
Female	80 (63.5%)	7 (58.3%)	31 (60.8%)	42 (66.7%)
Male	46 (36.5%)	5 (41.7%)	20 (39.2%)	21 (33.3%)
$\chi^2 = 2.772$, $p = 0.250$				

The distribution of males and females across the three groups was not statistically significant (p = 0.250), indicating that sex did not confound the group assignment.

Table 3: TMAO Linear Regression Result:

Variable	β Coefficient	95% CI	p-value
Sex (Male vs Female)	-0.087	-0.321 to 0.147	0.465
Age (Years)	0.012	-0.008 to 0.032	0.238

Males had slightly lower TMAO levels than females (β = -0.087), but this was not statistically significant (p = 0.465). This suggests that after adjusting for age, plasma TMAO levels do not differ significantly between males and females; The 95% CI for Sex (-0.321 to 0.147) crosses zero, indicating no statistically significant difference. The magnitude of sex difference is small and clinically negligible.

Table 4: Age Distribution Across Study Groups

Group	n	Median Age (IQR)
Gut Eubiosis	12	51 (47-58)
Gut Dysbiosis	51	54 (48-62)
Control	63	49 (43-56)
Kruskal-Wallis H = 6.456, p = 0.168		

Age distribution across the three groups was not significantly different (p = 0.168), indicating that age did not differ between groups.

Table 5: Age vs TMAO Correlation Results:

Correlation Type	Coefficient	p-value
Spearman's ρ	0.098	0.275
Pearson's r	0.112	0.214

The correlation between age and TMAO is weak and not statistically significant (p > 0.05). The positive direction (ρ = 0.098, r = 0.112) suggests TMAO tends to increase slightly with age, but this trend is not statistically meaningful. Age does not appear to be a significant confounder or predictor of TMAO levels in this cohort.

Sex and Age Analysis

Linear regression analysis, adjusted for age and sex (Table 3), revealed no significant difference in plasma TMAO levels between males and females (β = -0.087, 95% CI: -0.321 to 0.147, p = 0.465). Similarly, age did not correlate significantly with TMAO levels (Spearman's ρ = 0.098, p = 0.275). These findings suggest that the observed TMAO elevation in T2DM patients is independent of age and sex, underscoring the robustness of TMAO as a disease biomarker in this population.

TMAO levels across groups

Plasma TMAO concentrations were significantly different across the three groups (Kruskal-Wallis H = 93.87, p < 0.001, η^2H = 0.746). Both T2DM groups had markedly elevated TMAO compared to controls: median 7.22 ng/mL (IQR 5.88–8.94) in the eubiosis group, 6.80 ng/mL (IQR 4.93–9.27) in the dysbiosis group, and 2.62 ng/mL (IQR 2.12–3.32) in controls (Figure 1). There was no significant difference between the two T2DM subgroups (p = 0.34).

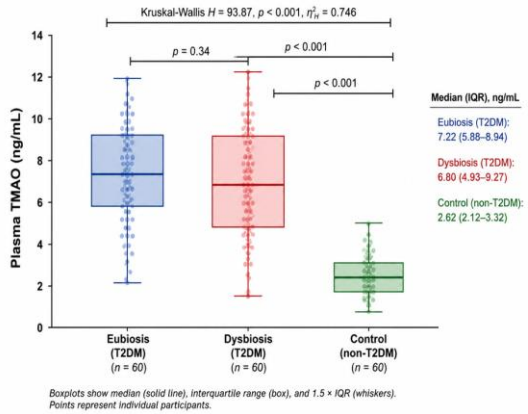

Figure 1: Plasma TMAO concentrations across study groups. Box plots show plasma trimethylamine N-oxide (TMAO) concentrations in non-diabetic control, gut eubiosis, and gut dysbiosis groups. The horizontal line represents the median; boxes indicate the interquartile range (IQR); whiskers represent 1.5×IQR. ***p < 0.001 vs control.

Association of TMAO with inflammatory markers and clinical parameters

Among dysbiotic patients (n = 51), TMAO showed a positive correlation with the F/B ratio (r = 0.407, p = 0.002). TMAO also showed strong positive correlations with TNF-α (r = 0.504, p < 0.001) and IL-6 (r = 0.386, p = 0.004) (Table 6). In eubiotic patients, correlations were not statistically significant, possibly due to small sample size (n = 12). In controls, no significant correlations were observed between TMAO and inflammatory markers.

Table 6. Correlations of TMAO with microbial ratio and inflammatory markers in dysbiotic T2DM patients (n = 51).

Variable	Spearman's r	p-value
F/B ratio	0.407	0.002
TNF-α (ng/L)	0.504	<0.001
IL-6 (pg/mL)	0.386	0.004

The logistic regression analysis showed that hyperglycaemia was independently associated with elevated TMAO (above the median) after adjustment for age and sex (AOR = 3.12, 95% CI 1.64–5.94, p = 0.001). Oral hypoglycaemic agent use (which in this cohort was universal among T2DM patients) was also associated with TMAO elevation, but this was confounded by diabetes status. BMI was not independently associated with TMAO levels (AOR = 1.07, 95% CI 0.99–1.16, p = 0.08).

DISCUSSION

To the best of our knowledge, this is the first study to characterise plasma TMAO and provide evidence that it is markedly elevated in Nigerian T2DM patients compared with healthy controls, and that it correlates with gut dysbiosis and systemic inflammation. The exceptionally large effect size for group differences in TMAO (η^2H = 0.746) underscores the strength of this association in our cohort.

The observation that TMAO levels are similarly elevated in both the eubiotic and dysbiotic T2DM groups with no significant difference between them represents a critical finding that challenges the conventional assumption that structural gut dysbiosis, as measured by the *Firmicutes/Bacteroidetes* (F/B) ratio, is the primary driver of elevated TMAO production in type 2 diabetes. This dissociation between microbial composition and functional output has significant implications for our understanding of the gut microbiome–metabolism axis.

The present data demonstrate that T2DM patients with a normal F/B ratio (eubiosis group, median F/B = 0.928) exhibited TMAO levels (7.22 ng/mL) that were not only significantly elevated compared to healthy controls (2.62 ng/mL, p < 0.001) but were also numerically higher than those in the dysbiosis group (6.80 ng/mL, F/B = 1.496). This finding reveals that structural eubiosis does not equate to functional eubiosis. While the F/B ratio has been widely used as a proxy for gut health in metabolic disease research (Olaleken et al., 2024; López-Tenorio et al., 2024), our data indicate that this phylum-level metric fails to capture the microbiota's functional capacity to produce TMAO.

This dissociation aligns with emerging literature suggesting that the relationship between gut microbial composition and metabolite production is far more complex than simple phylum-level ratios can capture. Dysbiosis is increasingly recognised as a phenomenon marked by changes in both microbial composition and function (Olaleken et al., 2024). In the context of T2DM, patients consistently exhibit marked gut dysbiosis, characterised by reduced microbial diversity and depletion of beneficial butyrate-producing taxa (Siptroth et al., 2023; Arora & Tremaroli, 2021). However, our findings suggest that functional alterations specifically enhanced TMA-producing capacity can occur independently of overt phylum-level compositional shifts.

Several mechanisms may explain why the presence of overt dysbiosis (elevated F/B ratio) does not further amplify TMAO production beyond the levels already observed in structurally eubiotic T2DM patients.

First, TMA production is driven by specific bacterial taxa rather than phylum-level abundance. The conversion of dietary choline, carnitine, and phosphatidylcholine to trimethylamine (TMA) is mediated by a relatively narrow range of bacteria possessing choline TMA-lyase (CutC) and carnitine oxygenase (CntA) enzymes (Tang et al, 2024; Zhang et al., 2026). These TMA-producing taxa are distributed across multiple phyla, including

Specific *Firmicutes* (e.g., *Clostridium* spp., *Eubacterium* spp.), *Proteobacteria*, and Actinobacteria (Cho & Caudill, 2017). An expansion of these specific functional guilds can occur without substantially altering the overall F/B ratio, particularly if reductions in non-TMA-producing Firmicutes offset the increase in TMA-producing Firmicutes. This functional enrichment

would explain why eubiotic T2DM patients with normal F/B ratios can exhibit TMAO levels comparable to those with overt dysbiosis.

Second, host factors in T2DM may enhance TMAO production independently of microbial composition**.** Insulin resistance, a hallmark of T2DM, has been shown to influence hepatic flavin-containing monooxygenase 3 (FMO3) activity, the enzyme responsible for oxidising TMA to TMAO in the liver (Jaworska et al., 2025). Hyperglycaemia and the associated metabolic dysregulation may upregulate FMO3 expression or activity, leading to increased conversion of TMA to TMAO even when TMA production by the gut microbiota remains unchanged. This suggests that TMAO may serve as a marker of hepatic insulin resistance (Jaworska et al., 2025), which could partially explain its elevation in T2DM independent of the degree of structural dysbiosis.

Third, the role of diet cannot be overlooked**.** TMAO precursors choline, carnitine, and phosphatidylcholine are abundant in animal-based foods such as eggs, red meat, and seafood (Hefni et al., 2025). Dietary patterns in this Nigerian cohort, characterised by high consumption of rice/swallow and refined carbohydrates, may provide a consistent substrate load for TMA production regardless of the underlying microbial composition. If dietary precursor supply is the primary limiting factor for TMAO production, then differences in microbial composition may have diminishing effects on overall TMAO levels once the substrate is abundant. This would explain why both eubiotic and dysbiotic T2DM patients exhibit similarly elevated TMAO.

Fourth, TMAO elevation may reflect a state of "functional dysbiosis" that is not captured by phylum-level metrics. Emerging evidence suggests that the functional capacity of the gut microbiome rather than its taxonomic composition may be the more clinically relevant determinant of metabolic disease risk (Fan & Pedersen, 2021; Alali & Shori, 2026). The present data support this paradigm: T2DM patients, regardless of their F/B ratio status, exhibit functional alterations in their gut microbial ecosystem that lead to increased TMA production. This functional shift may be driven by changes in the expression of microbial genes involved in TMA biosynthesis, alterations in gut transit time, or changes in the intestinal environment (e.g., pH, oxygen tension, bile acid composition) that favour TMA-producing metabolic activity.

The dissociation between TMAO elevation and conventional microbial composition metrics has several important clinical implications. First, it suggests that assessing the gut microbiome solely using phylum-level ratios, such as the F/B ratio, is insufficient to evaluate its metabolic impact on the host (Magne et al., 2020; Mohammadi et al., 2025). Functional biomarkers like TMAO provide critical, non-redundant information about microbial activity that is not captured by structural assessments.
Second, TMAO may represent a more direct and clinically actionable biomarker of gut microbial dysfunction in T2DM than taxonomic metrics. Its elevation in both eubiotic and dysbiotic patients suggests that TMAO reflects a fundamental shift in gut microbial function that is common to T2DM, regardless of the specific compositional changes present. This positions TMAO as a potentially valuable tool for risk stratification and for monitoring the efficacy of gut-targeted interventions.

Third, the finding that eubiotic T2DM patients have elevated TMAO highlights the importance of functional assessments in microbiome research. Interventions aimed at "normalising" the F/B ratio may be insufficient if they do not also address the microbiota's functional capacity to produce harmful metabolites such as TMAO. Therapeutic strategies should therefore target both compositional and functional restoration of the gut ecosystem.

The observation that TMAO is similarly elevated in eubiotic and dysbiotic T2DM patients underscores the dissociation between structural and functional assessments of the gut microbiome. While dysbiosis, defined by an elevated F/B ratio, is associated with elevated TMAO, overt dysbiosis does not significantly increase TMAO production beyond the levels observed in structurally "normal" microbiomes. This finding challenges the reliance on phylum-level metrics as proxies for microbial function and highlights the importance of metabolomic approaches in evaluating microbiome–host interactions in metabolic diseases. The elevation of TMAO in T2DM appears to reflect a functional shift in gut microbial activity, driven by a combination of specific TMA-producing taxa, host metabolic factors, and dietary substrate availability, rather than by phylum-level compositional changes alone.

The positive correlation between TMAO and the F/B ratio in dysbiotic patients suggests that, once dysbiosis is established, a higher proportion of Firmicutes may be associated with greater TMA-forming capacity. However, the absence of this correlation in eubiotic patients highlights the context-dependence of this relationship. Several bacterial taxa within *Firmicutes* (e.g., *Clostridium, Eubacterium*) and certain Proteobacteria are known to possess choline TMA-lyase activity (Rath et al., 2017). The specific composition of these TMA-producing species may determine TMAO levels more than the overall F/B ratio. However, the qPCR measurement was restricted to just two phyla. It cannot capture genus- or species-level expansions of specific TMA producers (e.g., *Klebsiella pneumoniae* or *Escherichia coli* from Proteobacteria).

TMAO was strongly correlated with the pro-inflammatory cytokines TNF-α and IL-6, supporting mechanistic studies showing that TMAO can activate inflammatory pathways. TMAO has been shown to activate the NLRP3 inflammasome, promote nuclear factor-κB (NF-κB) signalling, and induce cytokine production in macrophages and endothelial cells (Chen & Zhao, 2023; Seldin et al., 2022). In T2DM, this TMAO-driven inflammation likely contributes to the chronic low-grade inflammation that sustains insulin resistance and β-cell dysfunction.

Hyperglycaemia was independently associated with elevated TMAO, even after adjustment for potential confounders. This finding is consistent with previous studies that have linked poor glycaemic control to higher TMAO levels (Zhuang et al., 2019). Hyperglycaemia may alter gut microbiota composition and increase intestinal permeability, thereby enhancing TMA production and absorption (Allin et al., 202i). Alternatively, TMAO may directly impair insulin signalling, creating a bidirectional relationship between TMAO and glucose metabolism (Zhou et al., 2023).

In the present study, we observed no significant association between sex or age and plasma TMAO concentrations. This finding is consistent with recent literature suggesting that demographic influences on TMAO may be context-dependent and potentially less pronounced in non-Western populations without overt cardiovascular disease. A study from the CORDIOPREV cohort found that, while TMAO levels were higher in men with coronary heart disease than in women with the disease, no sex differences were observed in the non-cardiovascular disease population (Garcia-Fernandez et al., 2024). Similarly, a Chinese case-control study reported that the association between TMAO and coronary heart disease risk was significant only among older males (≥65 years) but not in other sex-age subgroups, indicating an age-dependent interaction between TMAO and CHD in men (Sun et al., 2024). Regarding age, while TMAO concentrations have been shown to increase from childhood to early adulthood (Almer et al., 2024), other research has demonstrated that the relationship between TMAO and clinical outcomes can be more pronounced in certain age groups, such as middle-aged and older women (Chen et al., 2024). The absence of significant sex or age associations in our cohort, which comprises a Nigerian population without overt cardiovascular disease, aligns with the finding that such demographic influences may be more salient in specific disease

contexts or populations (Garcia-Fernandez et al., 2024; Sun et al., 2024). This suggests that the marked elevation in TMAO observed in our T2DM patients is likely driven by diabetes-related metabolic dysregulation and shifts in gut microbial function, rather than by demographic factors alone.

The clinical implications of these findings are notable. TMAO is a well-established predictor of cardiovascular events, including myocardial infarction and stroke, in patients with T2DM (Schiattarella et al., 2021). In Nigeria, cardiovascular disease is a major cause of morbidity and mortality among diabetic patients. Measuring TMAO could identify individuals at elevated cardiovascular risk beyond traditional factors. Moreover, TMAO is modifiable through dietary interventions (reducing red meat, eggs, and other TMAO precursors) and possibly through probiotics or specific microbial inhibitors (Wang et al., 2021). Such strategies offer adjunctive benefits in the management of T2DM.

Several limitations should be acknowledged. The cross-sectional design precludes causal inference. Dietary intake of TMAO precursors was not assessed, limiting our ability to account for this important confounder. The study focused on only two bacterial phyla; metagenomic sequencing would allow identification of TMA-producing species. The sample size, although adequate for the primary analysis, limited subgroup analyses, particularly in the eubiosis group. Other limitations include the cross-sectional design and lack of detailed dietary data.

Finally, the single-centre design may limit generalisability to other Nigerian or African populations. Despite these limitations, this is the first study to characterise TMAO levels in relation to gut dysbiosis and inflammation in a Nigerian T2DM cohort. The strong associations observed warrant further investigation in larger, longitudinal studies that include detailed dietary and metagenomic data.

CONCLUSIONS

Plasma TMAO levels are significantly elevated in Nigerian patients with T2DM and are associated with gut dysbiosis and systemic inflammation. The dissociation between TMAO elevation and conventional microbial composition metrics underscores the importance of functional microbiome assessment. TMAO may represent a modifiable risk marker and a potential therapeutic target in this population. Prospective studies are needed to determine whether reducing TMAO levels improves glycaemic control and cardiovascular outcomes in African patients with T2DM.

ETHICS APPROVAL

Ethical approval for this study was granted by the Ethics and Research Committee of Usmanu Danfodiyo University, Sokoto, and the Sokoto State Ministry of Health (072025). Written informed consent was obtained from all study participants prior to enrollment. The study conformed to the principles of the Declaration of Helsinki.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The author acknowledges that AI-assisted technologies were partially used in the preparation of this manuscript. Specifically, generative AI tools (ChatGPT) were employed to support language refinement and grammar correction. All content was critically reviewed, edited, and approved by the author to ensure accuracy, originality, and alignment with academic standards. No AI tools were used to generate data, interpret results, or replace human intellectual contribution.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare no conflict of interest in this study

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AUTHOR'S CONTRIBUTION

D.M.K., G.J.I., O.K.A. and Y.H.D. contributed substantially to the conception and design of the work; J.M.B. and A.M.U. handled the acquisition and analysis of data, while G.J.I. and O.B.S. dealt with the interpretation of data for the work; J.M.L. and U.A.I. drafted the work, and J.M.B., A.M.U. and O.B.S. reviewed the manuscript for critical intellectual content. D.M.K., O.K.A., Y.H.D., and J.M.L. gave the final approval of the manuscript version to be published; All authors have agreed to be accountable for all aspects of the work and to ensure that questions regarding the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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