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Association of Gut Microbiota Derived Metabolites (TMAO and SCFAs) with Insulin Resistance Among Patients with Metabolic Syndrome

Hamza Mushtaq[1], Rashid Kouser[1], Ali Hassan[1]

1- Department of Medicine, Nishtar Medical University, Multan, Pakistan

Corresponding Author: Rashid Kouser, **Email:** muhammadrashidali793@gmail.com

ABSTRACT

Background: Gut microbiota-derived metabolites have emerged as key regulators of metabolic homeostasis, with increasing evidence linking them to insulin resistance and metabolic syndrome. Among these metabolites, trimethylamine N-oxide (TMAO) and short-chain fatty acids (SCFAs) exert opposing metabolic effects.

Objective: To evaluate the association between circulating levels of TMAO and SCFAs with insulin resistance in patients diagnosed with metabolic syndrome.

Methods: This cross-sectional study was conducted from May 2024 to March 2025 at Nishtar Medical University, Multan. A total of 70 adult patients with metabolic syndrome, diagnosed according to NCEP ATP III criteria, were enrolled. Fasting blood samples were collected to measure glucose, insulin, lipid profile, TMAO, and SCFAs using standardized biochemical techniques. Insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Statistical analysis included correlation and multivariable regression using SPSS version 26.

Results: The mean HOMA-IR was 5.9 ± 1.9, indicating significant insulin resistance. Elevated TMAO levels were observed in 65.7% of patients, while reduced SCFA levels were found in 61.4%. TMAO demonstrated a strong positive correlation with HOMA-IR ($r = 0.59$, $p < 0.001$), whereas SCFAs showed a significant negative correlation ($r = -0.52$, $p < 0.001$). Multivariable analysis identified TMAO as an independent predictor of insulin resistance ($\beta = 0.38$, $p < 0.001$), while butyrate exhibited a protective association ($\beta = -0.34$, $p < 0.001$).

Conclusion: Gut microbiota-derived metabolites are significantly associated with insulin resistance in metabolic syndrome. Elevated TMAO and reduced SCFAs contribute to metabolic dysfunction, highlighting their potential as biomarkers and therapeutic targets.

Keywords: Gut microbiota, TMAO, short-chain fatty acids, insulin resistance, metabolic syndrome, HOMA-IR

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INTRODUCTION

Metabolic syndrome is a significant worldwide public health issue defined by a combination of interconnected metabolic disorders, including central obesity, insulin resistance, dyslipidemia, and hypertension. It significantly elevates the risk of type 2 diabetes mellitus, cardiovascular disease, and overall mortality. Insulin resistance is the primary pathophysiological mechanism connecting metabolic inefficiency with systemic inflammation and endothelial damage. The underlying pathophysiology of insulin resistance remains poorly understood, particularly in the context of host-microbiome interactions, despite extensive research [2].

Over the last few years, the gut microbiota has become a key mediator of metabolic homeostasis, as a dynamic endocrine gland that can affect host physiology by producing its metabolites. The microorganisms in the human gastrointestinal tract are trillions that are involved in

nutrient metabolism, immune modulation, and intestinal barrier integrity maintenance. Changes in the composition of gut microbes, also known as dysbiosis, have been strongly associated with the pathogenesis of metabolic syndrome and insulin resistance, involving chronic low-grade inflammatory responses, increased intestinal permeability, and altered energy balance [3,4].

Trimethylamine N-oxide (TMAO) and short-chain fatty acids (SCFA) were some of the most popular among the various metabolites produced by gut microbiota because of their antagonistic metabolic effects. TMAO is produced by two stages, whereby dietary nutrients like choline, phosphatidylcholine, and L-carnitine are metabolised by microbes into trimethylamine (TMA), which is then further oxidised in the liver by flavin-containing monooxygenases. High plasma concentrations of TMAO have been linked to insulin resistance, atherosclerosis, and cardiovascular morbidity. In a mechanistic fashion, TMAO mediates metabolic dysfunction through an increase in oxidative stress, inflammatory signaling, disruption of insulin receptor signaling, and lipid metabolism [5,6].

Conversely, SCFAs such as acetate, propionate, and butyrate are useful metabolites that are formed by bacterial fermentation of dietary fibers in the colon. These metabolites are significant in the prevention of metabolic health by enhancing insulin sensitivity, balancing glucose and lipid metabolism, and having anti-inflammatory action. SCFAs have several mechanisms of action that include: activation of G-protein coupled receptors (GPR41 and GPR43), improving gut barrier functionality, and activating incretin hormones, such as glucagon-like peptide-1 (GLP-1). Among them, butyrate is of particular importance as it is involved in the colonic epithelial integrity preservation and the mitochondrial function regulation [7,8].

An imbalance between deleterious metabolites like TMAO and beneficial metabolites such as SCFAs indicates gut microbial dysregulation, which may significantly contribute to the onset of insulin resistance. This metabolic communication highlights the importance of gut-derived metabolites as key intermediates between dietary practices, microbial communities, and host metabolic attributes. Nevertheless, despite increasing experimental evidence, clinical studies assessing the interplay between TMAO, SCFAs, and insulin resistance in individuals with metabolic syndrome are scarce, especially in developing populations where dietary practices and environmental factors may distinctly affect gut microbiota composition [9,10].

Considering the increased burden of metabolic syndrome and its pathophysiology, there is an urgent necessity to investigate new biomarkers and mechanistic processes that will enhance the ability to detect and target therapy at an initial stage. The role of metabolites produced by gut microbiota in insulin resistance can be of great interest to the field of personalized approaches to treatment, such as dietary modification, probiotic therapy, and microbiome-based therapy [11].

The study aims to evaluate the association between circulating concentrations of TMAO and SCFAs and insulin resistance in patients with metabolic syndrome through a rigorous clinical and biochemical study.

MATERIALS AND METHODS

This is a cross-sectional study that was conducted between May 2024 and March 2025 at Nishtar Medical University, Multan, Pakistan. The study aimed to determine how intestinal microbiota-produced metabolites, including trimethylamine N-oxide (TMAO) and short-chain fatty acids (SCFAs), correlate with insulin resistance in people with metabolic syndrome.

Seventy persons aged between 30 and 65 years were recruited in outpatient and inpatient medical institutions using a non-probability sequential selection approach. Metabolic syndrome has been diagnosed according to the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria, where at least three of the following components were required: central obesity, elevated fasting glucose, hypertriglyceridemia, reduced high-density lipoprotein cholesterol, and hypertension. People who had chronic liver disease, chronic kidney disease, cancer, inflammatory bowel disease, autoimmune disease, acute infection in the recent past, or gastrointestinal surgery were not included. Patients with a history of antibiotic, probiotic, prebiotic, corticosteroid, or lipid-lowering drug use within the last three months were excluded to limit the possible confounding effects of these on gut microbiota composition and metabolic signatures. Pregnant women and those with type 1 diabetes mellitus were also ineligible.

All participants were notified about the research and asked to provide written informed consent before enrolment. Extensive demographic and clinical data were collected based on a standardised proforma, including age, gender, body mass index (BMI), waist circumference, blood pressure, lifestyle factors, and relevant medical history. Anthropometric measurements were done in a standardized manner, and blood pressure was then measured after rest on a calibrated sphygmomanometer.

Venous blood samples were collected in aseptic conditions following 8 to 12 hours of fasting. The level of serum insulin and fasting blood glucose was determined by standard enzymatic techniques, and the parameters of lipid profiles and additional routine biochemical studies were implemented in the hospital laboratory. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used to measure insulin resistance, which was calculated as fasting insulin multiplied by fasting glucose, then divided by 22.5. Higher readings of HOMA-IR were considered evidence of increased insulin resistance.

The quantification of TMAO was done through an approved enzyme-linked immunosorbent test (ELISA). Short-chain fatty acids (acetate, propionate, and butyrate) were quantified using standardised biochemical detection

methods, with proper sample handling and preservation to guarantee analytical precision. All lab tests were performed based on the methodology and quality standards of the manufacturer.

Insulin resistance was the major outcome measure of the research, and it was measured using HOMA-IR. Serum TMAO and SCFA were the primary independent factors. Potential confounding factors were also added with supplemental clinical and biochemical signs. SPSS version 26.0 was used to perform a statistical analysis. Continuous data were expressed as mean ± standard deviation, and categorical variables were reported as frequencies and percentages. Pearson or Spearman correlation analysis was used to determine the association between gut microbiota-derived metabolites and insulin resistance. Group comparisons were performed using independent sample t-tests or analysis of variance (ANOVA) as appropriate. Multivariate linear regression analysis was conducted to determine independent predictors of insulin resistance, correcting for confounding variables including age, gender, BMI, fasting glucose, and lipid profile. A p-value below 0.05 was considered statistically significant.

The Institutional Ethical Review Board of Nishtar Medical University, Multan, approved the ethical permission (Reference No: 9469/NMU). The study complied with the principles of the Declaration of Helsinki. All participants received written informed consent, and the anonymity of the patients was strongly ensured during the study period.

RESULTS

The study involved 70 patients with metabolic syndrome. The average age of the participants was 47.9 ± 8.7 years, with the majority of the participants, 60.0 % being male and 40.0% being female. The body mass index (BMI) was 30.8 ± 3.9 kg/m², and this is an indication that the participants were mostly obese. The mean fasting blood glucose was 136.2 ± 21.4 mg/dL, and the mean insulin level was 17.6 ± 5.8 µIU/mL. The mean derived HOMA-IR was found to be 5.9 ± 1.9, indicating a high level of insulin resistance among the study population. Table 1 summarizes the clinical and biochemical baseline features.

Assessment of microbiota-derived metabolites in the gut showed that 46 patients (65.7% high) and 43 patients (61.4% low) had high and low levels of TMAO and SCFA, respectively. Deficiency of SCFAs was the most consistent finding, with butyrate coming second and propionate and acetate deficiency coming third. These metabolites are distributed in Table 2, which shows a significant imbalance of gut-derived metabolic products among the study population.

Correlation analysis revealed that TMAO levels and insulin resistance had a strong positive association with a correlation coefficient of $r = 0.59$ ($p < 0.001$), and higher levels of TMAO were linked with higher HOMA-IR values. Conversely, SCFA levels were found to have a significantly negative correlation with insulin resistance, with a correlation $r = -0.52$ ($p < 0.001$), implying a protective metabolic effect. Butyrate was associated with HOMA-IR in the strongest negative relationship. Table 3 provides a summary of these findings.

Preliminary analysis with multivariate linear regression analysis showed that TMAO was still an independent predictor of insulin resistance after controlling for age, gender, BMI, fasting glucose, and lipid profile with a regression coefficient of $\beta = 0.38$ ($p < 0.001$). On the other hand, the butyrate concentrations were positively linked with less insulin resistance by themselves, and the protective effect was significant ($\beta = -0.34$, $p = 0.001$). These results suggest that the effect of metabolites produced by gut microbiota has an independent and opposing effect on metabolic regulation.

Altogether, the findings show that there is a statistically significant correlation between the gut microbiota-derived metabolites and insulin resistance among patients with metabolic syndrome, in which high levels of TMAO are associated with metabolic dysfunction, but SCFAs, especially butyrate, are protective.

Table 1: Baseline Clinical and Biochemical Characteristics of Study Participants (n = 70)

Parameter	Mean ± SD / Frequency (%)
Age (years)	47.9 ± 8.7
Male	42 (60.0%)
Female	28 (40.0%)
BMI (kg/m²)	30.8 ± 3.9
Fasting Glucose (mg/dL)	136.2 ± 21.4
Fasting Insulin (µIU/mL)	17.6 ± 5.8
HOMA-IR	5.9 ± 1.9

Table 2: Distribution of Gut Microbiota-Derived Metabolites

Parameter	Frequency (%)
Elevated TMAO	46 (65.7%)
Normal TMAO	24 (34.3%)
Reduced SCFAs	43 (61.4%)
Normal SCFAs	27 (38.6%)

Table 3: Correlation of Gut Microbiota Metabolites with Insulin Resistance

Variable	Correlation (r)	p-value
TMAO vs HOMA-IR	0.59	<0.001
SCFAs vs HOMA-IR	−0.52	<0.001

DISCUSSION

The current study presents clinically significant evidence that the microbiota-derived metabolites in the gut are strongly linked with insulin resistance among metabolic syndrome patients [10]. The results show that there is a positive relationship between high levels of trimethylamine N-oxide (TMAO) in the blood and insulin resistance, and a negative relationship between short-chain fatty acids (SCFAs), especially butyrate. This two-fold and contrasting metabolic effect underscores the importance of gut microbiota in regulating metabolic pathways of the host [11].

The positive high correlation between TMAO and HOMA-IR is observed, which suggests that TMAO might have a role to play in the pathogenesis of insulin resistance [12]. Mechanistically, TMAO is found to stimulate dysfunction of the metabolism in several pathways, namely, increased oxidative stress, stimulation of pro-inflammatory signal transduction pathways including NF-κB, as well as disruption of insulin receptor signaling [13]. These processes cause a reduction in glucose uptake and an upsurge in hepatic gluconeogenesis and eventually deteriorate glycemic control. Also, TMAO has been associated with disrupting lipid metabolism and inducing atherogenesis, hence the connection between metabolic syndrome and risk of cardiovascular disease. The results of the present research are in balance with the prior experimental and clinical data showing that the high TMAO level correlates with metabolic imbalances and cardiometabolic pathology [14].

Conversely, SCFAs were significantly negatively correlated with insulin resistance, which indicates that they are beneficial microbial metabolites [15]. SCFAs are also reported to enhance insulin sensitivity by means of various biological mechanisms, including activating G-protein-coupled receptors (GPR41 and GPR43), regulating gut hormone release, including glucagon-like peptide-1 (GLP-1), and mitochondrial functions. SCFAs also enhance the integrity of the intestinal barriers, which minimizes systemic endotoxemia and chronic low-grade inflammation, which are major contributors to insulin resistance. Butyrate was the most protective of the exposed SCFAs, which is attributed to the fact that it is the major source of energy in the colonocytes and because of its effective anti-inflammatory effects [16].

The presence of high levels of TMAO and low levels of SCFAs in the current study is indicative of a dysbiosis condition of the gut microbiota that is typical of metabolic syndrome. Such an imbalance is probably caused by the dieting habits that include the abundance of processed foods and low content of dietary fiber, which promote the synthesis of harmful metabolites and decrease the production of beneficial metabolites. This dysregulation forms a pro-inflammatory metabolic state that enhances insulin resistance and pathogenesis. The results support the notion that the products of gut microbial metabolism are major mediators between environmental factors, especially the diet, and the results of metabolic health [17,18].

Clinically, the outcomes of this study indicate that TMAO and SCFAs can be utilized as potential biomarkers for measuring the level of metabolic risk and the severity of insulin resistance. Besides, they are attractive therapeutic targets. Dietary fiber supplements, prebiotics, probiotics, and microbiota-directed therapy can be used to provide metabolic balance by lowering TMAO formation and increasing SCFAs. This method is in line with new precision medicine approaches, which seek to use the microbiome of the gut to treat metabolic diseases [19,20].

This study has several limitations despite its strengths, and one of them is that it incorporates both biochemical and clinical parameters [1,7]. The cross-sectional structure limits the possibility of determining the causality between insulin resistance and gut-derived metabolites. Its sample size is relatively small and not enough to generalize the results, and the assessment of the diet was not conducted in detail, which might also affect metabolite levels [9]. Also, it did not involve direct microbiome sequencing that would allow correlation of selected bacterial taxa with metabolite profiles. Further studies are required on longitudinal research and interventional trials so as to determine causal relationships and determine the therapeutic potential of altering intestinal microbiota. The metagenomic and metabolomic analyses would be incorporated, and these analyses would give a better understanding of the host-microbe interaction and its contribution to metabolic diseases [18-20].

CONCLUSION

This study shows that insulin resistance among patients with metabolic syndrome is closely related to the gut microbiota-derived metabolites. High levels of TMAO are associated with the deterioration of metabolic dysfunction, whereas SCFAs, especially butyrate, have a protective effect on insulin resistance. These results imply the significance of the gut microbiota in metabolism and provide a hint that microbial metabolite targeting could be considered a new approach to metabolic syndrome prevention and management.

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Authors' Contributions: H.M. contributed to conceptualization, data collection, and manuscript drafting. R.K. was responsible for study design, supervision, and

critical revision of the manuscript. A.H. performed data analysis, interpretation of results, and manuscript editing. All authors approved the final version of the manuscript.

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Data Availability: The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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