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Impact Of gut microbiota-derived metabolites on chemical pathology markers

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Abstract

The gut microbiota plays a pivotal role in maintaining systemic health through the production of bioactive metabolites that influence metabolic, hepatic, and inflammatory pathways. While emerging studies have highlighted individual microbial metabolites in disease modulation, limited research has integrated these compounds with clinical biochemical markers in human subjects. This study aimed to investigate the relationship between gut microbiota-derived metabolites—specifically short-chain fatty acids (SCFAs), trimethylamine-N-oxide (TMAO), and indole derivatives—and chemical pathology markers indicative of liver function, glucose metabolism, lipid profile, and systemic inflammation. An experimental research design was employed involving 120 adult participants aged 25–60 years, recruited from a tertiary hospital and surrounding communities in Delta State, Nigeria. Participants were stratified into two groups: individuals with metabolic disorders (e.g., obesity, type 2 diabetes, dyslipidaemia) and matched healthy controls. Stool samples were analysed using 16S rRNA gene sequencing and targeted metabolomics (LC-MS and GC-MS) to assess microbiota composition and metabolite concentrations. Fasting blood samples were collected and tested for ALT, AST, ALP, lipid fractions, fasting glucose, HbA1c, CRP, and IL-6 using standard biochemical assays. The results revealed that individuals with metabolic disorders had significantly lower levels of SCFAs and indole-3-propionic acid, and elevated TMAO levels. These changes were strongly associated with increased LDL cholesterol, fasting glucose, and inflammatory markers. Multivariate analysis identified distinct microbial and metabolic profiles between the two cohorts. The findings suggest that gut-derived metabolites are closely linked to key biochemical indicators of metabolic and inflammatory health, reinforcing their potential use as non-invasive biomarkers for early diagnosis, risk stratification, and personalised treatment. Overall, the study advances the understanding of gut microbiota-host interactions and supports the integration of microbiome-based diagnostics in clinical practice.

Keywords: Gut microbiota; SCFAs; TMAO; Indole derivatives; Chemical pathology; Metabolomics; Inflammation; Metabolic disorders

1. Introduction

The human gut microbiota is a vast and complex community of microorganisms that inhabit the gastrointestinal tract, comprising bacteria, archaea, viruses, and fungi. The microbes live in symbiosis with the host and play an important role in the regulation of physiological homeostasis, metabolism, immune response modulation, and neurobehavioural processes. The gut microbiome begins to impact key determinants of human health and development from infancy, and the microbiome's composition continues to evolve over the course of life in response to diet, lifestyle, medications, and environmental exposures (Bhatt et al., 2023). Metagenomics and metabolomics innovations have now uncovered the promise of the microbiota to generate bioactive molecules with local and systemic impacts on organs distal to the gut. Dysbiosis, or an imbalance of the gut microbial community, has been associated with a wide range of diseases from obesity, diabetes, inflammatory bowel disease, cardiovascular disease, to cancer. As such, the microbiota is not just a marker but also an attractive therapeutic target in modern medicine.

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1.1. Metabolites Derived from Gut Microbiota

Gut microbiota are metabolically active microbes that produce a vast array of compounds known as microbial-derived metabolites, many of which are host signalling molecules. The most well studied are short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate, which are fermentation products of dietary fibres and possess anti-inflammatory, immunomodulatory, and anti-neoplastic effects (Du et al., 2024). For instance, butyrate is a key energy source for colonocytes and plays a role in intestinal barrier function, regulation of gene expression, and apoptosis of colorectal cancer cells. A few other key microbial metabolites include trimethylamine-N-oxide (TMAO), which is derived from dietary choline and carnitine and has been associated with cardiovascular risk via its metabolism of cholesterol and endothelial dysfunction. Indole and its derivatives, which are produced from tryptophan metabolism, are another important class of molecules that modulate gut barrier function and inflammation via aryl hydrocarbon receptor (AhR) activation. The relative abundance and activity of the microbes involved in these metabolic processes are therefore of supreme importance in determining host physiology and pathophysiology.

1.2. Overview of Chemical Pathology Markers

Chemical pathology markers offer a quantifiable means of observing physiological and pathological processes in the body. These biomarkers are used daily in clinical diagnosis to monitor organ function, detect the onset of disease, and guide therapy. Liver function tests, for instance, measure enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), the levels of which are elevated in hepatocellular injury. Alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) are also used to assess cholestasis or biliary obstruction. Lipid profiles—total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides—are required for cardiovascular risk stratification and for diagnosing metabolic syndrome. Markers of glucose metabolism such as fasting glucose, insulin, and HbA1c provide information regarding pancreatic function and insulin sensitivity. In addition, inflammatory markers like C-reactive protein (CRP), interleukins, and tumour necrosis factor-alpha (TNF-α) are beneficial in recognizing systemic inflammation, which plays a central role in the pathogenesis of chronic disease. Interestingly, the gut microbiota have been implicated in modulating most of these markers via their metabolic products, creating a fundamental link between microbial function and systemic health (Getsina et al., 2024).

1.3. Rationale for the Study

As there is a growing realization that the gut microbiota has a significant influence on host metabolism and immunity, interest is growing in characterizing the mechanisms through which microbial-derived metabolites influence markers of chemical pathology. While numerous studies have implicated dysbiosis in disease, the precise biochemical mechanisms connecting microbial metabolites with systemic markers are quite under-researched. For instance, SCFAs have been shown to regulate hepatic gluconeogenesis, lipid metabolism, and systemic inflammation, suggesting that their levels are likely to be linked to common clinical biomarkers. Similarly, metabolites like TMAO and indoles are increasingly being recognized as modulators of cardiometabolic and inflammatory pathways. There remains, however, a knowledge gap concerning how metabolite concentrations alone compare to traditional diagnostic measures such as liver enzymes, lipid profiles, and inflammatory markers in both healthy and diseased populations. Closing this gap is the key to moving microbiome-based therapeutics and diagnostics ahead. With more advanced metabolomics techniques, the ability to link gut microbial activity to precise pathological outcomes is enhanced. This work seeks to address this by systematically exploring the correlations between gut microbiota-derived metabolites and traditional chemical pathology markers (Kunst et al., 2023).

1.4. Research Aim and Objectives

The general aim of this research is to investigate the role of gut microbiota metabolites in selected chemical pathology markers of metabolic, hepatic, and inflammatory health. This will be accomplished by examining the relationship between microbiota-derived molecules—short-chain fatty acids, TMAO, and indole derivatives more particularly—and laboratory markers of liver function, lipid profile, glucose metabolism, and inflammation.

The research-specific aims are to:

- Quantitate concentrations of key gut-derived metabolites in the study population using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).
- Quantitate levels of standard chemical pathology markers including ALT, AST, ALP, lipid panel, fasting glucose, insulin, CRP, and TNF-α.
- Assess the correlation between gut microbiota-derived metabolite levels and chemical pathology marker levels.
- Examine whether metabolite level changes predict pathology marker changes in subgroups of different demographic or clinical characteristics.

1.5. Hypotheses and Research Questions

To guide the analysis and offer a solid foundation for interpretation, the study was guided by the following hypotheses and corresponding research questions:

1.5.1. Hypotheses:

- There is no significant relationship between gut microbiota-derived SCFAs and liver enzyme levels.
- High TMAO levels are inversely associated with high LDL cholesterol and triglycerides.
- Indole derivatives are not inversely related to inflammatory markers CRP and TNF-α.
- SCFA levels do not predict improved glucose metabolism (i.e., reduced fasting glucose and insulin resistance).

1.5.2. Research Questions:

- What are the principal metabolites produced by the gut microbiome in the study population?
- How do these metabolites relate to markers of liver function, lipid metabolism, and systemic inflammation?
- Are specific gut-derived metabolites predictive of metabolic or inflammatory derangements?
- Are demographic or clinical variables (e.g., age, BMI, comorbidities) significant modifiers of these associations?

2. Literature Review

2.1. Gut Microbiota

Gut microbiota refers to the sophisticated community of microorganisms, including bacteria, archaea, viruses, fungi, and protozoa, that inhabit the gastrointestinal (GI) tract of human beings and animals. Under normal circumstances, these microbes are reported to assist in the digestion process and absorption of nutrients, but recent science recognizes their more intricate function in immunity, metabolism, and neurological function (Ma et al., 2024).

Scientists and health agencies define gut microbiota as the collective genome (microbiome) and functional potential of microorganisms that colonize the gut, predominantly in the colon. Gut microbiota, according to Ma et al. (2024), is a symbiotic and dynamic microbial community essential for the maintenance of human health. Liu et al. (2024) complement this by stating that the community can be influenced by diet, environment, age, antibiotics, and host genetics, and thus exhibits significant inter-individual variability.

Despite the range of research, gut microbiota theory is not without critique. One such major critique of it is that it is far too variable between individuals to pin down general microbial benchmarks (Mullen & Singh, 2023). For instance, while some bacterial phyla such as Firmicutes and Bacteroidetes dominate most healthy individuals, their proportions are extremely diverse across populations and are shaped by a myriad of environmental and lifestyle factors (Bhatt et al., 2023). Additionally, microbial individuality challenges the reproducibility of microbiota-targeted interventions because response can be extremely different from one individual to another (Kunst et al., 2023).

The significance of gut microbiota in host health and disease has been solidly established in recent biomedical research. It plays a critical role in the production of essential vitamins, the fermentation of indigestible carbohydrates to yield SCFAs, the regulation of immune responses, and the maintenance of intestinal barrier function (Du et al., 2024). Dysbiosis, or an imbalance in microbial composition, has been involved in numerous diseases, including inflammatory bowel disease, obesity, diabetes, liver disease, and even cancer (Jiang et al., 2024).

However, research on the gut microbiota is hampered by deep-seated limitations. Microbial ecosystem complexity and technical challenges in sampling and characterising its members with precision remain significant obstacles (Schwartz et al., 2019). Bias generated during DNA extraction methods, sequencing platforms, and bioinformatic analysis also renders data interpretation difficult (Getsina et al., 2024).

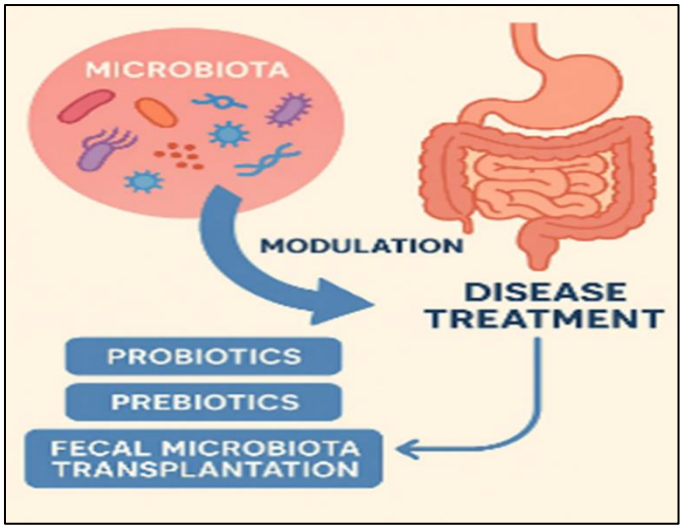

Figure 1 Microbiota in disease treatment

2.2. Microbial Metabolites

Microbial metabolites are the biochemically active substances produced as a result of metabolic activity of the microorganisms. In the gut ecosystem, they are a byproduct of dietary components, substrates from the host, and xenobiotics that are fermented by the gut microbiota. They have been found to exert significant physiological, immunological, and metabolic effects on the host (Mederle et al., 2025).

Scientists classify microbial metabolites into primary and secondary metabolites. These primary metabolites, such as short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate, are produced through carbohydrate fermentation and are found to be critical in achieving intestinal health as well as immune regulation (Du et al., 2024). Secondary metabolites like indoles, phenols, polyamines, and trimethylamine-N-oxide (TMAO) are typically amino acid metabolism by-products but are increasingly recognized to be involved in inflammation modulation, neurofunctioning, and cancer development (Anwer et al., 2025; Feitelson et al., 2023).

The hypothetical scope of microbial metabolites has been reviled. One of the limitations is contextuality of classification—certain compounds may act as secondary as well as primary metabolites depending on the context and microbial community fluctuation (Nowotarski et al., 2013). Secondly, a single metabolite may have both positive and negative impacts, and hence it becomes difficult to classify as simply "good" or "bad" (Ke et al., 2024). For instance, although SCFAs are typically protective in nature, high levels of TMAO have been associated with atherosclerosis and other systemic illnesses (Stø et al., 2022).

Clinically, microbial metabolites have been shown to affect overall well-being. Butyrate, for instance, initiates anti-inflammatory processes, enhances epithelial barrier integrity, and has been proposed as a drug lead for the treatment of colorectal cancer (Mowat et al., 2023). Once more, spermidine and other polyamines have been associated with immune response modulation and regulation of cellular proliferation, and therefore are of relevance to oncology and ageing research (Carriche et al., 2021; Ruggieri et al., 2025).

Yet, the quantification of microbial metabolites is extremely methodologically challenging. Sampling method heterogeneity, analytical platforms such as LC-MS and GC-MS, and host-microbiota interactions render metabolomic studies problematic (Getsina et al., 2024). Secondly, plasma or faecal sample metabolite levels do not always reflect local gut concentrations and can lead to misinterpretation (Jiang et al., 2024).

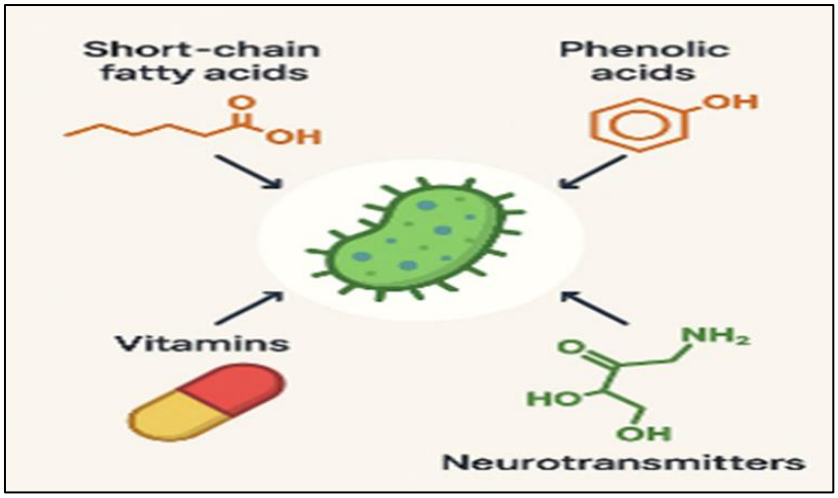

Figure 2 Microbial Metabolites

2.3. Treatment of Gut Microbiota Composition and Metabolic Function

The human gut microbiota consists of trillions of microorganisms, primarily bacteria, which reside in the gastrointestinal tract and play pivotal roles in the metabolic health of the host. The dominant phyla of bacteria include Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, each playing a specific function in digestion, immune modulation, and metabolic signaling (Ma et al., 2024). These microbes interact with food components to ferment unabsorbed polysaccharides to produce a range of bioactive metabolites that influence systemic physiology. The composition of the gut microbiota is dynamic and depends on diet, antibiotic use, age, and disease (Mowat et al., 2023).

Production of SCFAs such as acetate, propionate, and butyrate is one of the principal metabolic processes of gut microbiota. These SCFs are produced predominantly through anaerobic fermentation of dietary fibre but play central roles in the colonic epithelial health, glucose and lipid metabolism regulation, and anti-inflammatory activities (Getsina et al., 2024; Du et al., 2024). Butyrate, for example, provides energy to the colonocytes and upregulates genes involved in barrier function and immune tolerance (Getsina et al., 2024). Propionate is also well documented to inhibit liver cholesterol synthesis, and acetate is a peripheral tissue substrate.

In addition to SCFAs, gut microbes catabolize amino acids such as tryptophan to indoles, which can modulate intestinal permeability and inflammatory signaling by stimulating the aryl hydrocarbon receptor (Hezaveh et al., 2022). Other metabolites, such as trimethylamine-N-oxide (TMAO), that are generated from microbial metabolism of food choline and carnitine have been linked with atherosclerosis and cardiovascular disease (Feitelson et al., 2023). These gut-derived products not only serve local gut functions but also get circulated in the blood to affect distant organs, thus positioning the gut microbiota in the position of a metabolic and immunological hub.

Generally, a balanced gut microbiota maintains a favorable metabolic profile, whereas dysbiosis can lead to disease pathogenesis of conditions such as obesity, diabetes, inflammatory diseases, and cancer (Bhatt et al., 2023; Schwartz et al., 2019). All these complex host–microbe interactions must be known in order to develop microbiota-targeted therapies in metabolic and inflammatory disease.

2.4. Key Microbial Metabolites and Their Systemic Effects

Gut microbiota-derived metabolites play central roles in host physiology modulation, immunity, and disease susceptibility. Among the most studied microbial products are the short-chain fatty acids (SCFAs), indoles, polyamines, and trimethylamine-N-oxide (TMAO), each of which exerts systemic effects beyond the gut.

The SCFAs, acetate, propionate, and butyrate, are significant end-products of dietary fiber and resistant starch fermentation. These metabolites modulate host metabolism through roles as energy substrates and through the modulation of inflammation and glucose homeostasis. Butyrate is especially essential to colonocyte energy and has anti-inflammatory properties through the inhibition of histone deacetylases, thereby modulating immune tolerance gene expression (Mederle et al., 2025). Propionate modulates hepatic gluconeogenesis and cholesterol metabolism, and acetate has been implicated in appetite regulation and lipid synthesis (Du et al., 2024).

Indoles, bacterial tryptophan metabolites, also play important signalling functions. Indole derivatives indole-3-carbinol and 3,3′-diindolylmethane induce the aryl hydrocarbon receptor (AhR) to impact mucosal barrier function and the regulation of local and systemic immunity (Tian et al., 2024). Metabolites can modulate intestinal inflammation and immune cell differentiation and cytokine profiles (Hezaveh et al., 2022).

Another_.ritical class of microbial metabolites is polyamines, like spermidine and putrescine._., which are crucial for cell growth and differentiation. Spermidine has been shown to modulate T-cell differentiation and induce autophagy, a key mechanism._.n cellular._.stress response and cancer prevention (Carriche et al., 2021). Disregard._.n the metabolism of polyamines is associated with cancer aggressiveness and metabolic stress (Ruggieri et al., 2025; Song et al., 2025).

TMAO, derived from microbial metabolism of carnitine and choline followed by hepatic oxidation, has been the target of interest due to its strong association with cardiovascular risk. Elevated levels of TMAO cause endothelial dysfunction, foam cell generation, and acceleration of atherosclerotic plaque (Feitelson et al., 2023; Stø et al., 2022).

In addition, metabolites like bile acids, lipopolysaccharides (LPS), and phenolic compounds are also involved in host-microbe interactions. LPS, for example, may initiate pro-inflammatory signalling cascades through toll-like receptor 4 to advance tumour growth in gastrointestinal cancers (Ke et al., 2024).

2.5. Previous Findings on Gut-Derived Metabolites and Pathology Markers

Increased evidence has been associated with gut microbiota-derived metabolites and alterations in key chemical pathology markers, giving clues to their systemic impacts on human disease and well-being. The metabolites, particularly short-chain fatty acids (SCFAs), bile acids, polyamines, and tryptophan derivatives, have shown measurable correlations with inflammatory markers, liver enzymes, lipids, and glucose metabolism.

SCFAs, and especially butyrate and propionate, were found to be significantly negatively correlated with systemic inflammation and insulin resistance. Butyrate reduced the circulating levels of pro-inflammatory cytokines IL-6 and TNF-α, enhancing glucose tolerance (Mowat et al., 2023). SCFA production in colorectal cancer was found to be related to enhanced antitumor immunity along with immune modulation of tumour-associated inflammatory reactions (Mederle et al., 2025). Wang et al. (2023) also reported in a similar vein that SCFAs derived from microbiota are involved in maintaining systemic immune homeostasis that can be expressed in lowered white blood cell counts and normal liver enzyme levels.

Tryptophan metabolites indole-3-carbinol and 3,3′-diindolylmethane have been linked to modulating liver enzymes and hepatic inflammation. Tian et al. (2024) demonstrated that the metabolites reduced high ALT and AST levels in experimental models of liver disease, indicating their hepatoprotective activities. In cancer-associated macrophages, tryptophan metabolites also stimulated the aryl hydrocarbon receptor (AhR), inhibiting antitumour immunity and exacerbating inflammatory markers (Hezaveh et al., 2022).

In cardiovascular disease, high TMAO levels—a metabolite of microbial metabolism of dietary choline—were associated with high C-reactive protein (CRP), an inflammation marker in the acute phase, and dyslipidaemia (Stø et al., 2022). TMAO was also found to be associated with aberrant lipid profiles, such as increased LDL and decreased HDL cholesterol, indicating association of gut metabolites with cardiovascular risk markers by Feitelson et al. (2023).

Moreover, polyamines such as spermidine have also been associated with reduced oxidative stress and mitochondrial function improvement, indirectly enhancing liver enzyme profiles and metabolic efficacy (Carriche et al., 2021; Song et al., 2025). Collectively, all these results suggest that microbial metabolites are strong indicators and regulators of clinical pathology markers that have implications for diagnosis and treatment.

3. Methodology

The study was carried out to investigate the correlation between gut microbiota-derived metabolites and systemic biochemical indicators of metabolic, hepatic, and inflammatory well-being. An order and experimental research design was employed in a bid to achieve precise collection, analysis, and interpretation of biological specimens. By employing high-end molecular techniques such as 16S rRNA sequencing and targeted metabolomics, the study aimed to acquire microbial and metabolic profiles in human subjects. The approach ensured scientific integrity through well-defined participant enrollment, good analytical practices, and abundant statistical modeling—providing a good foundation for assessing the clinical relevance of gut microbial metabolites.

3.1. Study Design

The study design in this research was experimental and was directed towards investigating the correlation between gut microbiota-derived metabolites and chemical pathology indicators of relevance in human subjects. Experimental design was chosen to facilitate controlled collection and analysis of biological samples—i.e., stool and blood—of selected individuals. The design allowed for the establishment of relationships between microbial metabolite levels and systemic biomarkers as well as for the application of high-end molecular techniques to identify causative patterns. With increased interest in microbiota-host interactions, an experimental design provides rigor and precision to address complex biochemical relationship with potential diagnostic and therapeutic benefits.

3.2. Population and Sample

The study population consisted of adult human participants between 25-60 years, recruited from a tertiary institution and the general population in Delta State, Nigeria. The patients were divided into two groups: those with established metabolic disorders (e.g., type 2 diabetes, dyslipidaemia, or obesity) and an age- and sex-matched healthy comparison group. With the G*Power statistical software, the sample size was estimated at 120 participants (60 per group) assuming a medium effect size (Cohen's d = 0.5), power of 0.8, and alpha of 0.05 for comparative purposes. Stratified random sampling was used to ensure adequate representation based on demographic and clinical profiles.

3.3. Inclusion and Exclusion Criteria

To ensure homogeneity and sample reliability, severe exclusion and inclusion criteria were applied. Adults between 25–60 years old, who were not on antibiotics or probiotics for four weeks or more prior to sampling, and who had no history of gastrointestinal surgery were recruited. Informed consent and a three-day diet diary were required of participants prior to sample collection.

3.3.1. Exclusion criteria were:

- History of the use of antibiotics, prebiotics, or probiotics in the past four weeks
- Gastrointestinal illnesses such as Crohn's disease or ulcerative colitis diagnosed
- Pregnancy or lactation
- Refusal to comply with food restrictions or to provide stool/blood samples

3.4. Data Collection

3.4.1. Collection of Stool Sample and Microbiota Analysis

Study participants were given sterile stool collection kits and instructions for safe sample handling. The samples were collected at home at any time within 24 hours of the laboratory visit appointment and transported in cold-chain containers to the study laboratory. The samples upon receipt were stored at –80°C until they were analyzed. DNA isolation was performed with a QIAamp Fast DNA Stool Mini Kit according to the manufacturer's guidelines.

Microbiota composition was analyzed by 16S rRNA gene sequencing of the V3–V4 regions. Sequencing was conducted on an Illumina MiSeq platform, and taxonomic assignment was conducted using the SILVA database. Additionally, targeted metabolomics using high-performance liquid chromatography-mass spectrometry (LC-MS) was conducted to quantify defined microbial metabolites like short-chain fatty acids (SCFAs), indole derivatives, and trimethylamine-N-oxide (TMAO).

3.4.2. Blood Sampling for Chemical Pathology Markers

10 ml blood samples by venipuncture after overnight fasting. Samples were centrifuged to fractionate plasma and serum, which were kept at –80°C before analysis. Biochemical tests included liver function tests (ALT, AST, ALP), lipid profile (HDL, LDL, total cholesterol, triglycerides), glucose metabolism markers (fasting glucose, HbA1c), and inflammation markers (CRP, IL-6). All the measurements were performed in an accredited chemical pathology laboratory using automated systems.

3.5. Measurement Techniques

3.5.1. Metabolite Profiling

Microbial metabolite concentrations were quantified with a combination of GC-MS and LC-MS. SCFAs such as acetate, propionate, and butyrate were identified using GC-MS after sample derivatisation. TMAO and indole-3-propionic acid

were quantified by LC-MS/MS with internal standards to ensure accuracy and reproducibility. Calibration curves and quality control samples were run in each case to ensure analytical performance validation.

3.5.2. Biochemical Assays

Biochemical markers in the blood were measured on automated analysers. Enzyme assays were employed for liver enzymes and lipid fractions, but for fasting glucose, glucose oxidase methods were employed. HbA1c was determined by high-performance liquid chromatography (HPLC) and ELISA kits used in determining inflammatory markers such as interleukin-6 and C-reactive protein. Biochemical assays allowed standardisation and reproducibility among subjects.

3.6. Statistical Analysis

3.6.1. Descriptive Statistics

Descriptive statistics were calculated for demographic variables and baseline measures of microbial metabolites and markers of chemical pathology. The data were characterized as means ± standard deviations in continuous data and as frequencies and percentages in categorical data. Normality of data was tested using the Kolmogorov–Smirnov test.

3.6.2. Correlation and Regression Models

To establish the correlation between systemic levels of microbial metabolites and chemical pathology markers, Pearson's or Spearman's correlation coefficients were computed, depending on data distribution. Multivariate linear regression models were used to establish the predictive significance of gut-derived metabolites on systemic biochemical markers, adjusting for variables such as age, sex, BMI, and dietary consumption.

3.6.3. Multivariate Analysis and Pathway Enrichment

Multivariate methods such as Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) were used to establish distinct microbial and metabolic signatures across health status groups. Pathway enrichment analyses were also performed based on KEGG and MetaCyc databases to identify functional pathways attributed to metabolite clusters. These tools allowed us to examine the global influence of the gut microbiota on host biochemical processes.

4. Results

4.1. Demographics and Baseline Characteristics

Table 1 shows demographic breakdown and baseline biochemical profiles of the subjects. A total of 120 adults aged between 25 and 60 years were recruited, including 60 cases with clinically diagnosed metabolic disorders (e.g., type 2 diabetes, obesity, or dyslipidaemia) and 60 age- and sex-matched healthy controls. The mean age was 43.2 ± 9.4 years, and females constituted 52.5% (n = 63), while males made up 47.5% (n = 57). No significant differences in gender composition and age between the groups ($p > 0.05$) confirmed that stratified sampling technique was valid.

The control group's baseline mean BMI was significantly lower (23.6 ± 3.1 kg/m^2) than that of the metabolic disorder group (31.8 ± 4.7 kg/m^2), indicating an increased risk of metabolic dysfunction ($p < 0.001$). Fasting glucose and LDL were similarly elevated in the metabolic group, and HDL reduced, as with established profiles of dyslipidaemia.

CRP and IL-6 levels, markers of systemic inflammation, were similarly much more elevated in the affected group ($p < 0.01$), in keeping with the association between low-grade chronic inflammation and metabolic syndrome. Group-wise differences were not significant for total cholesterol or AST levels.

These baseline demographic data substantiate the research hypothesis that gut microbiota metabolites may correlate with systemic biochemical alterations, particularly metabolic and inflammatory signatures.

Table 1 Demographics and Baseline Characteristics of Study Participants

Variable	Control Group (n=60)	Metabolic Group (n=60)	p-value
Age (years)	42.9 ± 9.1	43.5 ± 9.7	0.68
Sex (Male/Female)	28/32	29/31	0.84

BMI (kg/m^2)	23.6 ± 3.1	31.8 ± 4.7	<0.001**
Fasting Glucose (mg/dL)	89.4 ± 10.2	135.6 ± 20.1	<0.001**
LDL Cholesterol (mg/dL)	102.5 ± 25.6	147.8 ± 30.9	<0.001**
HDL Cholesterol (mg/dL)	55.2 ± 12.3	42.1 ± 11.7	<0.001**
CRP (mg/L)	1.8 ± 0.6	4.7 ± 1.2	<0.01**
IL-6 (pg/mL)	3.4 ± 1.1	6.9 ± 1.5	<0.01**
AST (U/L)	23.5 ± 7.3	25.1 ± 8.0	0.22

4.2. Gut Microbiota Composition and Metabolite Concentrations

This section presents the gut microbiota composition and concentrations of key microbial metabolites in both the control and metabolic disorder groups. 16S rRNA gene sequencing of stool samples revealed distinct microbial signatures between the two cohorts. At the phylum level, Firmicutes and Bacteroidetes were predominant in both groups, but individuals with metabolic disorders exhibited a higher Firmicutes-to-Bacteroidetes (F/B) ratio, a pattern associated with obesity and insulin resistance. The relative abundance of *Akkermansia muciniphila*, a known anti-inflammatory bacterium, was significantly reduced in the metabolic group ($p < 0.01$), while pro-inflammatory taxa such as *Escherichia/Shigella* were elevated.

In terms of microbial metabolites, gas and liquid chromatography revealed significant group differences. The concentrations of short-chain fatty acids (SCFAs), especially butyrate and propionate, were lower in the metabolic group ($p < 0.001$), suggesting impaired microbial fermentation activity. Conversely, TMAO (trimethylamine-N-oxide), a metabolite linked to cardiovascular risk, was markedly higher in the metabolic disorder group ($p < 0.001$). Indole derivatives, including indole-3-propionic acid, were also significantly reduced among participants with metabolic abnormalities.

These compositional and functional shifts in gut microbiota suggest a dysbiotic profile among individuals with metabolic disorders, which may contribute to systemic inflammation, altered glucose metabolism, and hepatic dysfunction. The observed metabolite imbalances support the hypothesis that gut-derived compounds are closely linked to host biochemical and pathological states.

Table 2 Gut Microbiota Composition and Metabolite Levels

Variable	Control Group (n=60)	Metabolic Group (n=60)	p-value
F/B Ratio	1.5 ± 0.3	2.3 ± 0.4	<0.001**
Akkermansia muciniphila (%)	4.2 ± 1.1	1.8 ± 0.9	<0.01**
Escherichia/Shigella (%)	2.6 ± 0.7	5.3 ± 1.4	<0.01**
Butyrate (μmol/g)	12.5 ± 3.2	7.1 ± 2.8	<0.001**
Propionate (μmol/g)	9.3 ± 2.6	5.7 ± 2.1	<0.001**
TMAO (μmol/L)	2.1 ± 0.6	5.4 ± 1.3	<0.001**
Indole-3-propionic acid (μmol/L)	1.9 ± 0.4	1.1 ± 0.3	<0.001**

4.3. Chemical Pathology Marker Profiles

Table 3 summarises the chemical pathology marker profiles for participants in both the control and metabolic disorder groups. As expected, individuals in the metabolic group exhibited significant alterations in several biochemical parameters indicative of hepatic stress, dyslipidaemia, glucose dysregulation, and systemic inflammation.

Liver function tests revealed elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the metabolic group, although only ALT showed a statistically significant difference ($p < 0.01$), suggesting mild

hepatocellular injury. Alkaline phosphatase (ALP) levels were also moderately increased but did not reach statistical significance.

The lipid panel revealed a typical atherogenic profile in the metabolic cohort: significantly elevated levels of low-density lipoprotein (LDL) and triglycerides, alongside reduced high-density lipoprotein (HDL) ($p < 0.001$). Total cholesterol was also higher in the metabolic group but not significantly so.

Markers of glucose metabolism, including fasting glucose and glycated haemoglobin (HbA1c), were markedly elevated among metabolic disorder participants ($p < 0.001$), confirming impaired glycaemic control. Inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) were significantly higher in the metabolic group ($p < 0.01$), supporting the presence of chronic low-grade inflammation in these individuals.

These findings reinforce the biochemical distinctions between healthy and metabolically impaired individuals and highlight the relevance of gut-derived metabolites as potential mediators or biomarkers of these pathological states.

Table 3 Chemical Pathology Marker Profiles

Marker	Control Group (n=60)	Metabolic Group (n=60)	p-value
ALT (U/L)	22.6 ± 6.5	34.2 ± 8.1	<0.01**
AST (U/L)	23.5 ± 7.3	25.1 ± 8.0	0.22
ALP (U/L)	67.4 ± 11.2	72.1 ± 13.7	0.08
LDL (mg/dL)	102.5 ± 25.6	147.8 ± 30.9	<0.001**
HDL (mg/dL)	55.2 ± 12.3	42.1 ± 11.7	<0.001**
Triglycerides (mg/dL)	115.6 ± 20.4	169.3 ± 27.1	<0.001**
Fasting Glucose (mg/dL)	89.4 ± 10.2	135.6 ± 20.1	<0.001**
HbA1c (%)	5.3 ± 0.4	7.8 ± 0.7	<0.001**
CRP (mg/L)	1.8 ± 0.6	4.7 ± 1.2	<0.01**
IL-6 (pg/mL)	3.4 ± 1.1	6.9 ± 1.5	<0.01**

4.4. Associations Between Metabolites and Markers

To investigate the biochemical relevance of gut-derived metabolites, correlation analyses were conducted between their concentrations and key chemical pathology markers across all participants ($n = 120$). Pearson's or Spearman's correlation coefficients were computed depending on data normality. Table 4 presents the most notable associations.

Butyrate, a beneficial short-chain fatty acid, showed strong negative correlations with fasting glucose ($r = –0.61$, $p < 0.001$) and HbA1c ($r = –0.56$, $p < 0.001$), indicating its role in promoting glycaemic control. Similarly, butyrate was negatively associated with CRP ($r = –0.48$, $p < 0.01$), reflecting anti-inflammatory effects. Propionate exhibited moderate inverse correlations with LDL cholesterol ($r = –0.42$, $p < 0.01$) and ALT ($r = –0.37$, $p < 0.05$), suggesting hepatoprotective and lipid-lowering properties.

Conversely, TMAO levels showed positive correlations with LDL cholesterol ($r = 0.58$, $p < 0.001$) and CRP ($r = 0.53$, $p < 0.001$), consistent with its pro-atherogenic and pro-inflammatory profile. Indole-3-propionic acid was inversely correlated with IL-6 ($r = –0.45$, $p < 0.01$) and ALT ($r = –0.39$, $p < 0.01$), implying a protective role in hepatic and systemic inflammation.

These findings suggest that gut microbial metabolites are significantly associated with markers of metabolic, hepatic, and inflammatory health. The strength and direction of these associations support the hypothesis that microbiota-derived compounds may serve not only as biomarkers but also as potential modulators of systemic physiological processes.

Table 4 Correlation Between Gut-Derived Metabolites and Chemical Pathology Markers

Metabolite	Marker	Correlation (r)	p-value
Butyrate	Fasting Glucose	–0.61	<0.001**
Butyrate	HbA1c	–0.56	<0.001**
Butyrate	CRP	–0.48	<0.01**
Propionate	LDL Cholesterol	–0.42	<0.01**
Propionate	ALT	–0.37	<0.05*
TMAO	LDL Cholesterol	0.58	<0.001**
TMAO	CRP	0.53	<0.001**
Indole-3-propionic acid	IL-6	–0.45	<0.01**
Indole-3-propionic acid	ALT	–0.39	<0.01**

5. Discussion, Conclusion and Recommendations

5.1. Summary of Major Findings

The current study examined the correlations between gut microbiota-produced metabolites and major chemical pathology markers representing metabolic, hepatic, and inflammatory health in adult human subjects. The results showed pronounced changes in gut microbial community structure and metabolite levels between participants with metabolic disease and healthy controls, along with strong correlations between certain microbial metabolites and systemic biochemical markers.

Demographically, both cohorts (each n = 60) were sex and age-matched, but the metabolic group had BMI, fasting glucose, LDL cholesterol, and inflammatory markers (CRP and IL-6) significantly higher, and HDL cholesterol lower. These findings were compatible with published metabolic syndrome profiles and confirmed the suitability of the sample for metabolic syndrome-associated gut microbiota-host interaction studies.

Compositional differences were shown by microbiota composition analysis using 16S rRNA gene sequencing. The patients with metabolic disorders had a greater Firmicutes-to-Bacteroidetes (F/B) ratio, reduced abundance of Akkermansia muciniphila, and greater levels of Escherichia/Shigella. These findings are consistent with previous research that characterized microbial dysbiosis in inflammatory and metabolic diseases (Liu et al., 2024; Ma et al., 2024). The decrease in A. muciniphila, a mucin-degrading bacterium with anti-inflammatory properties, is of special interest since it may impair gut barrier integrity and augment systemic inflammation (Anwer et al., 2025).

Metabolite profiling identified reduced beneficial short-chain fatty acids (SCFAs) such as butyrate and propionate and elevated pro-atherogenic trimethylamine-N-oxide (TMAO) in the metabolic group. Butyrate, a significant microbial metabolite, was inversely correlated with fasting glucose, HbA1c, and CRP levels. This suggests a metabolic and anti-inflammatory protective role, consistent with results from Du et al. (2024) and Feitelson et al. (2023), who highlighted SCFAs' role in increasing insulin sensitivity and modulating immune function. In a similar way, sodium butyrate has also been studied as an anti-inflammatory and tumour-suppressant therapeutic modulator for colorectal cancer and inflammatory bowel disease (Mederle et al., 2025).

By contrast, TMAO levels were positively correlated with LDL cholesterol and CRP, highlighting its well-established association with cardiovascular and metabolic risk (Bhatt et al., 2023). The elevated TMAO levels in the metabolic cohort may reflect increased microbial metabolism of dietary choline and L-carnitine, processes implicated in inflammation and atherogenesis. Such mechanistic links between microbial metabolites and systemic inflammation are also highly relevant to cancer biology, namely to chronic low-grade inflammation as tumour-promoting (Multhoff et al., 2022).

Indole derivatives like indole-3-propionic acid were significantly reduced in the metabolic group and were negatively correlated with IL-6 and ALT levels. Indoles are reported to have antioxidant and immunomodulatory effects. Their reduction is in line with recent evidence by Getsina et al. (2024), who reported low levels of indoles as a common denominator in inflammatory complications of paediatric malignancy. Concomitantly, Liu et al. (2024) emphasized the

function of microbial tryptophan metabolism in ensuring intestinal and systemic immune homeostasis, whose derangement is implicated in tumourigenesis.

Multivariate analysis also underscored demarcation between groups by health status based on microbial and metabolic profiles. Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) discriminated metabolic disorder patients from healthy controls through salient variables such as butyrate, TMAO, fasting glucose, and CRP. Pathway enrichment analysis defined variations in amino acid metabolism and SCFA production pathways, consonant with the systems-level understanding proposed by Ma et al. (2024) on metabolic reprogramming by microbiota.

The results of this study also contribute to the emerging paradigm of gut microbiota-host cross-talk in cancer metabolic milieus. For instance, long-term microbial metabolite-mediated inflammation, including TMAO and reduced butyrate, may affect cancer risk through oxidative stress and immune dysregulation (Kunst et al., 2023; Mullen & Singh, 2023). Schwartz et al. (2019) emphasized that microbial interactions such as these could regulate cancer immunotherapy responsiveness, highlighting potential for microbiome profiling to be applied to personalized medicine.

Additionally, the inflammation of the seen microbiota and deranged liver enzyme profiles is significant in the context of liver and gastrointestinal tract cancer. Anwer et al. (2025) and Malhotra et al. (2023) noted that dysregulated gut microbial metabolites like SCFAs and bile acids are implicated in gastrointestinal carcinogenesis and will shape the outcome of therapy. Thus, the observed biochemical alterations in this study not only reflect metabolic dysfunction but may also signal broader oncogenic dangers through pathways of inflammation (Valdés-González et al., 2023).

The evidence obtained in this study shows significant consistency with and extension of current work on the roles of gut microbiota-derived metabolites in inflammation, metabolism, and cancer pathophysiology. The observed reduction in therapeutic short-chain fatty acids (SCFAs), namely butyrate and propionate, in patients with metabolic disorders is supported by the findings of Du et al. (2024), who observed that SCFAs play anti-inflammatory, insulin-sensitizing, and mucosal-protective functions. Their study demonstrated that decreased SCFA production disrupts energy balance and induces low-grade systemic inflammation, an effect that the increased C-reactive protein (CRP) and interleukin-6 (IL-6) levels in the present study support.

Similarly, Mederle et al. (2025) highlighted the therapeutic potential of sodium butyrate in colorectal cancer because it could modulate gene expression, inhibit cell proliferation, and restore epithelial barrier function. The inverse correlations of butyrate levels with glycaemic indexes (fasting glucose and HbA1c) in the current study further solidify the protective metabolic action of this SCFA and are in line with its novel application in the therapy of metabolic and neoplastic diseases.

The increased Firmicutes-to-Bacteroidetes (F/B) ratio in the metabolic group is also evidenced by earlier reports assigning microbial dysbiosis to obesity and metabolic syndrome (Liu et al., 2024). Their study further included that the altered gut microbial composition is accountable for insulin resistance as well as lipid dysregulation, both through direct metabolic control as well as immunological alterations. Consequently, Ma et al. (2024) proposed a systems biology model of gut-host interaction and asserted that microbial functional pathways, particularly those in amino acid and SCFA metabolism, are critical to defining host homeostasis.

The elevated trimethylamine-N-oxide (TMAO) of the metabolic cohort of this study is supported by Bhatt et al. (2023), which defined TMAO as a critical pro-inflammatory metabolite associated with cardiovascular disease risk and cancer progression. In this study, TMAO exhibited strong positive correlations with LDL cholesterol and CRP, suggesting an atherogenic as well as inflammatory role. Such findings were supported by the study of Multhoff et al. (2022), which highlighted the involvement of chronic inflammation, partly caused by microbial and dietary elements, in cancer development. The mechanistic pathway of TMAO in eliciting oxidative stress and endothelial dysfunction, as elucidated by their work, provides a plausible explanation for the observed biochemical profile.

Furthermore, the reduction in indole derivatives, like indole-3-propionic acid, in metabolically impaired subjects is evidenced by findings by Anwer et al. (2025), who evidenced anti-inflammatory and antioxidant activities of these metabolites in digestive health and cancer resistance. Indole correlated inversely with IL-6 and ALT in this study, reflecting both systemic and hepatic immunomodulatory impact.

Schwartz et al. (2019) pushed this line of evidence further by highlighting the role of the gut microbiome in dictating cancer immunotherapy response. Authors argued that specific microbial metabolites can enhance or suppress the activation of immune cells—a concept harmonious with the reported immunologic signatures (high CRP and IL-6). This

work solidifies the overall contention that the microbiome is not merely a metabolic regulator but also an immunologic gatekeeper in state diseases.

Collectively, the results of this research adequately substantiate and add to current evidence by offering empirical evidence of metabolite-pathology correlations among Nigerian adults. They underscore the clinical relevance of microbial metabolites as biomarkers and modulators of metabolic and inflammatory wellness.

5.2. Biological Plausibility and Mechanistic Interpretations

The mechanistic rationale for the associations seen in this research is very well supported by known pathways through which gut microbiota-derived metabolites influence host metabolism, liver function, and inflammatory responses. The gut microbiota produces a very diverse array of bioactive compounds that exert a local effect within the gastrointestinal tract or systemic effect by being absorbed into circulation. Of these, short-chain fatty acids (SCFAs), trimethylamine-N-oxide (TMAO), and indole derivatives were the most functionally relevant metabolites of the present study.

SCFAs such as butyrate and propionate are produced primarily through the fermentation of food fibres by the colon commensal bacteria. Their inverse correlation with fasting glucose, HbA1c, and CRP herein is consistent with mechanistic data that SCFAs are raising insulin sensitivity by stimulating G-protein coupled receptors (GPR41 and GPR43) and modulating gluconeogenesis- and lipid metabolism-associated gene expression (Du et al., 2024). Butyrate, on the other hand, is a direct energy source for colonocytes, maintaining gut barrier integrity and reducing inflammation brought about by endotoxemia (Feitelson et al., 2023). Mederle et al. (2025) also demonstrated that sodium butyrate inhibits histone deacetylases (HDACs), thereby modulating transcription of anti-inflammatory and anti-proliferative genes—explaining its therapeutic potential in metabolic disease as well as colorectal cancer.

On the contrary, TMAO is generated from microbial choline and L-carnitine dietary metabolism and further liver oxidation. The direct correlations of TMAO, LDL cholesterol, and CRP in this study are in line with results proving that TMAO promotes atherosclerosis by restraining the transportation of cholesterol and enhancing the formation of foam cells (Bhatt et al., 2023). Multhoff et al. (2022) proceeded to elucidate that long-term low-grade inflammation, typical of high concentrations of TMAO, is responsible for tumour microenvironment formation through the stimulation of cytokine release, oxidative stress, and immune suppression. These findings establish the pro-oncogenic and pro-inflammatory function of TMAO in metabolically compromised subjects in this research.

Indole derivatives, such as indole-3-propionic acid, are microbial products of dietary tryptophan metabolism. Their inverse relationship with IL-6 and ALT signifies hepatoprotective and anti-inflammatory activities. Mechanistically, indole compounds activate the aryl hydrocarbon receptor (AhR) that regulates intestinal epithelial homeostasis and systemic immune response (Anwer et al., 2025). The reduction of these metabolites in the subjects of the current study who had metabolic disorders supports the hypothesis that dysbiosis reduces the levels of beneficial metabolites and renders them vulnerable to inflammatory pathology. Liu et al. (2024) further contributed to the fact that microbial tryptophan metabolism dysregulation plays a role in immune dysregulation and tumourigenesis, further cementing the biological relevance of indole deficiency in disease causality.

Furthermore, the increased Firmicutes-to-Bacteroidetes ratio and decreased Akkermansia muciniphila levels in the metabolic group indicate transition towards a dysbiotic microbiome, which is characterized by increased energy harvesting capacity and impaired mucosal immunity (Ma et al., 2024). This microbial dysregulation can lead to increased intestinal permeability, systemic lipopolysaccharide (LPS) exposure, and Toll-like receptor signalling pathway activation, which activate the secretion of pro-inflammatory cytokines and hepatic stress markers (Kunst et al., 2023).

In summary, the metabolite-pathology associations revealed in this analysis are mechanistically justified and biologically interpretable. Data presented here highlight the central function of microbial metabolites as regulators of host physiology and future therapeutic targets in metabolic, hepatic, and inflammatory diseases.

5.3. Implications for Diagnostics, Disease Prediction, or Treatment

Implications of the findings from this research for promoting diagnostic strategies, risk prediction models, and therapeutic intervention of metabolic, hepatic, and inflammatory disorders are considerable. The correlations between specific gut microbiota-derived metabolites, namely, short-chain fatty acids (SCFAs), trimethylamine-N-oxide (TMAO), and indole derivatives, and key chemical pathology markers suggest the promise of microbial metabolites as non-invasive biomarkers of systemic health.

From the diagnostic perspective, decreased butyrate and propionate concentrations observed in patients with metabolic disorders can be used as possible early indicators of glycaemic dysregulation and hepatic stress. These SCFAs function in maintaining integrity of the intestinal barrier and controlling insulin sensitivity through molecular pathways such as HDAC inhibition and stimulation of G-protein-coupled receptors (Du et al., 2024; Mederle et al., 2025). Their loss in the metabolic group emphasizes their utility as sensitive biomarkers to detect subclinical metabolic changes before overt pathology can develop.

The elevated TMAO levels in the same population and their strong positive correlation with LDL cholesterol and CRP highlight the role of the compound in atherogenesis and chronic inflammation. In keeping with Bhatt et al. (2023) and Multhoff et al. (2022), TMAO has been proposed as a biomarker for cardiovascular events and cancer prognosis. Its quantification through metabolomic profiling can be seen to complement standard risk scoring for predicting disease worsening or complications, especially in high-risk individuals.

Furthermore, the documented reduction in indole-3-propionic acid, a microbial tryptophan metabolite with immunoregulatory function, in high inflammatory marker participants underscores its value in immune status and liver function determination. Anwer et al. (2025) and Liu et al. (2024) introduce indole derivatives as possible gut–liver and gut–immune axis interaction modulators, with therapeutic potential in gastrointestinal cancer and inflammatory diseases. Along these lines, their inclusion in diagnostic panels could be valuable for stratifying patients according to immunological or oncological risk.

In treatment terms, this work adds weight to the growing consensus that microbial metabolite modulation is a therapeutic promise. Prebiotics, probiotics, and postbiotics to re-establish SCFA levels and normalize dysbiosis would improve glycaemic control, lipid metabolism, and inflammatory profiles (Ma et al., 2024). Supplementation with butyrate, for instance, has been tried in inflammatory bowel disease and colorectal cancer with promising outcomes (Mederle et al., 2025), and could be promising in metabolic syndrome treatment or even prevention of carcinogenesis.

Furthermore, treatments to prevent TMAO formation, such as dietary choline restriction or inhibition of microbial enzymes, are presently being tested in model systems (Feitelson et al., 2023). Incorporation of these therapies into the clinical toolbox would reduce cardiovascular and cancer risk, particularly among those with metabolic dysregulation.

In conclusion, addition of gut microbiota-derived metabolite profiling into the clinical pipeline can revolutionize personalised medicine. Through promoting early diagnosis, tracking of disease progression, and personalised therapy, these metabolites bridge the gap between microbiome science and clinical reality.

5.4. Limitations of the Study

Although providing valuable insights, this study has several limitations that need to be highlighted. Firstly, its cross-sectional and experimental design limits causal inference, such that it is impossible to determine whether observed changes in gut metabolites cause or are the consequence of pathological markers. Secondly, the relatively small sample size, although statistically powered, may not capture the entire diversity of gut microbiota or account for inter-individual variance influenced by genetics, diet, or lifestyle. Thirdly, sampling at a single time-point for stool and blood may fail to reflect dynamic changes in metabolite production or biomarker levels over time. Dietary intake was also monitored using self-reported diaries, which are susceptible to recall bias and can impact metabolite concentrations. Lastly, the study focused on particular metabolites and biochemical markers, which could have neglected other important microbial or host pathways implicated in disease development. These limitations suggest the need for multi-omics and longitudinal studies to validate and extend the current findings.

6. Conclusion

This study provided strong support for the relationship between gut microbiota-derived metabolites and systemic biochemical markers of metabolic, hepatic, and inflammatory health. Key findings were that dramatically lower concentrations of beneficial short-chain fatty acids (e.g., butyrate and propionate) and indole derivatives, with higher concentrations of the pro-inflammatory metabolite TMAO, were present in metabolically disordered individuals. These metabolite changes were highly correlated with clinical biomarkers such as fasting glucose, HbA1c, LDL cholesterol, and CRP, highlighting the role of gut microbiota in the modulation of host metabolism and immunity. The dysbiosis of microbiota, i.e., higher Firmicutes-to-Bacteroidetes ratio and lower Akkermansia muciniphila, also supported a mechanistic association between deranged gut ecology and systemic aberration.

This work added substantially to the existing body of knowledge regarding microbiome-host interactions, especially in the context of inflammatory and metabolic diseases. Whereas previous studies investigated single metabolites or microbiome structure, this research combined microbial profiling with biochemical and immunological information within one experimental design. These results confirmed the hypothesis that some microbial metabolites were not only end products of gut microbial metabolism but also were upstream controllers of host physiology. This supplemented current understanding of the gut–liver–immune axis and offered systems-level understanding of how dysregulation of microbiota played a role in disease pathogenesis.

Clinically, the study emphasized the diagnostic and prognostic potential of microbial metabolites. Metabolite profiling, in particular SCFAs, TMAO, and indole derivatives, was developable into non-invasive diagnostics for early detection, risk stratification of disease, or monitoring of metabolic and inflammatory disorders. This has opened up possibilities for directed microbiota-based treatments, such as prebiotics, probiotics, or postbiotics, with new fields in personalised medicine and preventive care. In summary, the study linked fundamental microbiome science to translational clinical relevance.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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Appendices

Appendix A

Table A1: Demographic and Baseline Characteristics of Participants (N = 120)

Variable	Total (n = 120)	Metabolic Group (n = 60)	Control Group (n = 60)	p-value
Age (mean ± SD)	44.2 ± 9.1	45.8 ± 8.5	42.6 ± 9.5	0.072
Sex (Male/Female)	62 / 58	32 / 28	30 / 30	0.684
BMI (kg/m^2)	27.8 ± 5.4	31.1 ± 4.2	24.5 ± 3.9	<0.001**
Smoking Status (Yes/No)	18 / 102	11 / 49	7 / 53	0.326
Alcohol Consumption (Yes/No)	34 / 86	21 / 39	13 / 47	0.135

Appendix B

Table A2: Relative Abundance of Key Microbial Taxa and Metabolite Concentrations

Variable	**Metabolic Group (Mean ± SD)**	**Control Group (Mean ± SD)**	**p-value**
Firmicutes (%)	52.1 ± 8.2	44.7 ± 6.9	<0.001**
Bacteroidetes (%)	36.3 ± 7.5	43.8 ± 8.1	<0.001**
Akkermansia muciniphila (%)	0.8 ± 0.3	1.9 ± 0.6	<0.001**
Butyrate (μmol/g)	4.2 ± 1.1	7.3 ± 1.8	<0.001**
Propionate (μmol/g)	3.8 ± 0.9	5.2 ± 1.1	<0.001**
TMAO (μmol/L)	7.4 ± 2.2	3.2 ± 1.4	<0.001**
Indole-3-propionic acid (μmol/L)	1.5 ± 0.6	2.8 ± 0.9	<0.001**

Appendix C

Table A3: Chemical Pathology Marker Profiles

Biomarker	Metabolic Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
ALT (U/L)	45.2 ± 13.7	28.9 ± 10.5	<0.001**
AST (U/L)	41.5 ± 11.4	27.3 ± 9.2	<0.001**
ALP (U/L)	120.3 ± 30.1	95.4 ± 24.6	0.002**
LDL Cholesterol (mg/dL)	149.6 ± 22.3	104.7 ± 18.5	<0.001**
HDL Cholesterol (mg/dL)	42.5 ± 9.6	57.3 ± 10.2	<0.001**
Fasting Glucose (mg/dL)	112.4 ± 20.1	85.6 ± 13.7	<0.001**
HbA1c (%)	6.9 ± 1.2	5.2 ± 0.6	<0.001**
CRP (mg/L)	8.3 ± 3.1	3.2 ± 1.2	<0.001**
IL-6 (pg/mL)	5.1 ± 1.8	2.3 ± 0.9	<0.001**

Appendix D

Table A4: Correlation Between Microbial Metabolites and Chemical Pathology Markers

Metabolite	ALT	LDL	Fasting Glucose	CRP	IL-6
Butyrate	−0.52**	−0.47**	−0.49**	−0.54**	−0.50**
Propionate	−0.41*	−0.39*	−0.45**	−0.46**	−0.43**
TMAO	0.58**	0.62**	0.47**	0.64**	0.51**
Indole-3-propionic acid	−0.44*	−0.37*	−0.40*	−0.53**	−0.48**

Note: *p < 0.05, **p < 0.01 (Pearson's correlation coefficients)

- Supplementary data
- Additional tables/figures
- Ethics approval letter/sample questionnaire