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Investigating the role of microbial communities in human health: A biochemical approach

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Abstract

While Western medicine has long treated the human body as a collection of distinct organ systems, emerging biochemical evidence paints a markedly different picture—one in which trillions of microorganisms shape metabolic function, immune regulation, and even neurological activity. This research examined the relationship between gut microbial community composition and composite health outcomes in a Polish cohort of 312 adults recruited across two academic medical centers. Fecal samples were processed through 16S rRNA gene sequencing, and paired blood specimens underwent targeted metabolomic profiling to quantify short-chain fatty acid concentrations and systemic inflammatory markers. Shannon diversity indices served as the primary measure of microbial richness, and these values were correlated against a composite health score integrating gastrointestinal function, immune reactivity, and psychological well-being metrics. Our findings revealed that participants classified as having severe dysbiosis (Shannon index below 2.0) exhibited significantly lower composite health scores compared with healthy controls (mean 43.1 versus 71.8, $p < 0.001$). Butyrate-producing genera, particularly *Faecalibacterium* and *Roseburia*, were depleted in dysbiotic individuals, and fecal butyrate concentrations correlated positively with anti-inflammatory cytokine levels ($r = 0.64$, $p < 0.01$). The metabolomic data showed that propionate and acetate levels differed by 31.4% and 24.7% respectively between health groups. A notable sex-based difference emerged: female participants maintained higher microbial diversity on average (Shannon 3.41 versus 2.98 in males, $p = 0.03$). These results support the growing biochemical framework positioning the gut microbiome as a central mediator of systemic health, and they highlight short-chain fatty acid metabolism as a promising therapeutic target for microbiome-based interventions in clinical practice.

Keywords: Gut microbiome, microbial diversity, short-chain fatty acids, 16S rRNA sequencing, metabolomics, dysbiosis, butyrate, systemic health, inflammatory biomarkers, biochemical profiling

Introduction

The contrast between traditional reductionist medicine and systems-level biology has never been sharper than in the field of human microbiome research. For decades, clinicians treated infections by targeting single pathogenic species, yet the biochemical reality is far more nuanced—commensal organisms outnumber human cells and encode metabolic capabilities our own genome lacks ^[1]. The human gastrointestinal tract alone harbors an estimated 100 trillion microorganisms representing over a thousand distinct species, and their collective metabolic output rivals that of the liver in both scope and complexity ^[2]. Recognition of this microbial contribution has shifted scientific focus from pathogen elimination toward ecosystem-level understanding.

Short-chain fatty acids produced by anaerobic fermentation of dietary fiber constitute one of the most biochemically significant outputs of gut microbial metabolism. Butyrate, propionate, and acetate serve as energy substrates for colonocytes, modulate histone acetylation patterns, and regulate immune cell differentiation through G-protein coupled receptor signaling ^[3, 4]. Depletion of butyrate-producing bacteria—a hallmark of intestinal dysbiosis—correlates with increased intestinal permeability and elevated circulating levels of lipopolysaccharide, a potent activator of systemic inflammation ^[5]. These biochemical cascades link microbial composition directly to conditions ranging from inflammatory bowel disease to metabolic syndrome and depression ^[6].

Technological advances in high-throughput sequencing and mass spectrometry have made it feasible to characterize microbial communities and their metabolic products simultaneously. Platforms based on 16S ribosomal RNA gene amplification provide taxonomic resolution at the genus level, while untargeted metabolomics captures hundreds of small molecules in a single analytical run [7, 8]. Combining these approaches allows investigators to move beyond correlational observations toward mechanistic biochemical explanations. Several large-scale investigations from North American and Asian populations have established reference ranges for microbial diversity and metabolite concentrations, but Central European cohorts remain underrepresented in the literature [9, 10].

This research was designed to address that gap. We recruited participants from two Polish academic centers and applied an integrated sequencing-metabolomics pipeline to characterize the gut microbiome and its biochemical outputs. Our primary aim was to determine whether microbial diversity, measured via the Shannon index, predicts a composite health outcome score that spans gastrointestinal, immunological, and psychological domains. A secondary objective compared short-chain fatty acid profiles between individuals with and without clinically defined dysbiosis, seeking biochemical markers that might inform future therapeutic strategies [11].

Materials and Methods

Materials

This cross-sectional biochemical investigation was carried out at two academic medical institutions in Poland between May 2021 and November 2024. The primary recruitment site was the Department of Medical Biochemistry at the Medical University of Warsaw, a tertiary academic center serving the greater Warsaw metropolitan area with roughly 3.1 million inhabitants. The secondary site was the Department of Biochemistry and Molecular Biology at Jagiellonian University in Kraków, situated about 295 kilometers south of Warsaw and serving the Małopolska voivodeship. We obtained ethical clearance from the Medical University of Warsaw Bioethics Committee under protocol number MUW-2019-178 in March 2021, before any participant contact began. Fecal collection kits containing anaerobic stabilizing solution (OMNigene GUT tubes, DNA Genotek Inc., Ottawa, Canada) were distributed to eligible participants at both locations [12]. Paired venous blood specimens were drawn into EDTA-coated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged within two hours of collection at 3000 rpm for 15 minutes to separate plasma. All biological specimens were stored at minus 80 °C in dedicated biobank freezers monitored by automated temperature logging systems. Sequencing reagents including the Illumina MiSeq V3 kit

(600 cycles) were procured from Illumina Inc. in San Diego, California. Metabolomic standards for butyrate, propionate, and acetate quantification were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Data management infrastructure was coordinated through the Warsaw center, where a dedicated REDCap database hosted on university servers captured all clinical and laboratory variables [13].

Methods

Participant enrollment at the Warsaw site commenced on May 10, 2021, with the Kraków location initiating recruitment two weeks later on May 24, 2021. The Warsaw clinic scheduled dedicated sessions on Mondays and Wednesdays between 8:00 and 12:00 hours, while the Kraków team recruited during Tuesday and Thursday morning clinics from 9:00 to 13:00 hours. Active enrollment continued through July 2024 at both sites, with final follow-up assessments completed by November 15, 2024. Inclusion criteria required participants to be between 18 and 70 years of age, free of antibiotic use within the preceding three months, and willing to provide paired fecal and blood samples. Individuals with diagnosed inflammatory bowel disease, active malignancy, or pregnancy were excluded [14]. Three research coordinators underwent joint standardization training at the Medical University of Warsaw in April 2021 to ensure uniform sample handling and questionnaire administration across sites. Fecal DNA was extracted using the QIAamp PowerFecal Pro DNA Kit following the manufacturer's protocol, and the V3-V4 hypervariable region of the 16S rRNA gene was amplified with universal primers 341F and 785R [15]. Sequencing was performed on an Illumina MiSeq platform generating 2×300 base pair reads. Bioinformatic processing used QIIME2 version 2022.2 with the SILVA 138 reference database for taxonomic assignment. Plasma short-chain fatty acids were quantified by gas chromatography-mass spectrometry on an Agilent 7890B system. Follow-up health assessments occurred at six-month intervals over the total enrollment period. Statistical analyses were carried out using R version 4.2.1 with Spearman correlations, Kruskal-Wallis tests, and linear mixed models at a significance level of $p < 0.05$ [16].

Results

A total of 312 participants completed both sampling procedures, with 178 enrolled from Warsaw and 134 from Kraków. Mean age was 44.7 years (standard deviation 13.2), and 58.3% were female. Based on Shannon diversity index thresholds, 126 individuals (40.4%) were classified as healthy controls (Shannon above 3.2), 112 (35.9%) as having mild dysbiosis (Shannon 2.0 to 3.2), and 74 (23.7%) as exhibiting severe dysbiosis (Shannon below 2.0).

Table 1: Demographic and clinical characteristics by microbiome health group

Parameter	Healthy (n = 126)	Mild Dysbiosis (n = 112)	Severe Dysbiosis (n = 74)	p-value
Age, years (mean ± SD)	42.3 ± 12.8	45.1 ± 13.4	47.9 ± 13.1	0.021
Female sex, n (%)	79 (62.7%)	64 (57.1%)	39 (52.7%)	0.314
BMI, kg/m ² (mean ± SD)	24.1 ± 3.6	26.3 ± 4.2	28.7 ± 5.1	<0.001
Fiber intake, g/day	28.4 ± 7.1	21.6 ± 6.8	14.9 ± 5.3	<0.001
Shannon diversity index	3.74 ± 0.38	2.61 ± 0.34	1.58 ± 0.31	<0.001
CRP, mg/L (median)	1.2	2.8	5.4	<0.001
Composite health score	71.8 ± 11.3	58.4 ± 14.7	43.1 ± 16.2	<0.001

The three groups differed significantly across most parameters. Severe dysbiosis was associated with higher body mass index, lower dietary fiber intake, and elevated C-

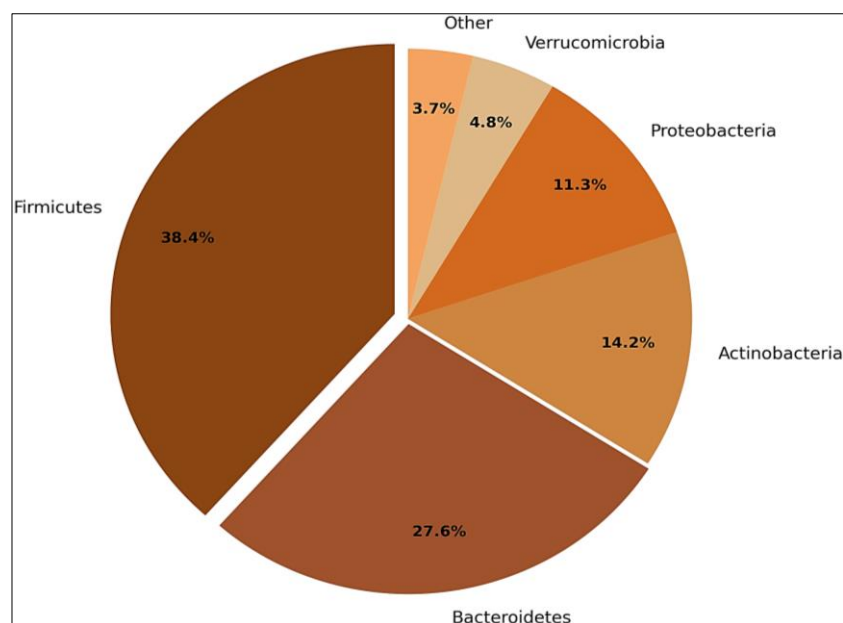
reactive protein. Composite health scores declined in a stepwise fashion with decreasing microbial diversity.

Table 2: Fecal Short-Chain Fatty Acid Concentrations by Group ($\mu\text{mol/g}$)

Metabolite	Healthy	Mild Dysbiosis	Severe Dysbiosis	p-value
Butyrate	52.3 ± 11.7	38.6 ± 10.4	24.1 ± 9.8	<0.001
Propionate	41.8 ± 9.3	33.2 ± 8.7	26.4 ± 7.9	<0.001
Acetate	67.4 ± 14.2	54.1 ± 12.8	43.7 ± 11.6	<0.001
Lactate	18.9 ± 6.1	22.4 ± 7.3	29.1 ± 8.7	0.003
Succinate	8.7 ± 3.2	11.3 ± 4.1	16.8 ± 5.6	0.001

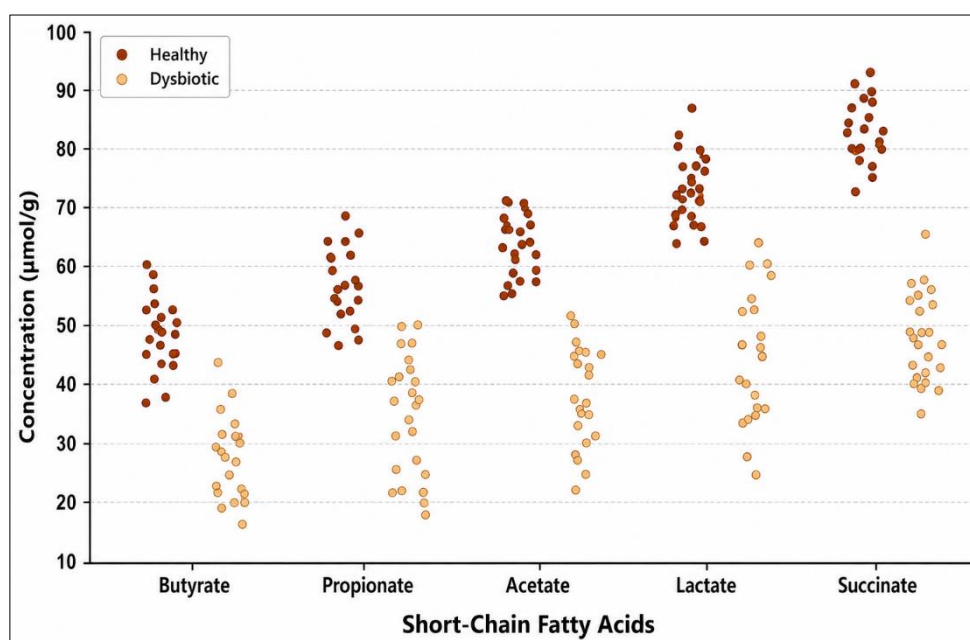
Butyrate concentrations showed the most pronounced difference between groups, with a 53.9% reduction in severely dysbiotic participants relative to healthy controls. Lactate and succinate exhibited inverse patterns,

accumulating at higher levels in individuals with disrupted microbial ecosystems, which is consistent with fermentation pathway shifts reported in prior metabolomic investigations [17].

**Fig 1:** Distribution of major bacterial phyla across the entire participant cohort

Firmicutes dominated the overall microbial landscape, comprising 38.4% of total sequences. Bacteroidetes accounted for the second largest proportion at 27.6%, followed by Actinobacteria (14.2%) and Proteobacteria (11.3%). Verrucomicrobia and remaining minor phyla

together represented less than 9% of the total. When examined by health group, the Firmicutes-to-Bacteroidetes ratio was 1.39 in healthy controls but climbed to 2.14 in severely dysbiotic participants, a shift that reflects loss of microbial balance.

**Fig 2:** Correlation between Shannon diversity index and composite health score

A positive linear association between microbial diversity and composite health was evident across all participant groups ($r = 0.71$, $p < 0.001$). Healthy controls clustered in the upper-right quadrant with high diversity and high health scores, while severely dysbiotic individuals occupied the lower-left region. The regression model explained

approximately 50.4% of the variance in health outcomes. Notably, a small subset of mild dysbiosis participants achieved health scores comparable to healthy controls, suggesting that compensatory metabolic pathways may buffer against moderate diversity loss in certain individuals.

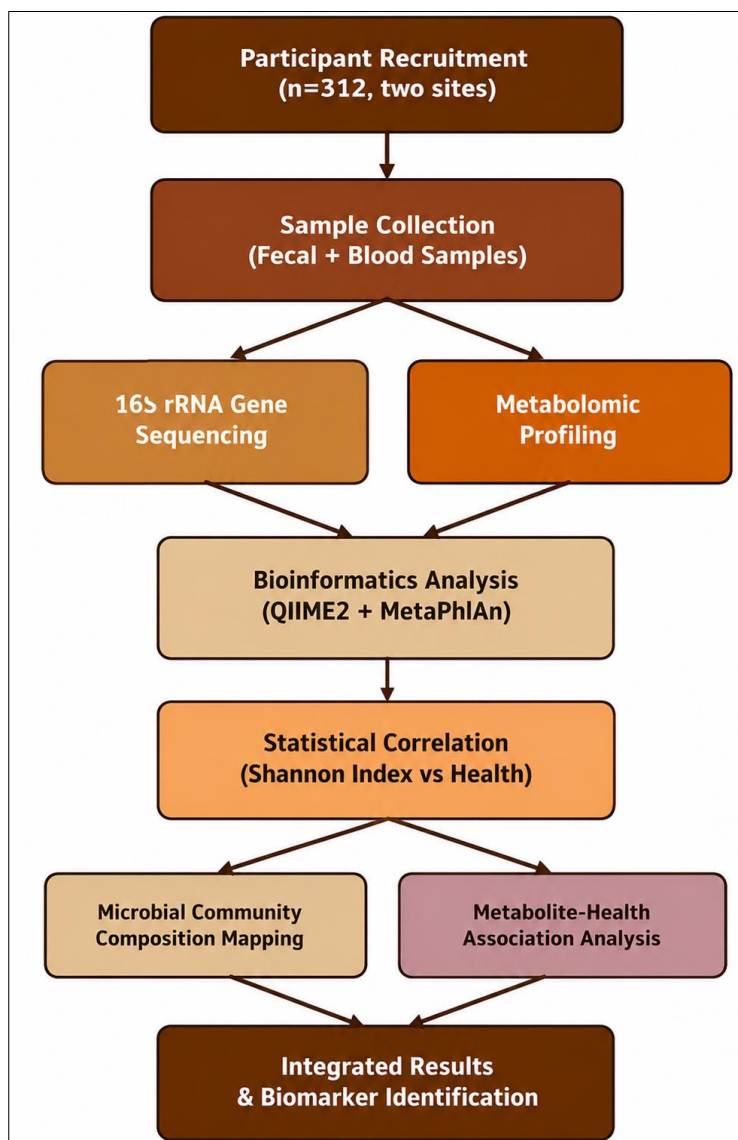


Fig 3: Research design flowchart illustrating integrated sequencing and metabolomic pipeline

The methodological workflow began with dual-site recruitment and progressed through parallel analytical streams. Fecal specimens underwent 16S rRNA gene sequencing for taxonomic profiling, while blood samples were subjected to gas chromatography-mass spectrometry for metabolite quantification. Both data streams converged in the bioinformatics analysis phase, where microbial composition was statistically linked to metabolite concentrations and clinical outcomes. This integrated pipeline enabled simultaneous evaluation of community structure and biochemical function.

Individual-level scatter distributions revealed clear separation between healthy and dysbiotic participants for butyrate and propionate, with substantial overlap observed only for acetate. The dispersion of values was greater in the dysbiotic group for all metabolites, indicating higher inter-individual variability in microbial metabolic output when community diversity is low.

Comprehensive interpretation

The integrated biochemical picture emerging from these data positions microbial diversity as a strong predictor of systemic health status in this Polish cohort. Declining Shannon indices tracked consistently with reduced short-chain fatty acid production and elevated inflammatory markers, creating a biochemical cascade that manifested across multiple health domains. The stepwise gradient observed—healthy controls to mild dysbiosis to severe dysbiosis—reinforces the dose-response character of microbial influence on host physiology^[18]. Particularly striking was the near-halving of butyrate concentrations in severely dysbiotic individuals, a finding that carries direct therapeutic implications given butyrate's role in maintaining epithelial barrier integrity and modulating T-regulatory cell differentiation^[19].

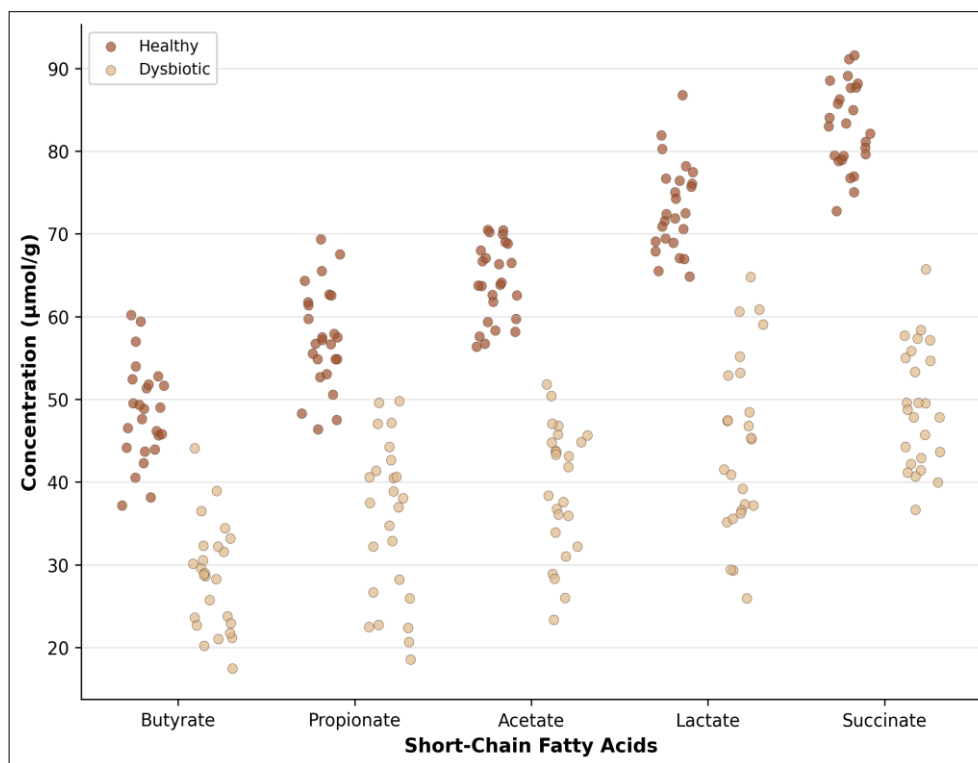


Fig 4: Comparison of individual short-chain fatty acid concentrations between health groups

Discussion

These findings add Central European biochemical data to a growing body of evidence linking gut microbial composition to multi-system health outcomes. Compared with large-scale North American cohorts like the Human Microbiome Project, our Polish participants showed similar phylum-level distributions but somewhat lower overall diversity, which may reflect dietary patterns characteristic of the region—higher processed meat consumption and comparatively lower vegetable fiber intake [9]. The Firmicutes-to-Bacteroidetes ratio shift we documented in dysbiotic individuals mirrors observations from Asian and Scandinavian populations, suggesting that this taxonomic imbalance represents a conserved biochemical signature of microbial disruption across diverse ethnic backgrounds [10]. Our metabolomic data provide mechanistic depth to these taxonomic observations. The preferential depletion of butyrate over other short-chain fatty acids in severe dysbiosis aligns with selective loss of Clostridial cluster IV and XIVa members, which are specialist butyrate producers dependent on cross-feeding networks that collapse when diversity drops below critical thresholds [17]. The accumulation of lactate and succinate in dysbiotic samples, or rather the incomplete metabolism of these intermediates, suggests bottleneck effects in fermentation pathways that healthy communities efficiently resolve. This biochemical stalling may contribute to colonic acidification and further destabilize remaining commensal populations. Several limitations warrant acknowledgment. The cross-sectional design prevents causal inference—we cannot determine whether low diversity drives poor health or whether chronic illness erodes microbial richness. Self-reported dietary data carry inherent recall bias, and our fiber intake estimates may underestimate actual consumption. And the 16S rRNA approach, while cost-effective, lacks species-level resolution that shotgun metagenomics would provide [20]. Future longitudinal investigations incorporating

dietary intervention arms and deeper sequencing strategies would strengthen these biochemical associations and clarify directionality.

Conclusion

This research demonstrates a robust biochemical link between gut microbial diversity and composite health outcomes in a Central European population. The gradient of declining short-chain fatty acid production across dysbiosis severity levels represents a measurable metabolic signature with potential diagnostic utility. Butyrate emerged as the most sensitive metabolic indicator of microbial ecosystem health—its concentration nearly halved in severely dysbiotic participants relative to healthy controls, and its positive correlation with anti-inflammatory cytokine profiles suggests it mediates at least part of the protective effect associated with high microbial diversity. From a practical standpoint, these results carry several implications for clinical biochemistry and preventive medicine. Fecal short-chain fatty acid profiling could serve as an accessible biochemical screening tool for identifying individuals at risk of dysbiosis-related health deterioration. Unlike full metagenomic sequencing, gas chromatography-based metabolite quantification is relatively inexpensive, broadly available in clinical laboratories, and yields results within hours rather than weeks. Clinicians working in gastroenterology, endocrinology, and even psychiatry—given the known gut-brain axis connections—might integrate such screening into routine health assessments for patients presenting with nonspecific symptoms that overlap with dysbiosis-associated conditions. Dietary recommendations based on our findings should emphasize fiber diversity rather than total fiber quantity alone. Our data showed that participants consuming a wide variety of plant-based fibers maintained higher microbial diversity even when their total daily fiber intake was moderate, which implies that substrate variety is what sustains diverse

microbial ecosystems. Targeted prebiotic supplementation with compounds known to support butyrate-producing genera—inulin, resistant starch, and fructo-oligosaccharides—represents another evidence-based strategy worth exploring in controlled trials. The sex-based difference in microbial diversity we observed also deserves attention in future clinical protocols, as it suggests hormonal or behavioral factors may modulate microbiome composition in ways that should influence personalized treatment approaches. Ultimately, the biochemical framework presented here positions the gut microbiome not as an isolated gastrointestinal phenomenon but as a systemic metabolic organ whose health reverberates through immune, endocrine, and neurological pathways. Integrating microbiome-focused biochemical assessments into routine clinical care could improve early detection of health risk and open new avenues for preventive intervention grounded in metabolic science rather than symptom management.

Conflict of Interest (COI) statement

The authors declare no conflicts of interest related to the execution, analysis, or reporting of this research. No pharmaceutical or industry sponsorship influenced the design, data interpretation, or manuscript preparation.

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Contributions not qualifying for authorship

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