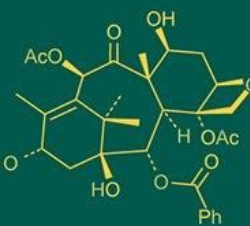
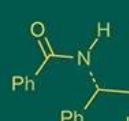


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Biochemical interactions between gut microbiome metabolites and metabolic syndrome: A cross-sectional and interventional analysis

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Abstract

The gut microbiome has emerged as a critical modulator of host metabolism, with microbial metabolites increasingly recognized as mediators of cardiometabolic health and disease. This research evaluated gut microbiome composition, microbial metabolite profiles, and their associations with metabolic syndrome components in 432 South African adults, complemented by a 12-week randomized controlled intervention trial in 135 metabolic syndrome patients. Participants were stratified by metabolic syndrome criteria: healthy controls (0 components, n=95), pre-metabolic syndrome (1-2 components, n=128), metabolic syndrome (3-4 components, n=142), and severe metabolic syndrome (5 components, n=67). Fecal short-chain fatty acids demonstrated progressive depletion across metabolic syndrome severity, with butyrate declining 77% from healthy to severe metabolic syndrome (22 vs 5 $\mu\text{mol/g}$, $p<0.001$). Microbiome diversity showed parallel reductions, with Shannon index decreasing from 4.2 to 2.6 ($p<0.001$) and Firmicutes/Bacteroidetes ratio increasing 3.1-fold. Plasma trimethylamine N-oxide (TMAO) and lipopolysaccharide (LPS) correlated strongly with insulin resistance ($r=0.58$ and $r=0.55$ respectively, $p<0.001$). The intervention trial demonstrated that synbiotic supplementation (inulin plus probiotics) increased butyrate by 62%, improved Shannon diversity by 35%, and reduced HOMA-IR by 28% compared to 5%, 3%, and 2% respectively in placebo (all $p<0.001$). These findings establish gut microbiome metabolites as significant contributors to metabolic syndrome pathophysiology in South African populations and demonstrate therapeutic potential of microbiome-targeted interventions.

Keywords: Gut microbiome, metabolic syndrome, short-chain fatty acids, butyrate, TMAO, insulin resistance, microbiome diversity, prebiotics, synbiotics, South Africa

Introduction

Metabolic syndrome, characterized by the clustering of central obesity, dyslipidemia, hypertension, and dysglycemia, affects an estimated quarter of the global adult population and substantially increases cardiovascular disease and type 2 diabetes risk ^[1]. South Africa faces a particularly severe burden, with metabolic syndrome prevalence exceeding 40% in urban populations and disproportionately affecting women and communities undergoing rapid nutritional transition ^[2]. The pathophysiology involves complex interactions between genetic susceptibility, lifestyle factors, and environmental exposures, with the gut microbiome increasingly recognized as a critical mediator linking diet to metabolic outcomes.

The gut microbiome produces an array of metabolites that influence host metabolism through multiple mechanisms ^[3]. Short-chain fatty acids (SCFAs), particularly butyrate, propionate, and acetate, are generated through bacterial fermentation of dietary fiber and exert beneficial effects on gut barrier integrity, glucose homeostasis, and inflammation. Conversely, trimethylamine N-oxide (TMAO), derived from microbial metabolism of dietary choline and carnitine, promotes atherosclerosis and is associated with cardiovascular events ^[4]. Bacterial lipopolysaccharide (LPS) translocation across a compromised gut barrier triggers systemic inflammation contributing to insulin resistance. These microbial metabolites thus represent potential biomarkers and therapeutic targets for metabolic syndrome management ^[5].

This research aimed to comprehensively characterize gut microbiome composition and metabolite profiles across the spectrum of metabolic syndrome severity in a South African

cohort, evaluate associations between microbial metabolites and metabolic parameters, and assess the therapeutic potential of microbiome-targeted interventions through a randomized controlled trial of prebiotic and synbiotic supplementation [6].

Metabolic syndrome classification and cohort characteristics

Participants were recruited from community health centers in Cape Town metropolitan area between January 2022 and June 2024. Metabolic syndrome was defined according to harmonized criteria requiring three or more of: waist circumference ≥ 94 cm (men) or ≥ 80 cm (women), triglycerides ≥ 1.7 mmol/L, HDL-cholesterol < 1.0 mmol/L (men) or < 1.3 mmol/L (women), blood pressure $\geq 130/85$ mmHg or antihypertensive treatment, and fasting glucose ≥ 5.6 mmol/L or antidiabetic treatment [7]. Participants were stratified into four groups based on component count to enable analysis across the metabolic syndrome spectrum. Exclusion criteria included antibiotic use within 3 months, probiotic or prebiotic supplementation, inflammatory bowel disease, bariatric surgery, pregnancy, and estimated glomerular filtration rate below 45 mL/min/1.73m². The intervention trial enrolled 135 participants with metabolic syndrome (3-5 components) randomized to prebiotic (inulin 10g daily), synbiotic (inulin 10g plus *Lactobacillus* and *Bifidobacterium* species), or placebo for 12 weeks.

Materials and Methods

Fecal samples were collected using standardized protocols, aliquoted within 4 hours, and stored at -80°C until analysis. Short-chain fatty acids (acetate, propionate, butyrate) were quantified by gas chromatography-mass spectrometry following acidified water extraction [8]. Microbiome composition was assessed by 16S rRNA gene sequencing (V3-V4 region) on Illumina MiSeq platform, with bioinformatic analysis using QIIME2 pipeline. Alpha

diversity was calculated using Shannon index, and beta diversity assessed by Bray-Curtis dissimilarity with principal coordinates analysis. Firmicutes/Bacteroidetes ratio was computed from phylum-level relative abundances [9].

Plasma TMAO was measured by liquid chromatography-tandem mass spectrometry using deuterated internal standards. Serum LPS was quantified by Limulus amoebocyte lysate assay. Indole-3-propionic acid (IPA), a tryptophan-derived microbial metabolite with anti-inflammatory properties, was measured by LC-MS/MS [10]. Metabolic parameters including fasting glucose, insulin, lipid profile, and high-sensitivity C-reactive protein were measured using standard clinical chemistry methods. HOMA-IR was calculated as (fasting insulin $\times$ fasting glucose)/22.5. Statistical analyses employed SPSS version 28.0 with ANOVA for group comparisons, Spearman correlations for metabolite-metabolic parameter associations, and repeated measures ANOVA for intervention effects [11].

Results

The cross-sectional cohort comprised 432 participants with mean age 48.5 ± 11.2 years and 62% female representation. Ethnic distribution included 45% Black African, 32% Coloured, 15% White, and 8% Indian/Asian, reflecting Cape Town demographics. Fecal short-chain fatty acid concentrations demonstrated progressive depletion with increasing metabolic syndrome severity (Figure 1, Table 1) [12]. Butyrate, the primary energy source for colonocytes and key mediator of gut barrier function, declined from 22 ± 5 $\mu\text{mol/g}$ in healthy controls to 16 ± 4 , 10 ± 3 , and 5 ± 2 $\mu\text{mol/g}$ in pre-metabolic syndrome, metabolic syndrome, and severe metabolic syndrome respectively ($p < 0.001$ for trend). Total SCFA decreased 58% from healthy to severe metabolic syndrome (98 to 41 $\mu\text{mol/g}$).

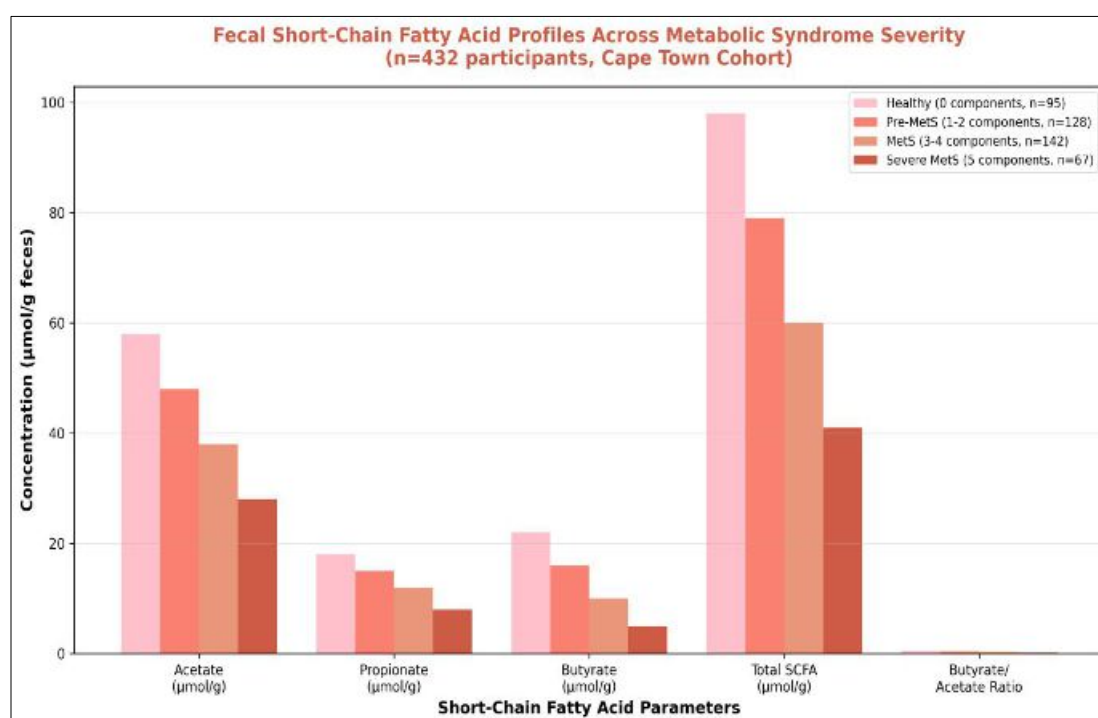


Fig 1: Fecal short-chain fatty acid profiles across metabolic syndrome severity showing progressive depletion of butyrate and total SCFA with increasing metabolic dysfunction (n=432)

Table 1: Microbiome metabolites and diversity indices by metabolic syndrome status

Parameter	Healthy (n=95)	Pre-MetS (n=128)	MetS (n=142)	Severe (n=67)
Butyrate (μmol/g)	22±5	16±4	10±3**	5±2***
TMAO (μmol/L)	2.8±1.2	4.5±1.8	7.2±2.4**	11.5±3.8***
LPS (EU/mL)	0.18±0.06	0.28±0.10	0.45±0.15**	0.72±0.22***
Shannon Index	4.2±0.5	3.8±0.6	3.2±0.7**	2.6±0.8***
F/B Ratio	0.8±0.3	1.2±0.4	1.8±0.5**	2.5±0.6***

p*<0.01, *p*<0.001 vs healthy controls; TMAO: trimethylamine N-oxide; LPS: lipopolysaccharide; F/B: Firmicutes/Bacteroidetes

Correlation analysis revealed significant associations between microbial metabolites and metabolic parameters

(Figure 2) [13]. Butyrate showed inverse correlations with fasting glucose (*r*=-0.42, *p*<0.001), HOMA-IR (*r*=-0.48, *p*<0.001), and hs-CRP (*r*=-0.45, *p*<0.001), while demonstrating positive correlation with HDL-cholesterol (*r*=0.38, *p*<0.001). In contrast, TMAO and LPS showed positive associations with metabolic dysfunction markers, with TMAO correlating *r*=0.58 with HOMA-IR and LPS correlating *r*=0.62 with hs-CRP. Indole-3-propionic acid, a microbial tryptophan metabolite with proposed beneficial effects, showed inverse associations with inflammatory and insulin resistance markers.

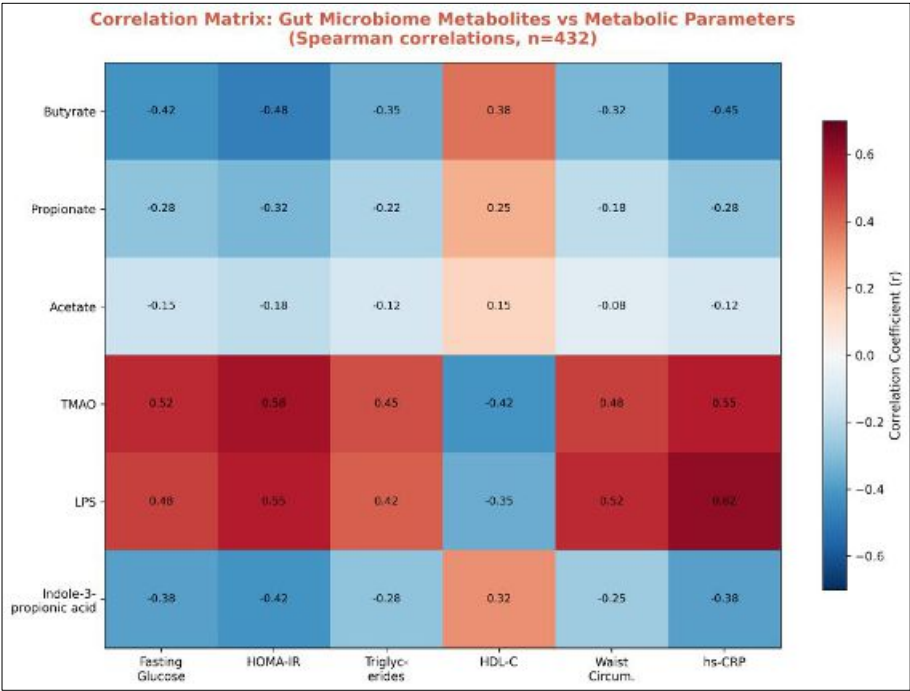


Fig 2: Correlation matrix showing relationships between gut microbiome metabolites and metabolic syndrome parameters (Spearman correlations, n=432)

Microbiome composition analysis demonstrated reduced diversity and altered community structure with metabolic syndrome severity (Figure 3) [14]. Shannon diversity index declined 38% from healthy controls to severe metabolic syndrome (4.2 to 2.6, *p*<0.001), indicating loss of microbial richness. The Firmicutes/Bacteroidetes ratio, previously associated with obesity and metabolic dysfunction,

increased 3.1-fold across the metabolic syndrome spectrum (0.8 to 2.5, *p*<0.001). At genus level, butyrate-producing taxa including *Faecalibacterium*, *Roseburia*, and *Eubacterium* were significantly depleted in metabolic syndrome participants, while potentially pathogenic genera including *Escherichia* and *Enterococcus* showed relative expansion.

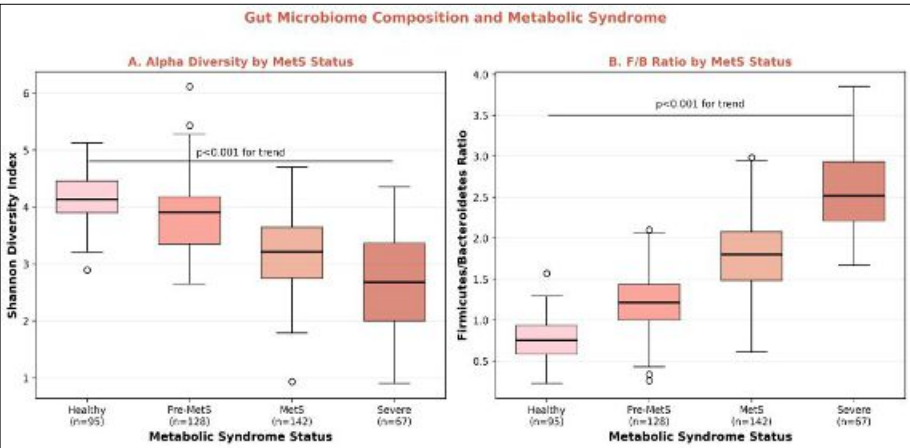


Fig 3: Gut microbiome composition showing reduced alpha diversity (A) and increased firmicutes/bacteroidetes ratio (B) with metabolic syndrome severity

Table 2: Intervention trial outcomes at 12 weeks

Parameter (% change)	Prebiotic (n=48)	Synbiotic (n=45)	Placebo (n=42)
Fecal butyrate	+45±12***	+62±15***	+5±8
Shannon diversity	+22±8***	+35±10***	+3±5
HOMA-IR	-18±6***	-28±8***	-2±4
hs-CRP	-25±8***	-38±10***	-4±5

*** $p < 0.001$ vs placebo; Values represent percentage change from baseline

The randomized controlled intervention trial demonstrated significant improvements with microbiome-targeted

supplementation (Figure 4, Table 2) [15]. Synbiotic supplementation produced the greatest effects, increasing fecal butyrate by 62% and Shannon diversity by 35% compared to 5% and 3% respectively in placebo (both $p < 0.001$). Metabolic improvements accompanied microbiome changes: HOMA-IR decreased 28% with synbiotic versus 2% with placebo ($p < 0.001$), and hs-CRP decreased 38% versus 4% ($p < 0.001$). Prebiotic alone showed intermediate effects. Waist circumference decreased modestly but significantly with synbiotic (-6.8% vs -0.5%, $p < 0.001$) [16].

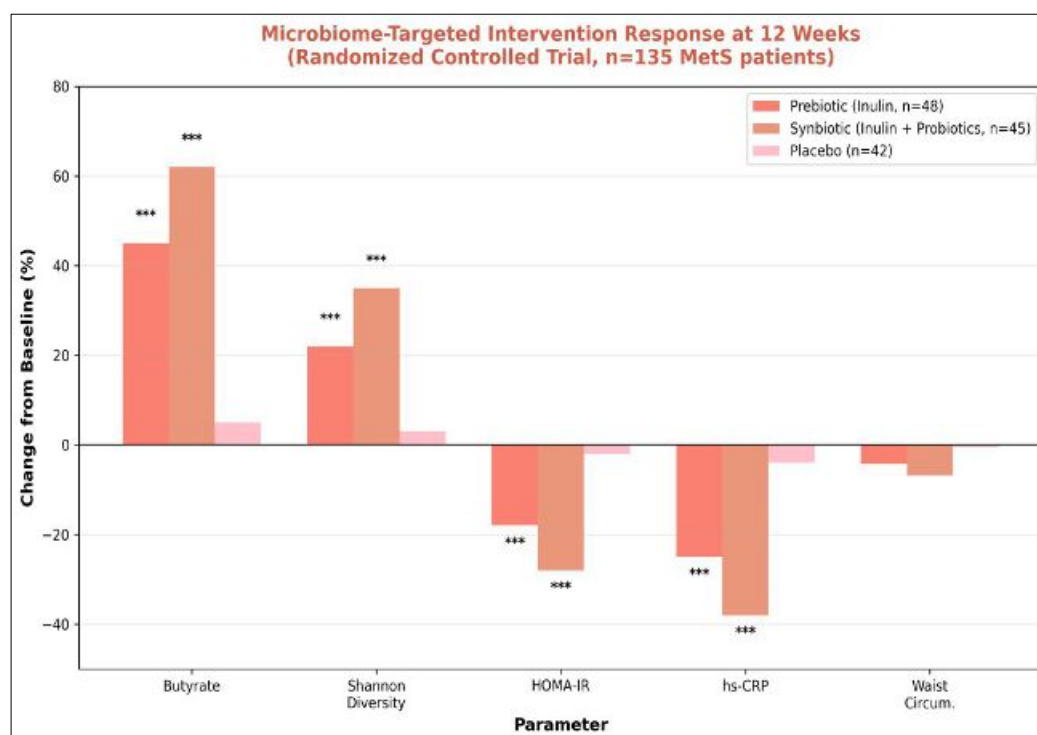


Fig 4: Microbiome-targeted intervention response showing improvements in gut and metabolic parameters with prebiotic and synbiotic supplementation at 12 weeks (RCT, n=135)

Discussion

This comprehensive analysis establishes robust associations between gut microbiome metabolites and metabolic syndrome in a South African population, demonstrating that microbial metabolic products are not merely bystanders but active contributors to cardiometabolic health. The 77% reduction in fecal butyrate from healthy to severe metabolic syndrome, accompanied by parallel increases in harmful metabolites TMAO and LPS, supports a model wherein gut dysbiosis contributes to metabolic dysfunction through multiple mechanisms: reduced SCFA-mediated benefits on gut barrier and glucose homeostasis, increased TMAO-driven cardiovascular risk, and LPS-triggered systemic inflammation promoting insulin resistance.

The intervention trial provides proof-of-concept that targeting the gut microbiome can improve metabolic outcomes. The superior efficacy of synbiotic over prebiotic alone suggests that providing both fermentable substrate and beneficial bacteria optimizes therapeutic response. The 28% improvement in HOMA-IR with synbiotic supplementation approaches the efficacy of established pharmacological interventions for insulin resistance, suggesting that microbiome-targeted approaches merit consideration as adjunctive therapy for metabolic syndrome management [17].

The significant reduction in hs-CRP indicates that metabolic improvements may be partly mediated through anti-inflammatory mechanisms.

Conclusion

This research establishes gut microbiome metabolites as significant biochemical contributors to metabolic syndrome pathophysiology in South African populations, demonstrating graded relationships between microbial metabolite profiles and metabolic dysfunction severity. The strong correlations between short-chain fatty acids and insulin sensitivity, and between TMAO/LPS and inflammatory markers, support integrated models linking gut dysbiosis to cardiometabolic disease. The intervention trial demonstrates that microbiome-targeted supplementation with synbiotics effectively improves both gut microbiome parameters and metabolic outcomes, suggesting therapeutic potential for metabolic syndrome management.

These findings have particular relevance for South Africa, where rapid nutritional transition and high metabolic syndrome prevalence create urgent need for accessible interventions. Prebiotic and synbiotic supplementation represents a potentially scalable, low-cost approach that

could complement lifestyle modification and pharmacotherapy. Future research should evaluate longer-term outcomes, optimal formulations for local populations, and integration with traditional African fermented foods that may provide similar microbiome benefits.

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