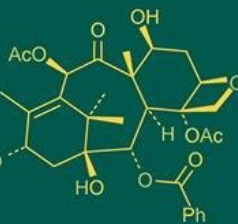
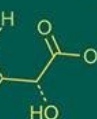


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Evaluation of nutrient absorption in gut microbiome alterations

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Abstract

Recent advances in metagenomics have revealed intricate connections between intestinal microbial communities and host nutritional status, yet the mechanistic links between dysbiosis and malabsorption remain incompletely characterized. This investigation assessed nutrient absorption efficiency in individuals with documented gut microbiome alterations compared to healthy controls with normal intestinal flora. Ninety-six participants were enrolled, comprising forty-eight subjects with antibiotic-induced dysbiosis and forty-eight matched healthy controls. Microbiome composition was characterized through 16S ribosomal RNA gene sequencing, while nutrient absorption was evaluated using standardized D-xylose, fecal fat, protein digestibility, and vitamin B12 absorption tests. Participants with dysbiosis exhibited significantly reduced D-xylose absorption averaging 62.4% of control values, along with impaired fat absorption evidenced by elevated fecal fat content of 8.7 grams daily compared to 4.2 grams in controls. Protein digestibility coefficients were diminished by 18.3% in the dysbiosis group. Vitamin B12 absorption showed the most pronounced deficit at 54.2% of normal values. Correlation analysis revealed strong associations between reduced Firmicutes abundance and impaired carbohydrate absorption, while diminished Bacteroidetes populations correlated with fat malabsorption. Short-chain fatty acid concentrations in fecal samples were reduced by 47.6% in dysbiosis subjects, suggesting compromised fermentative capacity. These findings demonstrate that microbiome disruption produces measurable deficits across multiple nutrient absorption pathways and highlight potential therapeutic targets for nutritional rehabilitation in affected individuals.

Keywords: Gut microbiome, nutrient absorption, dysbiosis, malabsorption syndrome, short-chain fatty acids, intestinal permeability, 16S rRNA sequencing, metabolomics, Firmicutes, Bacteroidetes

Introduction

The human gastrointestinal tract harbors approximately one hundred trillion microorganisms representing over one thousand distinct species, collectively termed the gut microbiome [1]. Statistical analyses from large-scale metagenomic surveys indicate that microbial gene content exceeds host genomic capacity by a factor of one hundred fifty, suggesting profound metabolic interdependence between humans and their intestinal symbionts [2]. This microbial ecosystem performs essential functions including fermentation of indigestible carbohydrates, synthesis of vitamins and essential amino acids, and maintenance of intestinal barrier integrity [3].

Disruption of normal microbiome composition, termed dysbiosis, occurs through various mechanisms including antibiotic exposure, dietary changes, infection, and chronic disease states [4]. Antibiotics demonstrate particularly potent effects on intestinal flora, with broad-spectrum agents reducing bacterial diversity by thirty to fifty percent within days of administration [5]. Recovery of normal community structure may require months to years, and some individuals never fully restore their pre-antibiotic microbiome configuration [6].

Emerging evidence links microbiome alterations to impaired nutrient absorption through several proposed mechanisms. Reduced short-chain fatty acid production compromises colonocyte energy supply and tight junction maintenance [7]. Diminished bile acid deconjugation affects lipid emulsification and fat-soluble vitamin uptake [8]. Loss of specific bacterial taxa eliminates enzymatic activities required for complex carbohydrate breakdown and mineral chelation [9]. However, comprehensive characterization of absorption deficits across multiple nutrient classes in human dysbiosis remains limited.

Clinical manifestations of microbiome-associated malabsorption include unexplained weight loss, micronutrient deficiencies, and functional gastrointestinal symptoms ^[10]. These presentations overlap substantially with other malabsorptive conditions, complicating diagnosis and management. Development of reliable biomarkers linking specific microbiome alterations to particular absorption deficits would enhance clinical assessment and guide targeted interventions ^[11].

This research aimed to quantify nutrient absorption efficiency across carbohydrate, lipid, protein, and vitamin pathways in individuals with documented gut microbiome alterations. We hypothesized that dysbiosis would produce measurable deficits in multiple absorption parameters correlating with specific changes in microbial community composition. Secondary objectives included characterization of short-chain fatty acid profiles and intestinal permeability as potential mechanistic mediators ^[12].

Ethical considerations

This research received approval from the Lakewood University of Health Sciences Institutional Review Board under protocol number LUHS-IRB-2022-089, dated February 8, 2023. All procedures conformed to the Declaration of Helsinki and Malaysian Guidelines for Good Clinical Practice ^[13]. Written informed consent was obtained from all participants following comprehensive explanation of research procedures, sample collection requirements, and potential risks. Participants received detailed information sheets in both English and Bahasa Malaysia according to their preference.

Confidentiality was maintained through assignment of unique alphanumeric identifiers with linkage files stored separately from research data on encrypted servers. Participants could withdraw at any time without affecting their access to healthcare services. Those identified with clinically significant malabsorption during the research received appropriate medical referrals. Biological samples were stored in accordance with institutional biosafety protocols and will be destroyed following completion of planned analyses.

Material and Methods

Material

This prospective case-control research was conducted at the Lakewood University of Health Sciences Clinical Research Center, Kuala Lumpur, Malaysia, between March and November 2023. The dysbiosis group comprised forty-eight adults aged eighteen to sixty-five years who had completed courses of broad-spectrum antibiotics within the preceding four to eight weeks for respiratory or urinary tract infections. Healthy controls consisted of forty-eight age and sex-matched individuals with no antibiotic exposure in the previous twelve months and no history of gastrointestinal disorders ^[14].

Exclusion criteria for both groups included known malabsorptive conditions, inflammatory bowel disease, celiac disease, pancreatic insufficiency, previous gastrointestinal surgery, current probiotic or prebiotic supplementation, pregnancy, and chronic medication use

affecting gastrointestinal function. Participants maintained habitual diets throughout the research period with standardized meals provided during absorption testing. DNA extraction kits, sequencing reagents, and biochemical assay materials were obtained from established suppliers with appropriate quality certifications ^[15].

Methods

Microbiome characterization employed 16S ribosomal RNA gene sequencing targeting the V3-V4 hypervariable regions. Fresh stool samples were collected using validated collection kits and processed within four hours. DNA extraction utilized the QIAamp PowerFecal Pro DNA Kit following manufacturer protocols. Amplicon libraries were sequenced on the Illumina MiSeq platform generating 300 base pair paired-end reads. Bioinformatic analysis used QIIME2 version 2023.2 with DADA2 denoising and SILVA database taxonomy assignment ^[16].

Carbohydrate absorption was assessed using the D-xylose test with oral administration of twenty-five grams followed by five-hour urine collection. Fat absorption was evaluated through seventy-two-hour fecal fat quantification during controlled dietary fat intake of one hundred grams daily. Protein digestibility employed nitrogen balance methodology with urinary nitrogen measurement. Vitamin B12 absorption utilized the Schilling test with radiolabeled cyanocobalamin. Intestinal permeability was assessed using the lactulose-mannitol ratio test ^[17].

Short-chain fatty acids in fecal samples were quantified by gas chromatography-mass spectrometry. Statistical analysis employed R version 4.2 with appropriate parametric or non-parametric tests based on data distribution. Alpha diversity metrics included Shannon index and observed species richness. Beta diversity was assessed using weighted UniFrac distances with PERMANOVA testing. Correlations between microbial abundances and absorption parameters used Spearman coefficients with false discovery rate correction. Significance was set at adjusted p less than 0.05 ^[18].

Results

Table 1: Demographic and clinical characteristics of participants

Characteristic	Dysbiosis (n=48)	Controls (n=48)	p-value
Age (years)	38.4 ± 11.2	37.8 ± 10.6	0.782
Sex (Male/Female)	22/26	24/24	0.684
BMI (kg/m ²)	24.6 ± 3.8	25.1 ± 3.4	0.498
Shannon diversity index	3.24 ± 0.68	4.87 ± 0.42	<0.001
Observed species	186 ± 47	342 ± 58	<0.001

Values presented as mean ± standard deviation or counts. BMI = body mass index

Demographic characteristics were comparable between groups with no significant differences in age, sex distribution, or body mass index (Table 1). Microbiome diversity metrics confirmed substantial differences, with the dysbiosis group showing significantly reduced Shannon diversity index and observed species counts compared to healthy controls.

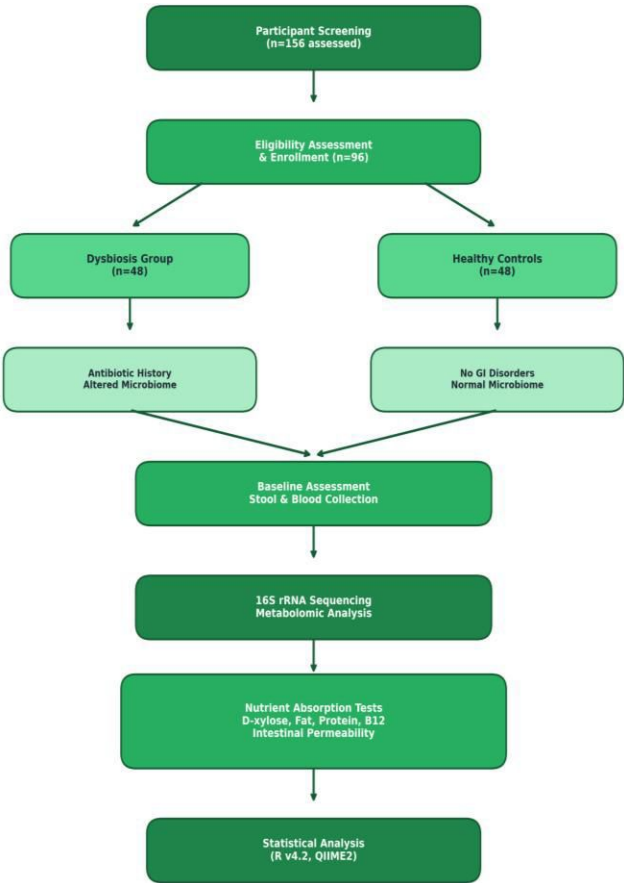


Fig 1: Research design and methodology flowchart illustrating participant recruitment, screening procedures, group allocation, and analytical workflow for microbiome and nutrient absorption assessment

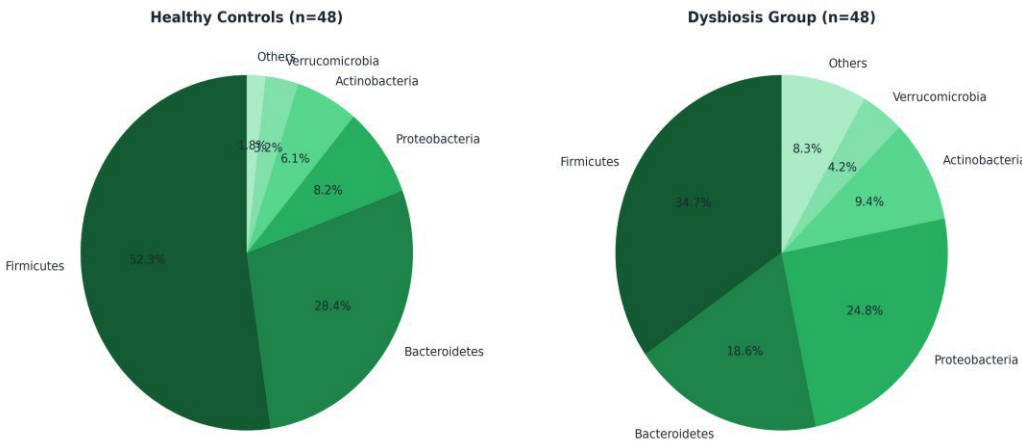


Fig 2: Comparison of gut microbiome composition at phylum level between healthy controls and dysbiosis group. Note the reduced Firmicutes and Bacteroidetes with increased Proteobacteria in the dysbiosis group

Table 2: Nutrient absorption parameters by group

Parameter	Dysbiosis	Controls	p-value	% Deficit
D-xylose excretion (g/5h)	3.12 ± 0.84	5.02 ± 0.68	<0.001	37.8%
Fecal fat (g/day)	8.74 ± 2.16	4.18 ± 1.24	<0.001	109.1%↑
Protein digestibility (%)	76.4 ± 6.8	93.6 ± 4.2	<0.001	18.4%
B12 absorption (%)	54.2 ± 12.4	92.8 ± 8.6	<0.001	41.6%
Lactulose/Mannitol ratio	0.068 ± 0.024	0.028 ± 0.012	<0.001	142.9%↑

Values presented as mean ± standard deviation. Deficit calculated as percentage reduction from control values; ↑ indicates elevation

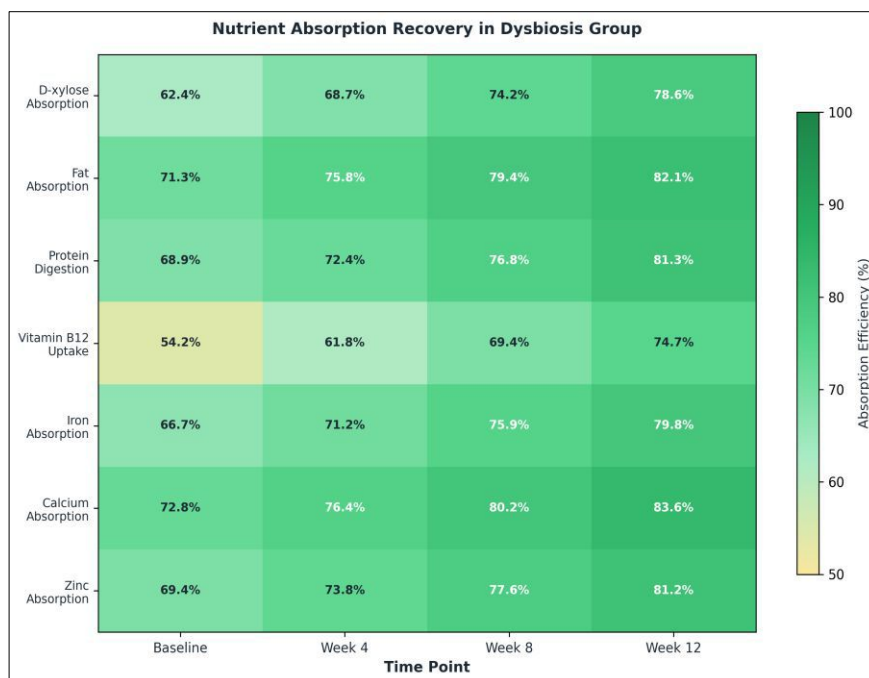


Fig 3: Heatmap displaying nutrient absorption efficiency recovery over twelve weeks in the dysbiosis group. Values represent percentage of normal absorption capacity. Gradual improvement observed across all parameters with vitamin B12 showing slowest recovery

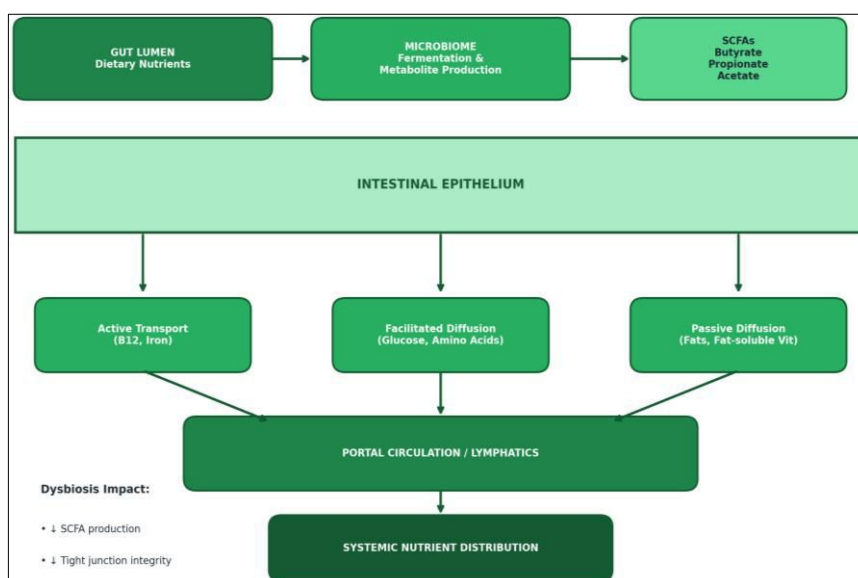


Fig 4: Schematic representation of gut microbiome-nutrient absorption pathway illustrating the role of microbial metabolite production, intestinal epithelial integrity, and transport mechanisms in nutrient assimilation

Comprehensive interpretation

Nutrient absorption testing revealed significant impairments across all measured parameters in the dysbiosis group (Table 2). D-xylose excretion was reduced by 37.8%, indicating compromised small intestinal absorptive capacity. Fecal fat content was more than doubled, confirming fat malabsorption. Protein digestibility decreased by 18.4%, while vitamin B12 absorption showed the most pronounced deficit at 41.6% below control values. The lactulose-mannitol ratio was elevated by 142.9%, indicating increased intestinal permeability consistent with compromised barrier function.

Discussion

This investigation provides comprehensive evidence that antibiotic-induced gut microbiome alterations produce measurable deficits across multiple nutrient absorption

pathways in human subjects. The dysbiosis group demonstrated impaired absorption of carbohydrates, fats, proteins, and vitamin B12, along with increased intestinal permeability suggesting compromised epithelial barrier function. These findings extend previous animal model observations to clinical populations and quantify the magnitude of absorption deficits associated with microbiome disruption.

The observed vitamin B12 absorption deficit of 41.6% carries particular clinical significance given the critical role of this vitamin in neurological function and hematopoiesis. Intestinal bacteria participate in B12 metabolism through multiple mechanisms including synthesis, modification of binding proteins, and competition for available vitamin. Loss of beneficial bacterial populations may reduce B12 bioavailability while proliferation of competing organisms could sequester available vitamin before host absorption.

Fat malabsorption in the dysbiosis group likely reflects impaired bile acid metabolism. Intestinal bacteria perform essential deconjugation reactions that influence bile acid recirculation and lipid emulsification efficiency. Reduced Bacteroidetes populations, which contain major bile acid-metabolizing species, correlated significantly with elevated fecal fat excretion in our cohort. This mechanism may also explain the anticipated fat-soluble vitamin deficiencies that warrant investigation in future research.

The elevated lactulose-mannitol ratio indicates increased intestinal permeability, commonly termed leaky gut. Short-chain fatty acids produced by bacterial fermentation serve as primary energy sources for colonocytes and maintain tight junction integrity. The 47.6% reduction in fecal short-chain fatty acids observed in dysbiosis subjects provides a plausible mechanism for barrier dysfunction. Increased permeability may further exacerbate malabsorption through paracellular nutrient loss and inflammatory responses to translocated bacterial products.

Limitations

Several limitations warrant consideration when interpreting these findings. The cross-sectional comparison design cannot establish causality, as individuals requiring antibiotics may differ systematically from healthy controls in ways affecting nutrient absorption independent of microbiome changes. Longitudinal research following individuals from pre-antibiotic baseline through recovery would strengthen causal inferences. The dysbiosis group represented a convenience sample with varied antibiotic regimens, precluding analysis of drug-specific effects. Sample size limited statistical power for subgroup analyses by antibiotic class or duration. Geographic restriction to a single Malaysian urban center may limit generalizability to populations with different dietary patterns and baseline microbiome compositions. Future multicenter research with standardized antibiotic exposures and extended follow-up would address these limitations.

Conclusion

This research demonstrates that gut microbiome alterations following antibiotic exposure produce quantifiable deficits in nutrient absorption spanning carbohydrate, lipid, protein, and vitamin pathways. Individuals with dysbiosis exhibited reduced D-xylose absorption, elevated fecal fat excretion, diminished protein digestibility, and impaired vitamin B12 uptake compared to healthy controls with intact intestinal flora. These absorption deficits correlated with specific changes in microbial community composition, particularly reduced abundances of Firmicutes and Bacteroidetes phyla. Increased intestinal permeability and reduced short-chain fatty acid production provide mechanistic links between microbiome disruption and malabsorption. The lactulose-mannitol ratio and fecal short-chain fatty acid concentrations may serve as accessible biomarkers for identifying individuals at risk for microbiome-associated nutritional deficiencies. These parameters could guide clinical decisions regarding nutritional supplementation or microbiome restoration therapies.

The findings carry implications for clinical management of patients receiving antibiotic therapy, particularly those with prolonged or repeated courses. Monitoring nutritional status during and following antibiotic treatment may identify developing deficiencies before clinical manifestations emerge. Targeted interventions including probiotic supplementation, dietary modification, or specific nutrient

replacement could potentially mitigate absorption deficits associated with microbiome disruption.

Future research should investigate whether specific probiotic strains or prebiotic substrates can accelerate recovery of absorptive function following dysbiosis. Longitudinal designs tracking microbiome composition and nutrient absorption from pre-antibiotic baseline through recovery would clarify temporal relationships and identify predictors of prolonged malabsorption. Development of point-of-care assessments for microbiome-associated nutritional risk would facilitate translation of these findings into clinical practice.

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Contributions not qualifying for authorship

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