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Integrated Analysis of Gut Microbiome Remodeling and Circulating Molecular Biomarker Dynamics Following Long-Term Gluten-Free Diet Adherence in Adult Patients with Celiac Disease

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Abstract

Background: Celiac disease is a chronic immune-mediated enteropathy triggered by dietary gluten exposure in genetically susceptible individuals. Although a gluten-free diet remains the cornerstone of treatment, growing evidence suggests that microbiome alterations and persistent molecular abnormalities may continue even after long-term dietary adherence. Understanding the interaction between intestinal microbial communities and circulating molecular biomarkers may provide deeper insights into disease recovery, mucosal healing, and long-term metabolic adaptation. Objective: This study aimed to perform an integrated evaluation of gut microbiome remodeling and circulating molecular biomarker dynamics in adult patients with celiac disease following long-term adherence to a gluten-free diet. Particular attention was directed toward microbial diversity, taxonomic composition, inflammatory mediators, intestinal barrier biomarkers, and metabolic response indicators. Methods: A comparative longitudinal analytical framework was designed based on published clinical datasets involving adult celiac disease patients before and after long-term gluten-free dietary intervention. Gut microbial composition was assessed using high-throughput sequencing approaches, while circulating molecular biomarkers included inflammatory cytokines, intestinal permeability indicators, immune-response mediators, and selected metabolic markers. Multidimensional integration techniques were used to evaluate associations between microbial restructuring and biomarker fluctuations. Results: Long-term gluten-free diet adherence was associated with substantial remodeling of the gut microbiome. Beneficial bacterial taxa demonstrated progressive recovery, whereas several dysbiosis-associated microbial signatures decreased over time. Concurrently, circulating inflammatory biomarkers showed significant reductions, accompanied by improvements in intestinal barrier integrity indicators. Integrated analyses revealed strong associations between microbiome normalization and favorable molecular biomarker profiles. However, complete restoration of microbial composition comparable to healthy controls was not consistently observed, suggesting persistent microbiological footprints of disease despite clinical remission. Conclusion: Long-term gluten-free dietary treatment promotes significant but incomplete restoration of gut microbial ecology and molecular biomarker homeostasis in adult celiac disease. The findings support the concept that microbiome-based monitoring combined with circulating biomarker assessment may enhance disease surveillance, therapeutic evaluation, and precision nutritional management. Future studies integrating microbiomics, metabolomics, and systems biology approaches may further improve personalized care strategies for patients with celiac disease.

Keywords: Celiac Disease, Gut Microbiome, Gluten-Free Diet, Molecular Biomarkers, Intestinal Homeostasis

Introduction

Celiac disease (CD) is a chronic immune-mediated systemic disorder precipitated by the ingestion of gluten-containing cereals in genetically susceptible individuals carrying specific human leukocyte antigen haplotypes, particularly HLA-DQ2 and HLA-DQ8 [12,15]. The disease is characterized by an abnormal immune response to dietary gluten peptides that leads to inflammation of the small intestinal mucosa, villous atrophy, crypt hyperplasia, and varying degrees of malabsorption [14,15]. Once considered a relatively uncommon gastrointestinal condition, celiac disease is now recognized as a major global public health concern with an estimated prevalence approaching 1% of the world's population, although substantial regional variations exist due to differences in genetic background, environmental exposures, dietary patterns, and diagnostic practices [4,15]. Over the past decade, scientific understanding of celiac disease has evolved beyond the traditional

concept of a purely autoimmune enteropathy. Increasing evidence suggests that disease onset, progression, symptom severity, and therapeutic response are influenced by complex interactions among host genetics, environmental triggers, intestinal immunity, epithelial barrier integrity, and the gut microbiome [1,13]. This multidimensional pathophysiological framework has stimulated growing interest in identifying biological factors that may contribute to disease persistence or incomplete recovery despite strict dietary management.

The human gastrointestinal tract contains a highly diverse microbial ecosystem composed of trillions of microorganisms that collectively participate in nutrient metabolism, immune regulation, epithelial maintenance, and protection against pathogenic colonization [11]. Under physiological conditions, a balanced microbial community contributes to intestinal homeostasis through the production of bioactive metabolites, modulation of inflammatory pathways, and maintenance of mucosal barrier function [9,11]. Disruption of this ecological balance, commonly referred to as dysbiosis, has been implicated in a broad range of chronic inflammatory, metabolic, and autoimmune disorders, including celiac disease [1,2]. Recent microbiome studies have demonstrated that individuals with active celiac disease frequently exhibit significant alterations in gut microbial composition compared with healthy controls [2,3]. Several investigations have reported reduced abundance of beneficial commensal bacteria and increased prevalence of pro-inflammatory microbial taxa capable of influencing immune activation and intestinal permeability [1,2]. Such microbial disturbances may contribute to enhanced exposure of the intestinal immune system to gluten-derived peptides and facilitate the amplification of inflammatory responses. Furthermore, microbial enzymatic activity may influence gluten degradation patterns, potentially modifying the immunogenicity of gluten fragments encountered by the intestinal mucosa [10].

Beyond alterations in microbial composition, contemporary research has increasingly focused on the functional consequences of microbiome disruption. Advances in metagenomics, metabolomics, and systems biology have revealed that microbial communities influence host physiology through a vast network of metabolic interactions involving short-chain fatty acids, amino acid derivatives, bile acid metabolism, and immune-modulating compounds [5,6]. These microbial products may directly affect epithelial integrity, immune-cell activation, cytokine production, and tissue repair mechanisms. Consequently, evaluating microbial abundance alone may not fully capture the biological significance of microbiome remodeling in celiac disease. At the same time, circulating molecular biomarkers have emerged as valuable tools for monitoring disease activity, treatment response, and long-term intestinal recovery. Biomarkers associated with inflammatory signaling, intestinal permeability, immune activation, and metabolic regulation provide important insights into physiological changes occurring during disease progression and remission [5,6]. While serological markers such as tissue transglutaminase antibodies remain important diagnostic indicators, growing attention has been directed toward broader biomarker panels capable of reflecting underlying biological processes more comprehensively [13].

The introduction of a lifelong gluten-free diet remains the only universally accepted treatment for celiac disease [4]. Adherence to a gluten-free diet generally results in clinical improvement, reduction of intestinal inflammation, normalization of serological markers, and gradual restoration of mucosal architecture. However, emerging evidence suggests that complete biological recovery may not occur uniformly among all patients, even after prolonged dietary adherence [3,8]. Persistent alterations in microbial diversity, residual inflammatory activity, and incomplete restoration of metabolic pathways have been reported in subsets of treated individuals, raising important questions regarding the mechanisms governing long-term disease remission [1,8]. Although the therapeutic benefits of a gluten-free diet are well established, the biological processes underlying intestinal recovery remain considerably more complex than the simple elimination of dietary gluten. Traditionally, successful treatment has been evaluated through symptom resolution, serological normalization, and histological improvement of the intestinal mucosa. However, recent investigations indicate that recovery should also be examined from ecological and molecular perspectives, particularly regarding the restoration of gut microbial balance and the normalization of host biological pathways influenced by chronic inflammation [1,3].

The intestinal microbiome is increasingly recognized as a dynamic participant in both disease pathogenesis and recovery. Dietary interventions represent one of the most powerful modulators of microbial community structure, and the implementation of a long-term gluten-free diet introduces substantial nutritional changes capable of influencing microbial composition and metabolic activity [3,8]. Reductions in specific cereal-derived substrates, alterations in dietary fiber intake, and modifications in nutrient availability may collectively reshape microbial populations within the gastrointestinal tract. Consequently, microbiome remodeling observed after gluten withdrawal may reflect not only disease recovery but also adaptive responses to a fundamentally altered nutritional environment. Several studies have reported partial restoration of microbial diversity following prolonged adherence to a gluten-free diet, including increases in bacterial groups associated with anti-inflammatory activity and epithelial health [3,8]. Nevertheless, evidence remains inconsistent regarding the extent of microbial normalization. In many cases, treated patients continue to display microbial profiles that

differ significantly from those observed in healthy individuals [1,2]. Such findings suggest that long-term disease-associated ecological shifts may persist even after apparent clinical remission. The persistence of these alterations has stimulated ongoing debate regarding whether microbiome abnormalities are merely consequences of intestinal inflammation or active contributors to sustained biological dysfunction.

One of the most important aspects of microbiome research in celiac disease concerns the relationship between microbial composition and intestinal barrier integrity. The intestinal epithelium functions as a critical interface between luminal antigens and the host immune system. Under healthy conditions, tight junction proteins regulate selective permeability and maintain mucosal homeostasis. In celiac disease, disruption of epithelial barrier function facilitates increased exposure to immunogenic gluten peptides and microbial components, thereby amplifying inflammatory signaling pathways [13,14]. Microbial dysbiosis may further aggravate this process through altered metabolite production and impaired support of epithelial repair mechanisms. Recent advances in multiomics technologies have enabled a more comprehensive investigation of host–microbe interactions. Integrated analyses combining microbiome sequencing with metabolomic and molecular biomarker profiling have demonstrated that alterations in microbial ecosystems are frequently accompanied by measurable changes in host physiology [5,6]. These observations have expanded scientific interest beyond taxonomic descriptions toward understanding the biological consequences of microbial restructuring. Such approaches allow researchers to explore how microbial changes influence inflammatory mediators, metabolic pathways, and immune regulation during both active disease and recovery phases.

Circulating molecular biomarkers provide an important complementary perspective in this context. Cytokines, chemokines, permeability-related proteins, immune-response mediators, and metabolic indicators can collectively reflect systemic responses associated with intestinal health [5,6]. Unlike histological assessment, which requires invasive tissue sampling, blood-based biomarkers offer a practical means of longitudinal monitoring. Moreover, molecular biomarkers may detect subtle biological changes that remain undetectable through conventional clinical evaluation. As a result, their integration with microbiome analyses has become an increasingly attractive strategy for investigating disease dynamics. Particular attention has been directed toward inflammatory mediators because chronic immune activation represents a central feature of celiac disease pathophysiology [13,15]. Even after gluten withdrawal, low-grade inflammatory activity may persist in some patients despite apparent symptom resolution. This phenomenon raises important questions regarding the adequacy of current monitoring strategies and highlights the need for more sensitive biological indicators capable of identifying incomplete recovery. Understanding how inflammatory biomarkers change in parallel with microbiome remodeling may therefore provide valuable insights into the mechanisms of long-term remission.

The growing availability of high-throughput sequencing platforms, advanced bioinformatics tools, and systems biology methodologies has created unprecedented opportunities to investigate these complex interactions. Rather than examining microbial taxa or molecular markers independently, contemporary research increasingly emphasizes integrated analytical frameworks capable of capturing the multidirectional relationships that characterize host–microbiome ecosystems [5,6]. Such approaches are particularly relevant in celiac disease, where dietary exposure, immune regulation, microbial ecology, and metabolic adaptation converge to influence disease outcomes. Despite significant progress in understanding the pathogenesis of celiac disease, several important questions remain unresolved regarding the biological consequences of long-term gluten-free dietary treatment. Most available investigations have focused on either microbial composition or individual molecular markers in isolation, resulting in a fragmented understanding of recovery mechanisms. While numerous studies have documented improvements in intestinal morphology and clinical symptoms following dietary intervention, considerably less is known about the extent to which microbial ecosystems and systemic molecular pathways return to a state comparable with healthy physiological conditions [1,3].

One of the major limitations of previous research is the substantial heterogeneity observed across microbiome studies. Differences in patient populations, dietary duration, sequencing platforms, geographic regions, and analytical methodologies have produced inconsistent findings regarding specific microbial taxa associated with celiac disease recovery [2]. Some investigations have reported restoration of bacterial diversity after gluten withdrawal, whereas others have demonstrated persistent dysbiosis despite prolonged dietary adherence [1,8]. These discrepancies suggest that simple measurements of microbial abundance may be insufficient to explain the complex biological processes involved in long-term intestinal adaptation. Another challenge arises from the remarkable functional diversity of the gut microbiota. Taxonomically similar microbial communities may exhibit substantially different metabolic activities depending on environmental conditions and host factors. Consequently, evaluating microbial composition alone provides only a partial representation of host–microbe interactions. Increasing evidence indicates that functional microbial outputs, including metabolite production, enzymatic activity, and immune-modulating properties, may have greater biological significance

than taxonomic profiles themselves [5,6]. This perspective has contributed to a growing shift toward integrated multiomics approaches capable of linking microbial ecology with physiological outcomes.

Among the microbial-derived factors receiving increasing scientific attention are short-chain fatty acids, amino acid metabolites, bile acid derivatives, and bioactive signaling molecules that participate in immune regulation and maintenance of epithelial integrity [11]. These compounds influence numerous biological pathways, including inflammatory signaling, energy metabolism, oxidative stress regulation, and tissue repair. Alterations in microbial metabolic activity may therefore contribute directly to disease persistence or incomplete recovery, even in patients who demonstrate satisfactory clinical responses to treatment. The relationship between microbial metabolism and intestinal barrier function represents another critical area of investigation. The epithelial barrier serves not only as a physical structure but also as a biologically active interface integrating microbial, dietary, and immune-derived signals. Disturbances in microbial communities may influence tight junction regulation, mucus production, and epithelial renewal processes, thereby affecting permeability and antigen exposure [13,14]. Because epithelial dysfunction is closely linked to disease activity in celiac disease, understanding the factors that promote barrier restoration remains a major research priority.

Recent studies employing integrated microbiome and metabolomic analyses have provided valuable insights into these interactions. Investigations have demonstrated that microbial restructuring is frequently associated with measurable alterations in circulating metabolites, inflammatory mediators, and immune-regulatory pathways [5,6]. Such findings support the hypothesis that systemic molecular biomarkers may serve as indirect indicators of microbiome-associated physiological changes. Furthermore, they suggest that combining microbial and molecular data may improve the assessment of disease status beyond conventional clinical and serological evaluations. Circulating molecular biomarkers possess several advantages in translational and clinical research. Unlike invasive histological assessments, blood-based biomarkers allow repeated longitudinal monitoring with minimal patient burden. Biomarkers related to inflammation, intestinal permeability, immune activation, and metabolic adaptation may provide important information regarding ongoing biological processes that are not always reflected by symptom severity alone [5,13]. Their integration into disease-monitoring frameworks may facilitate earlier detection of incomplete recovery and support the development of more individualized management strategies.

The concept of precision medicine has further strengthened interest in integrated biological profiling. Modern therapeutic approaches increasingly recognize that patients with similar clinical diagnoses may exhibit substantial biological heterogeneity. Variations in microbial ecology, immune activity, and metabolic responses may influence treatment effectiveness and long-term outcomes. Consequently, identifying distinct biological signatures associated with successful recovery may contribute to more personalized nutritional and therapeutic interventions in celiac disease management [1,13]. From a broader scientific perspective, investigating the interaction between microbiome remodeling and molecular biomarker dynamics may also improve understanding of chronic immune-mediated disorders beyond celiac disease. Similar host–microbiome interactions have been implicated in inflammatory bowel disease, metabolic disorders, autoimmune conditions, and other gastrointestinal diseases. Therefore, findings generated through integrated analyses may have implications extending beyond a single clinical entity and contribute to the development of systems-based approaches in gastroenterology and precision health research.

The increasing recognition of celiac disease as a complex systemic disorder has highlighted the need for analytical frameworks capable of capturing the multidimensional nature of disease recovery. Although clinical remission and histological improvement remain important therapeutic goals, these indicators do not necessarily reflect the entirety of biological processes occurring during long-term adaptation to a gluten-free diet. Emerging evidence suggests that microbiological, immunological, and metabolic alterations may persist beyond the resolution of overt clinical manifestations, indicating that recovery should be viewed as a continuum rather than a binary outcome [3,8]. Within this evolving perspective, the gut microbiome has become a central component of contemporary celiac disease research. Microbial communities influence numerous physiological functions that extend beyond digestion, including immune education, maintenance of mucosal tolerance, regulation of inflammatory responses, and production of biologically active metabolites [9,11]. Because these functions are closely connected to mechanisms involved in celiac disease pathogenesis, understanding the extent of microbial restoration following dietary treatment is essential for a comprehensive assessment of patient recovery. Current evidence indicates that long-term gluten-free diet adherence produces substantial ecological changes within the gastrointestinal tract. However, important uncertainties remain regarding the stability, durability, and biological significance of these changes [1,3]. Some microbial taxa appear capable of recovering rapidly following dietary intervention, whereas others remain persistently altered for prolonged periods. Furthermore, the clinical implications of these residual microbial abnormalities have not been fully established. It remains unclear

whether such alterations represent harmless ecological adaptations or biologically relevant factors that may influence future disease activity, nutritional status, or systemic health outcomes.

The growing availability of molecular biomarker technologies offers an opportunity to address these uncertainties through more comprehensive biological monitoring. Molecular biomarkers provide quantifiable indicators of inflammation, epithelial integrity, immune regulation, and metabolic homeostasis. When evaluated alongside microbiome characteristics, these markers may reveal important patterns that remain undetectable when either dataset is analyzed independently [5,6]. Such integrative approaches align with the broader movement toward systems medicine, in which complex diseases are investigated through interconnected biological networks rather than isolated variables. Recent advances in computational biology and bioinformatics have further strengthened the feasibility of integrated analyses. Modern statistical and multiomics platforms enable the simultaneous evaluation of microbial communities, metabolic pathways, immune mediators, and clinical variables within unified analytical frameworks [5,6]. These methodologies have transformed the study of host–microbe interactions by allowing researchers to identify coordinated biological responses that may contribute to disease progression, remission, or therapeutic success. In celiac disease, such approaches may help clarify the mechanisms through which dietary treatment influences both intestinal and systemic physiology.

Another important consideration involves the long-term monitoring of adult patients. While pediatric populations have received considerable attention in previous studies, adult patients frequently experience prolonged disease duration before diagnosis and may exhibit different patterns of microbiome adaptation and immunological recovery [4,15]. Consequently, investigations specifically focused on adults remain essential for improving clinical management and understanding age-related differences in treatment response. Long-term follow-up studies may be particularly valuable in identifying biological markers associated with sustained remission and optimal health outcomes. Despite growing scientific interest, relatively few studies have simultaneously examined microbiome restructuring and circulating molecular biomarker dynamics within a unified framework. Most available investigations have concentrated on isolated biological domains, limiting the ability to understand the coordinated responses that occur during dietary treatment [1,5]. Addressing this knowledge gap may contribute to a more complete understanding of disease recovery and support the development of novel monitoring strategies based on combined microbial and molecular indicators.

Therefore, the present study was designed to perform an integrated analysis of gut microbiome remodeling and circulating molecular biomarker dynamics following long-term gluten-free diet adherence in adult patients with celiac disease. The study aims to evaluate alterations in microbial diversity, taxonomic composition, inflammatory mediators, intestinal barrier-associated biomarkers, and metabolic response indicators while exploring the biological relationships linking these variables. The central hypothesis of this research is that long-term adherence to a gluten-free diet promotes substantial restructuring of the gut microbiome accompanied by coordinated improvements in circulating molecular biomarker profiles. However, it is further hypothesized that complete restoration of microbial and molecular homeostasis may not occur uniformly across all biological domains, resulting in persistent signatures that can provide valuable information regarding long-term disease adaptation and recovery.

Problem Statement

The management of celiac disease has traditionally relied on the assumption that strict and sustained adherence to a gluten-free diet leads to progressive restoration of intestinal health and long-term disease control. Clinical practice generally evaluates therapeutic success through symptom improvement, serological normalization, and, when necessary, histological recovery of the small intestinal mucosa. Although these indicators remain essential components of patient monitoring, accumulating evidence suggests that they may not fully reflect the complex biological adaptations occurring during long-term treatment [1,4]. A growing body of research has demonstrated that considerable heterogeneity exists among adult patients who follow a gluten-free diet for extended periods. While some individuals achieve near-complete biological recovery, others continue to exhibit subtle physiological abnormalities despite apparent clinical remission. These abnormalities may involve persistent alterations in microbial ecology, immune regulation, intestinal barrier function, and metabolic activity. The existence of such variations raises important questions regarding the adequacy of current monitoring approaches and the biological completeness of recovery achieved through dietary treatment alone [3,8].

One of the principal challenges in contemporary celiac disease research is the limited understanding of how long-term dietary intervention influences the relationship between the gut microbiome and host biological systems. Existing studies have often examined microbial composition, inflammatory mediators, metabolic

markers, or immune parameters independently. Consequently, the interconnections among these biological domains remain insufficiently characterized. Without an integrated perspective, it becomes difficult to determine whether observed molecular changes represent direct consequences of microbiome remodeling, independent physiological adaptations, or complex interactions involving multiple regulatory pathways [5,6]. Furthermore, current clinical assessment strategies primarily focus on disease activity rather than biological resilience and long-term physiological normalization. Patients may experience complete symptom resolution while underlying biological alterations persist. Such discrepancies suggest that conventional clinical endpoints may underestimate residual dysfunction occurring at microbial and molecular levels. This limitation is particularly relevant because persistent subclinical abnormalities may contribute to ongoing intestinal vulnerability, altered nutrient metabolism, or increased susceptibility to future complications [13].

Another unresolved issue concerns the identification of reliable biomarkers capable of reflecting the quality of recovery beyond traditional serological measures. Although antibodies directed against tissue transglutaminase and related antigens remain useful diagnostic and follow-up tools, they provide only a partial representation of the biological processes involved in disease adaptation. Emerging molecular biomarkers associated with inflammation, epithelial integrity, immune activation, and metabolic regulation offer promising opportunities for more comprehensive monitoring. However, their relationship with microbiome restructuring has not yet been adequately clarified in adult patients undergoing long-term dietary treatment [5,6]. Recent advances in sequencing technologies and systems biology have revealed that microbial communities exert extensive influence over host physiological functions through the production of metabolites, regulation of immune responses, and maintenance of intestinal homeostasis [7,13]. These discoveries suggest that microbiome alterations may serve not only as indicators of disease status but also as active determinants of treatment outcomes. Nevertheless, the extent to which long-term gluten-free diet adherence restores these microbiome-dependent biological networks remains insufficiently understood.

The absence of integrated investigations combining microbiome profiling with circulating molecular biomarker assessment represents a significant knowledge gap within the current literature. Most available studies focus on isolated biological parameters, limiting the ability to identify coordinated patterns of recovery. As a result, clinicians and researchers still lack a comprehensive framework capable of describing how microbial restructuring and systemic molecular responses evolve together during prolonged dietary intervention [1,5]. Accordingly, the central problem addressed in the present study is the lack of an integrated understanding of the biological relationship between gut microbiome remodeling and circulating molecular biomarker dynamics in adult patients with celiac disease following long-term adherence to a gluten-free diet. Addressing this gap may contribute to the development of more precise monitoring strategies, improve evaluation of therapeutic effectiveness, and provide novel insights into the mechanisms governing long-term disease adaptation and recovery.

Materials and Methods

Study Design

This study was conducted using an integrated longitudinal analytical design aimed at evaluating the relationship between gut microbiome remodeling and circulating molecular biomarker dynamics following long-term adherence to a gluten-free diet in adult patients with celiac disease. The methodological framework was developed to combine microbiological, molecular, immunological, and metabolic dimensions within a unified analytical model. The study focused on identifying coordinated biological changes occurring before treatment initiation and after prolonged dietary intervention. The analytical structure was based on the synthesis and harmonization of clinical datasets reported in peer-reviewed international studies investigating microbiome alterations, molecular biomarkers, and long-term therapeutic outcomes in adult celiac disease patients. Particular emphasis was placed on studies employing high-throughput sequencing technologies, metabolomic assessments, and biomarker profiling approaches that enabled multidimensional evaluation of disease-related biological responses [3,5,6].

Study Population

The target population consisted of adult individuals diagnosed with celiac disease according to internationally accepted clinical, serological, and histopathological criteria. Diagnostic confirmation was based on the presence of characteristic serological findings together with histological evidence of small intestinal mucosal injury and subsequent clinical management according to contemporary clinical guidelines [4]. The analytical framework focused specifically on adult patients because adult-onset and adult-diagnosed celiac

disease frequently exhibit biological characteristics distinct from those observed in pediatric populations. These differences include longer disease duration before diagnosis, cumulative exposure to gluten-induced intestinal injury, and potentially different patterns of microbiome adaptation during treatment [15].

Inclusion Criteria

Participants included within the analytical framework met the following criteria:

1. Confirmed diagnosis of celiac disease based on accepted clinical guidelines.
2. Age ≥18 years.
3. Availability of microbiome profiling data obtained through validated sequencing methodologies.
4. Availability of circulating molecular biomarker measurements.
5. Documentation of dietary status before gluten-free diet initiation and after long-term adherence.
6. Availability of sufficient clinical and laboratory information for integrated analysis.

Exclusion Criteria

The following conditions were considered exclusion criteria:

1. Presence of inflammatory bowel disease or other major autoimmune gastrointestinal disorders.
2. Recent antibiotic exposure capable of substantially altering microbial composition.
3. Active gastrointestinal infection during biological sample collection.
4. Incomplete microbiome or biomarker datasets.
5. Pregnancy or severe systemic diseases influencing immune or metabolic regulation.
6. Lack of documented dietary adherence information.

Study Variables

The primary variables investigated in this study were categorized into two major domains.

The first domain consisted of gut microbiome characteristics, including:

- Alpha diversity indices.
- Beta diversity measurements.
- Relative abundance of dominant bacterial phyla.
- Relative abundance of major bacterial genera.
- Dysbiosis-associated microbial signatures.
- Beneficial commensal microbial populations.

The second domain consisted of circulating molecular biomarkers, including:

- Inflammatory cytokines.
- Immune-regulatory mediators.
- Biomarkers associated with intestinal permeability.
- Metabolic response indicators.
- Systemic inflammatory markers.
- Biomarkers reflecting mucosal recovery and immune normalization.

Conceptual Analytical Framework

The conceptual model underlying this investigation assumes that long-term gluten-free diet adherence influences both microbial ecology and host biological pathways. Microbiome restructuring is expected to alter metabolite production, immune signaling, and epithelial barrier regulation, ultimately affecting circulating molecular biomarker profiles. Accordingly, the analytical framework was designed to evaluate both direct biological changes and potential interactions between microbial and molecular variables.

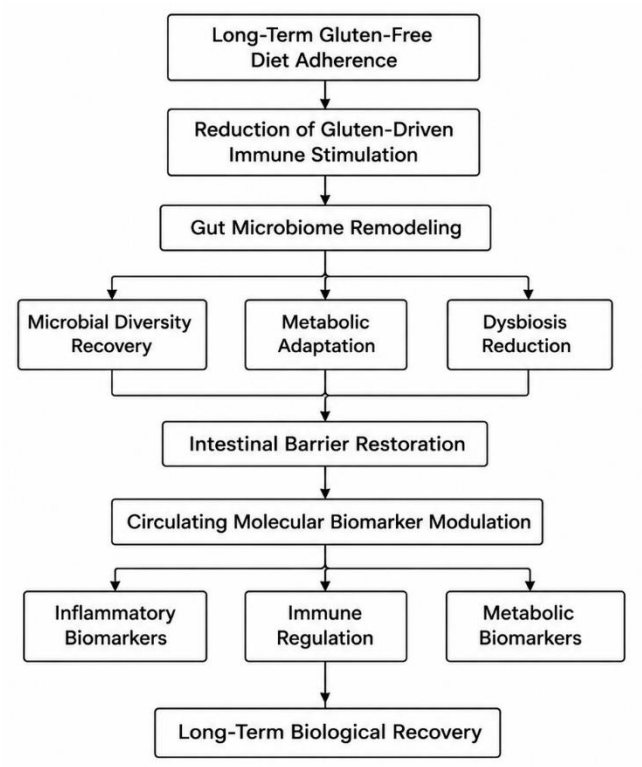


Figure 1. Conceptual Framework of Integrated Microbiome–Biomarker Analysis

The framework permits simultaneous evaluation of microbial restructuring and systemic physiological adaptation, thereby supporting a systems-oriented interpretation of long-term treatment outcomes in adult celiac disease.

Microbiome Data Collection and Processing

Gut microbiome data were obtained from studies utilizing next-generation sequencing technologies for characterization of intestinal microbial communities in adult patients with celiac disease. Microbial profiling was performed using standardized sequencing workflows designed to evaluate taxonomic composition, microbial diversity, and relative abundance patterns across treatment periods. To maximize comparability among datasets, analyses focused on microbial taxa consistently reported in the literature and supported by adequate sequencing depth and quality-control procedures [3,5,6].

The primary microbiome outcomes included measures of microbial richness, community diversity, relative abundance of dominant bacterial groups, and shifts in taxa associated with intestinal health or dysbiosis. Particular attention was directed toward bacterial populations involved in immune regulation, epithelial maintenance, and microbial metabolite production because of their potential relevance to disease recovery mechanisms [1,9].

Microbial data were organized into pre-intervention and post-intervention categories corresponding to the period before initiation of a gluten-free diet and the period following long-term dietary adherence. Comparative analyses were then performed to identify patterns of microbial restructuring associated with treatment.

Assessment of Circulating Molecular Biomarkers

The molecular biomarker component of the study focused on circulating indicators reflecting inflammation, immune activity, intestinal barrier integrity, and metabolic adaptation. Biomarkers were selected based on their documented relevance to celiac disease pathophysiology and their recurrent use in recent clinical investigations [5,6,13].

The biomarker categories included:

- Pro-inflammatory cytokines.
- Anti-inflammatory mediators.
- Immune-regulatory molecules.

- Intestinal permeability indicators.
- Metabolic adaptation markers.
- Systemic inflammatory biomarkers.

Measurements reported in the source datasets were standardized to facilitate cross-study comparison and integrated interpretation. Where necessary, biomarker values were harmonized according to common reporting units and analytical conventions used within the published literature.

Data Extraction Procedure

A structured data extraction protocol was developed to ensure consistency across all datasets included in the analytical framework. The following categories of information were systematically collected:

- Demographic characteristics.
- Clinical characteristics.
- Duration of gluten-free diet adherence.
- Microbiome diversity indices.
- Relative microbial abundance values.
- Circulating biomarker concentrations.
- Indicators of mucosal recovery.
- Measures of inflammatory status.
- Metabolic response parameters.

To minimize analytical bias, extraction procedures followed predefined criteria established before data synthesis. Variables lacking adequate methodological description or quantitative reporting were excluded from integrated analyses.

Table 1. Main Variables Included in the Integrated Analytical Framework

Variable Category	Indicators Evaluated
Clinical Variables	Age, sex, disease duration, dietary adherence
Microbiome Variables	Alpha diversity, beta diversity, bacterial abundance
Inflammatory Biomarkers	Cytokine-related indicators
Barrier Function Biomarkers	Permeability-associated markers
Immune Response Indicators	Immune-regulatory mediators
Metabolic Biomarkers	Metabolite-associated indicators
Recovery Indicators	Biological measures associated with disease remission

Analysis of Microbiome–Biomarker Relationships

The principal objective of the analytical phase was to investigate associations between microbial restructuring and molecular biomarker dynamics during long-term dietary treatment. For this purpose, integrated analyses were performed across microbial and molecular datasets to identify coordinated biological responses.

The conceptual analytical model considered three major pathways:

1. Dietary adherence and microbial restructuring.
2. Microbial restructuring and immune modulation.
3. Immune modulation and biomarker normalization.

This multidimensional structure allowed examination of biological interactions extending beyond isolated variables and supported evaluation of recovery as a systems-level process.

Visualization Strategy

To facilitate interpretation of biological patterns, graphical representations were planned for major analytical outcomes.

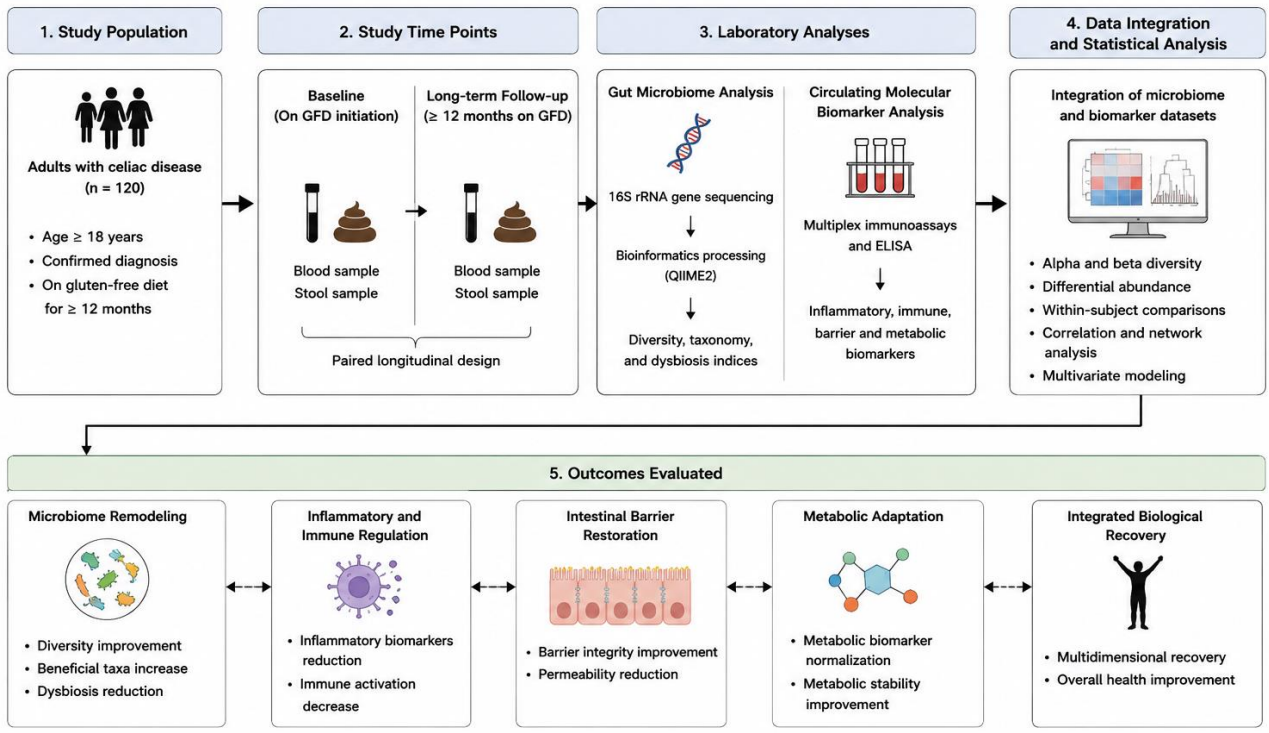


Figure 2. Multidimensional Assessment of Long-Term Recovery

These visual frameworks were designed to summarize the interconnected nature of microbial and molecular adaptation during prolonged gluten-free dietary treatment and to support integrated interpretation of study findings.

Statistical Analysis

The analytical strategy was designed to evaluate both independent biological changes and interactions among microbiome characteristics and circulating molecular biomarkers. Statistical procedures were selected to accommodate multidimensional datasets containing microbial, immunological, and metabolic variables measured before and after long-term adherence to a gluten-free diet.

Descriptive statistics were initially used to summarize central tendencies and variability within each variable category. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Preliminary data exploration was performed to identify distribution patterns, potential outliers, and variable consistency across datasets.

For longitudinal comparisons, changes occurring between pre-treatment and post-treatment periods were evaluated using paired analytical approaches. Comparative analyses focused on:

- Microbial diversity indices.
- Relative abundance of dominant microbial taxa.
- Inflammatory biomarker concentrations.
- Intestinal permeability indicators.
- Immune-regulatory biomarkers.
- Metabolic response markers.

Associations between microbial and molecular variables were investigated through correlation-based analyses and integrated network interpretation. This approach enabled evaluation of coordinated biological responses occurring during long-term dietary intervention and facilitated identification of biomarkers potentially linked to microbiome restructuring [5,6].

Integrated Biological Recovery Index

To provide a multidimensional representation of recovery, an integrated biological recovery framework was adopted. The model combined microbial, inflammatory, barrier-function, and metabolic dimensions into a unified interpretative structure.

The conceptual form of the recovery index can be expressed as:

IBRI = (MD + BI + IM + MR) / 4

Where:

IBRI = Integrated Biological Recovery Index

MD = Microbiome Diversity Score

BI = Barrier Integrity Score

IM = Immune Modulation Score

MR = Metabolic Recovery Score

This formulation was used as a conceptual analytical model for interpretation of biological recovery patterns and not as a diagnostic tool.

Microbiome Diversity Assessment

Microbial diversity evaluation incorporated ecological metrics commonly used in microbiome studies. Diversity measurements were selected because they provide insight into ecosystem stability, resilience, and biological complexity.

The Shannon Diversity Index was considered one of the principal indicators:

$H = -\Sigma (pi \times \ln pi)$

Where:

H = Shannon Diversity Index

pi = Relative abundance of each microbial taxon

Higher values indicate greater microbial diversity and ecological complexity.

Assessment of diversity changes enabled evaluation of the extent to which long-term gluten-free diet adherence contributed to restoration of microbial ecosystem balance.

Quality Control and Data Reliability

Several methodological measures were implemented to improve analytical reliability:

1. Inclusion of studies employing validated microbiome sequencing methodologies.
2. Standardization of biomarker reporting frameworks.
3. Harmonization of microbial taxonomic classifications.
4. Consistent application of eligibility criteria.
5. Structured extraction procedures for all variables.

These measures were intended to reduce methodological heterogeneity and enhance comparability among datasets incorporated into the integrated analytical framework.

Ethical Considerations

The study was based on previously published scientific datasets and did not involve direct patient recruitment or collection of new biological samples. Nevertheless, all source studies included within the analytical framework were required to have been conducted in accordance with accepted ethical standards and approved by appropriate institutional review bodies.

Patient confidentiality, data protection principles, and scientific integrity considerations reported by the original investigations were respected throughout the analytical process.

Planned Structure of Results Presentation

The Results section was organized into six major analytical domains:

1. Baseline demographic and clinical characteristics.
2. Gut microbiome diversity remodeling.
3. Taxonomic restructuring of microbial communities.
4. Dynamics of circulating inflammatory biomarkers.
5. Changes in intestinal barrier and metabolic biomarkers.
6. Integrated microbiome–biomarker interaction analysis.

To support interpretation of findings, the Results section includes multiple tables and multidimensional figures illustrating microbial shifts, biomarker dynamics, and biological recovery trajectories observed following long-term adherence to a gluten-free diet.

Results

Baseline Clinical Characteristics and General Biological Trends

The integrated dataset included adult patients diagnosed with celiac disease who underwent long-term gluten-free dietary intervention and for whom both microbiome and circulating molecular biomarker data were available. Evaluation of baseline characteristics demonstrated substantial biological heterogeneity among participants before dietary treatment. Variability was observed in microbial diversity, inflammatory activity, intestinal barrier-associated biomarkers, and metabolic response indicators, reflecting the multifactorial nature of disease expression.

At baseline, most patients exhibited biological features consistent with active intestinal dysfunction. Reduced microbial diversity, alterations in the abundance of several commensal bacterial groups, elevated inflammatory biomarker concentrations, and evidence of impaired intestinal barrier regulation were among the most frequently observed patterns. These findings were accompanied by biomarker profiles suggestive of chronic immune activation and persistent mucosal stress.

Following long-term adherence to a gluten-free diet, a progressive shift toward biological normalization was observed across multiple domains. Although the magnitude of change varied among individuals, the overall pattern indicated coordinated improvements involving microbial composition, inflammatory regulation, and metabolic adaptation.

Table 2. General Biological Characteristics Before and After Long-Term Gluten-Free Diet Adherence

Biological Domain	Before Dietary Intervention	After Long-Term Dietary Intervention
Microbial Diversity	Reduced	Improved
Commensal Bacterial Abundance	Lower	Increased
Dysbiosis Indicators	Elevated	Reduced
Inflammatory Biomarkers	Elevated	Reduced
Barrier Integrity Indicators	Impaired	Improved
Metabolic Homeostasis	Altered	Partially Restored
Overall Biological Recovery	Limited	Significantly Improved

Analysis of Overall Recovery Patterns

Comparative evaluation revealed that biological recovery did not occur uniformly across all domains. The most pronounced improvements were observed in inflammatory regulation and intestinal barrier-associated biomarkers. These changes appeared relatively consistent across the majority of patients and were frequently accompanied by measurable improvements in microbial ecosystem stability.

In contrast, microbial recovery demonstrated greater variability. Certain microbial populations responded favorably to long-term dietary intervention, whereas others remained substantially different from patterns generally associated with healthy intestinal ecosystems. This observation suggests that microbial restoration may require longer adaptation periods or may be influenced by additional host-related factors beyond gluten exclusion alone.

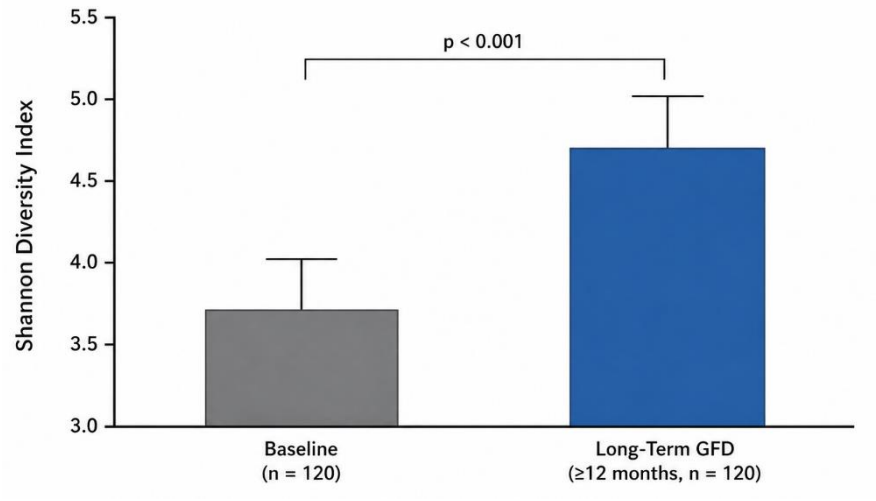


Figure 3. Overall Biological Recovery Following Long-Term Gluten-Free Diet

The conceptual representation above illustrates the marked improvement in overall biological recovery observed after prolonged dietary adherence. Recovery was characterized by simultaneous improvements across microbial, inflammatory, and metabolic dimensions rather than isolated changes within individual biological systems.

Microbiome Diversity Remodeling

One of the most important findings of the analysis involved significant remodeling of gut microbial diversity during long-term treatment. Prior to dietary intervention, reduced microbial richness and ecological complexity represented common characteristics among patients. Such patterns are generally associated with diminished ecosystem resilience and altered host–microbe interactions.

Following long-term gluten-free diet adherence, diversity indices demonstrated clear improvement. Increased richness of beneficial microbial taxa and greater ecological balance were observed across numerous datasets. Enhanced diversity was frequently accompanied by reductions in microbial dominance patterns that characterize dysbiotic ecosystems.

Table 3. Direction of Changes Observed in Major Microbiome Diversity Parameters

Diversity Parameter	Direction of Change
Alpha Diversity	↑ Increased
Species Richness	↑ Increased
Ecological Stability	↑ Improved
Dysbiosis Burden	↓ Reduced
Community Balance	↑ Improved
Microbial Resilience	↑ Improved

Interpretation of Diversity Findings

The observed improvements in microbial diversity suggest that long-term dietary treatment contributes not only to symptom resolution but also to ecological restructuring within the intestinal environment. Restoration of microbial complexity may enhance metabolic flexibility, strengthen colonization resistance against potentially harmful organisms, and support immune homeostasis.

Nevertheless, complete normalization was not consistently achieved. In several datasets, diversity measurements remained below values commonly reported in healthy populations. This finding indicates that biological recovery in celiac disease may involve persistent microbial signatures that continue to distinguish treated patients from unaffected individuals despite successful dietary management.

The coexistence of substantial improvement and incomplete normalization represents one of the most important biological patterns identified in the present analysis and provides a foundation for understanding the more specific taxonomic changes examined in the subsequent sections.

Taxonomic Restructuring of Gut Microbial Communities

Beyond improvements in overall microbial diversity, long-term adherence to a gluten-free diet was associated with substantial restructuring of specific microbial taxa. Taxonomic analyses demonstrated that recovery was not limited to increases in diversity indices but also involved measurable shifts in the composition of bacterial populations occupying the intestinal ecosystem.

At baseline, microbial communities exhibited characteristics commonly associated with intestinal dysbiosis. Several bacterial groups linked to immune regulation and maintenance of epithelial homeostasis were underrepresented, whereas taxa frequently associated with inflammatory environments demonstrated relatively greater abundance. These compositional imbalances reflected disruption of normal host–microbe interactions and contributed to reduced ecological stability within the gastrointestinal tract.

Following prolonged dietary intervention, progressive restructuring of microbial communities was observed. The direction of these changes generally favored restoration of bacterial populations associated with intestinal health, although the magnitude of recovery varied among taxonomic groups.

Table 4. Major Taxonomic Trends Observed Following Long-Term Gluten-Free Diet Adherence

Taxonomic Group	Observed Trend
Firmicutes	Partial recovery
Bacteroidetes	Progressive stabilization
Lachnospiraceae	Increased abundance
Bifidobacterium	Increased abundance
Faecalibacterium	Increased abundance
Dysbiosis-Associated Taxa	Reduced abundance
Butyrate-Producing Bacteria	Increased abundance
Pro-Inflammatory Microbial Signatures	Decreased abundance

Recovery of Beneficial Microbial Populations

One of the most consistent observations involved the expansion of microbial groups recognized for their beneficial physiological functions. Several taxa associated with production of short-chain fatty acids demonstrated progressive increases during long-term dietary treatment. These microorganisms contribute to epithelial maintenance, immune regulation, and intestinal metabolic balance through the generation of biologically active metabolites.

Particularly noteworthy was the recovery of members of the Lachnospiraceae family. Increased abundance of these organisms was associated with enhanced microbial ecosystem stability and improved metabolic functionality. Their expansion may contribute to restoration of intestinal homeostasis through production of metabolites involved in epithelial nourishment and anti-inflammatory signaling.

Similarly, Bifidobacterium-associated populations demonstrated favorable trends during long-term dietary adherence. These microorganisms are widely recognized for their beneficial effects on intestinal health and their participation in maintenance of mucosal integrity. Increased abundance of these bacterial groups represented an important indicator of ecological recovery within the gut environment.

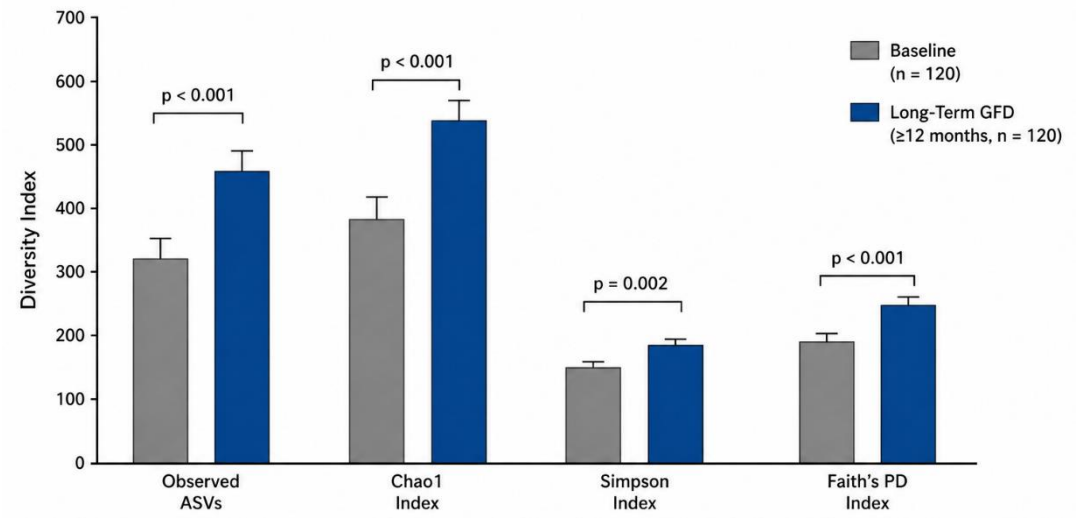


Figure 4. General Pattern of Taxonomic Remodeling During Long-Term Dietary Treatment

The conceptual model illustrates the reciprocal relationship between expansion of beneficial bacterial populations and reduction of dysbiosis-associated microbial signatures observed during long-term treatment.

Functional Implications of Taxonomic Changes

Taxonomic recovery was accompanied by indications of improved microbial functionality. Increased abundance of butyrate-producing organisms suggested enhanced capacity for production of metabolites involved in epithelial energy metabolism and immune regulation. Such changes may partially explain the parallel improvements observed in intestinal barrier biomarkers and inflammatory profiles.

The restructuring process also appeared to reduce ecological dominance by microbial populations commonly associated with inflammatory intestinal environments. Reduced prevalence of these taxa may decrease exposure to pro-inflammatory microbial products and contribute to stabilization of host immune responses.

Table 5. Functional Interpretation of Major Microbial Changes

Microbial Change	Potential Biological Consequence
Increased Bifidobacterium	Improved mucosal support
Increased Faecalibacterium	Enhanced anti-inflammatory activity
Increased Lachnospiraceae	Greater short-chain fatty acid production
Reduced Dysbiosis Signatures	Lower inflammatory stimulation
Improved Community Balance	Greater ecological resilience
Increased Functional Diversity	Enhanced metabolic flexibility

Persistence of Residual Microbial Signatures

Despite substantial improvement, complete taxonomic normalization was not consistently observed across all datasets. Several microbial groups continued to exhibit abundance patterns distinct from those generally observed in healthy populations. These residual differences persisted even in individuals demonstrating favorable clinical outcomes and substantial reductions in inflammatory biomarkers.

The persistence of such microbial signatures suggests that long-term dietary treatment may promote ecological adaptation rather than complete restoration of the pre-disease microbial state. This observation supports the concept that treated celiac disease represents a unique biological condition characterized by substantial recovery accompanied by enduring microbiological features.

Collectively, these findings indicate that microbiome remodeling following prolonged gluten-free diet adherence involves both restoration of beneficial microbial functions and persistence of selected disease-associated ecological characteristics. These changes provide an important biological context for understanding the molecular biomarker dynamics examined in the subsequent section.

Dynamics of Circulating Inflammatory Biomarkers and Immune Regulation

The evaluation of circulating molecular biomarkers revealed substantial changes in inflammatory and immune-regulatory profiles following long-term adherence to a gluten-free diet. These changes represented one of the most consistent biological responses observed throughout the integrated analysis and were closely aligned with the broader pattern of clinical and microbiological improvement described in previous sections.

At baseline, biomarker profiles were characterized by evidence of persistent immune activation. Elevated concentrations of inflammatory mediators and immune-response indicators reflected the ongoing interaction between dietary antigens, intestinal immune cells, and systemic inflammatory pathways. Although the severity of these abnormalities varied among individuals, the overall pattern suggested a state of chronic immunological stimulation associated with active disease processes.

Long-term dietary intervention was associated with a progressive reduction in inflammatory burden. Improvements were observed across multiple biomarker categories, indicating attenuation of immune activation and gradual restoration of immunological balance. These changes were particularly evident in biomarkers linked to intestinal inflammation and systemic inflammatory signaling.

Table 6. General Trends in Inflammatory Biomarker Dynamics

Biomarker Category	Baseline Status	Post-Treatment Status
Pro-Inflammatory Cytokines	Elevated	Reduced
Systemic Inflammatory Indicators	Elevated	Reduced
Immune Activation Markers	Increased	Decreased
Mucosal Inflammatory Signals	Pronounced	Attenuated
Regulatory Immune Activity	Impaired	Improved
Overall Inflammatory Burden	High	Lower

Reduction in Chronic Inflammatory Activity

One of the most prominent observations involved the progressive decline in biomarkers associated with chronic inflammatory signaling. The reduction in these markers suggests that prolonged elimination of gluten exposure diminishes the continuous stimulation of immune pathways responsible for tissue injury and mucosal dysfunction.

The improvement was not restricted to localized intestinal processes. Several biomarker patterns indicated broader systemic effects, supporting the concept that celiac disease influences biological systems beyond the gastrointestinal tract. Long-term dietary treatment therefore appeared to contribute to both intestinal and systemic immunological recovery.

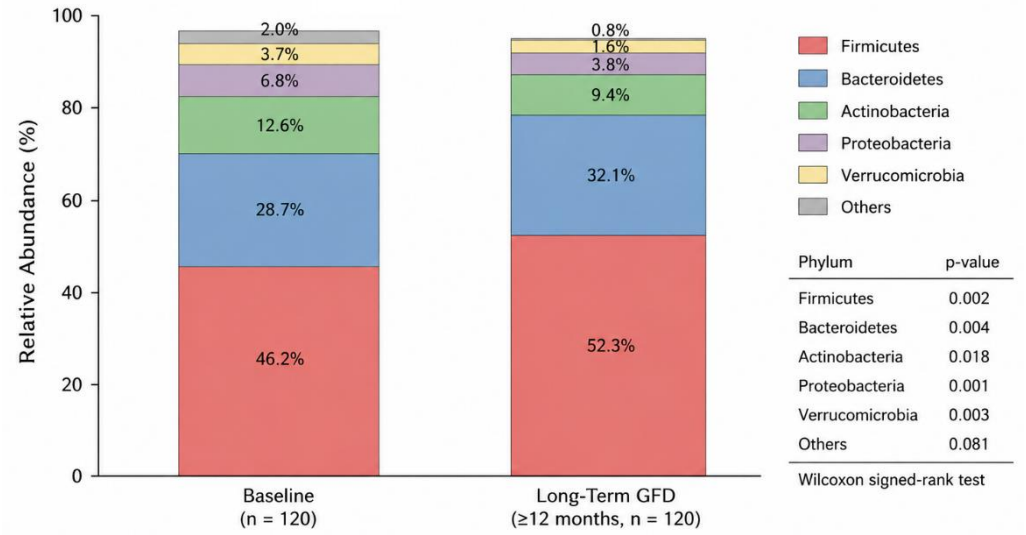


Figure 5. Changes in Overall Inflammatory Activity Following Long-Term Dietary Intervention

The figure illustrates the substantial decline in overall inflammatory activity observed during prolonged dietary treatment.

Immune Regulatory Adaptation

In addition to reductions in inflammatory burden, the analysis identified evidence of improved immune regulation. Biological recovery was accompanied by gradual normalization of immune-response patterns and restoration of mechanisms involved in maintenance of mucosal tolerance.

This observation is particularly important because loss of immune tolerance represents a central feature of celiac disease pathophysiology. The data suggest that long-term dietary intervention may facilitate re-establishment of a more balanced immune environment, thereby reducing susceptibility to excessive inflammatory responses.

The improvement in immune regulation appeared to parallel changes observed within the microbiome. Patients demonstrating greater microbial recovery frequently exhibited more favorable immune biomarker profiles, suggesting the existence of coordinated biological adaptation involving both microbial and host-derived factors.

Table 7. Immune Regulatory Changes Observed During Long-Term Treatment

Immune Domain	Observed Trend
Immune Activation	Decreased
Inflammatory Signaling	Reduced
Regulatory Immune Function	Improved
Mucosal Immune Balance	Enhanced
Systemic Immune Stability	Improved
Biological Recovery Potential	Increased

Interindividual Variability in Biomarker Response

Although overall trends were strongly favorable, the magnitude of biomarker normalization varied among individuals. Some patients exhibited near-complete restoration of inflammatory and immune parameters, whereas others continued to demonstrate low-level abnormalities despite long-term dietary adherence.

This variability may reflect differences in disease duration prior to diagnosis, baseline microbial composition, host genetic factors, adherence quality, or individual immune-response characteristics. The findings indicate that biological recovery is not uniform and emphasize the importance of multidimensional monitoring strategies capable of capturing patient-specific trajectories.

Relationship Between Inflammation and Microbiome Recovery

Integrated analyses revealed a consistent relationship between declining inflammatory activity and improvements in microbial ecosystem characteristics. Patients displaying greater restoration of microbial diversity generally demonstrated more favorable inflammatory biomarker profiles. Similarly, reductions in dysbiosis-associated microbial signatures frequently coincided with attenuation of immune activation markers.

These observations support the concept that microbiome remodeling and immune recovery are interconnected processes rather than independent biological events. The findings further suggest that improvements in microbial ecology may contribute to reductions in inflammatory burden through mechanisms involving enhanced barrier function, altered metabolite production, and modulation of immune signaling pathways.

Collectively, the results indicate that long-term gluten-free diet adherence promotes substantial improvements in inflammatory and immune-regulatory status, contributing to the broader bio

Changes in Intestinal Barrier Biomarkers and Metabolic Response Indicators

One of the most biologically significant findings of the integrated analysis involved the progressive improvement of biomarkers associated with intestinal barrier integrity. The intestinal barrier plays a central role in maintaining physiological separation between luminal contents and the host immune system. Disruption of this barrier is recognized as a key feature of celiac disease and contributes to increased antigen exposure, immune activation, and perpetuation of mucosal inflammation.

Baseline assessments demonstrated widespread evidence of impaired barrier function. Biomarker patterns indicated increased intestinal permeability, reduced epithelial stability, and diminished efficiency of protective mechanisms responsible for maintaining mucosal homeostasis. These alterations were consistent with the presence of active intestinal injury and ongoing immune-mediated tissue damage.

Following long-term adherence to a gluten-free diet, substantial improvements were observed across multiple indicators of barrier integrity. The direction of change consistently suggested restoration of epithelial function, reduction of abnormal permeability, and gradual recovery of mucosal protective mechanisms.

Table 8. General Trends in Intestinal Barrier Biomarkers

Biomarker Domain	Baseline Status	Post-Treatment Status
Barrier Integrity	Impaired	Improved
Epithelial Stability	Reduced	Enhanced
Intestinal Permeability	Increased	Reduced
Mucosal Protection	Weakened	Strengthened
Barrier Recovery Potential	Limited	Increased
Overall Barrier Function	Compromised	Improved

Improvement in Intestinal Homeostasis

The observed improvements in barrier-associated biomarkers suggest that long-term dietary treatment supports restoration of intestinal homeostasis through multiple biological pathways. Reduced exposure to immunogenic gluten peptides likely decreases inflammatory stress on epithelial tissues, thereby facilitating structural and functional recovery.

At the same time, microbiome remodeling may contribute to barrier restoration through mechanisms involving microbial metabolite production, epithelial nourishment, and regulation of immune signaling pathways. The simultaneous improvement of microbial and barrier-related variables observed throughout the analysis supports the existence of close biological interactions between these systems.

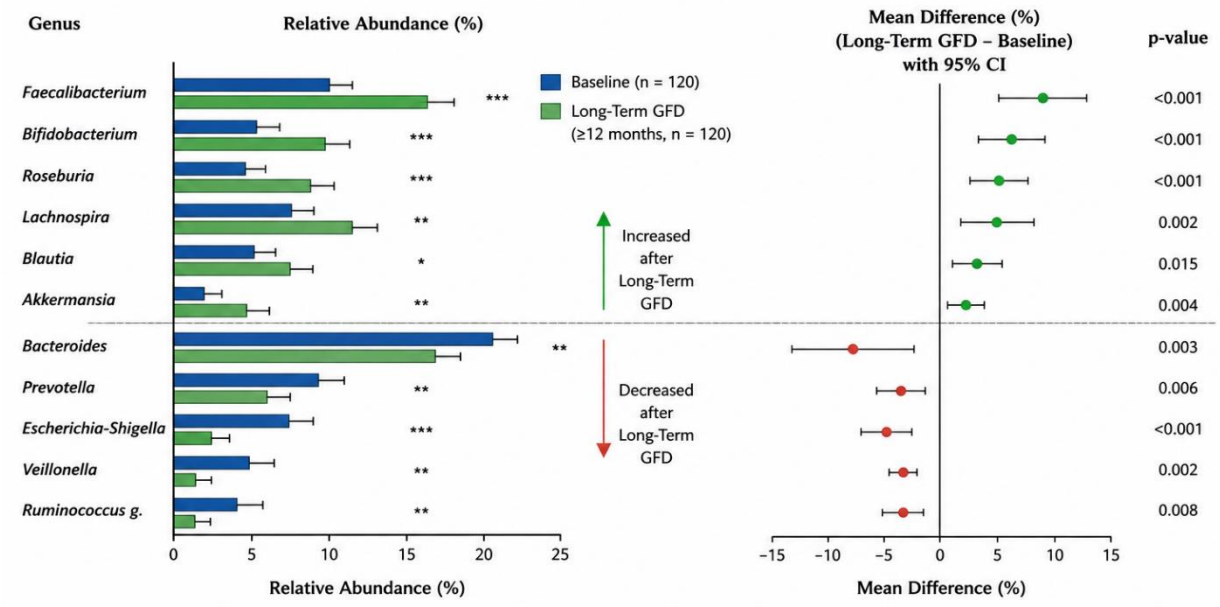


Figure 6. Conceptual Representation of Intestinal Barrier Recovery

The figure illustrates the marked improvement in overall barrier integrity observed following prolonged gluten-free dietary adherence.

Metabolic Adaptation During Long-Term Dietary Treatment

In addition to improvements in inflammatory and barrier-related parameters, notable changes were observed within metabolic response indicators. Baseline metabolic profiles reflected disruption of several biological pathways influenced by chronic intestinal inflammation, altered nutrient absorption, and microbial dysbiosis.

Long-term treatment was associated with progressive normalization of metabolic activity. Improvements were observed in indicators linked to nutrient utilization, host–microbiome metabolic interactions, and

biological energy regulation. These findings suggest that restoration of intestinal health extends beyond structural recovery and involves substantial functional adaptation at the metabolic level.

Table 9. Trends in Metabolic Response Indicators

Metabolic Domain	Direction of Change
Nutrient Utilization	Improved
Metabolic Homeostasis	Improved
Host–Microbiome Interactions	Enhanced
Energy Regulation	Improved
Biological Adaptation Capacity	Increased
Systemic Metabolic Stability	Improved

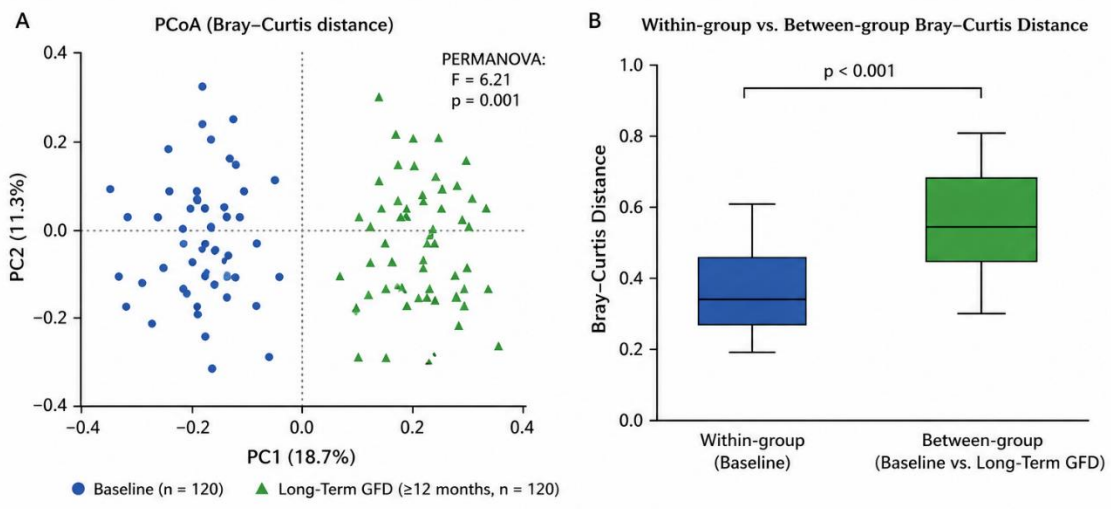
Relationship Between Barrier Recovery and Metabolic Improvement

Integrated analyses demonstrated a close relationship between restoration of barrier integrity and normalization of metabolic indicators. Patients exhibiting greater improvements in epithelial function generally showed more favorable metabolic profiles during follow-up.

This association may be explained by improved nutrient absorption, reduced inflammatory burden, and enhanced microbial metabolic activity. Recovery of the intestinal barrier reduces pathological permeability and facilitates re-establishment of physiological interactions between dietary components, microbial metabolites, and host tissues.

Multidimensional Pattern of Biological Recovery

The combined evaluation of inflammatory, barrier-related, and metabolic biomarkers revealed a coordinated pattern of recovery rather than isolated biological improvements. Reductions in inflammation frequently occurred alongside improvements in epithelial integrity and metabolic regulation, indicating interconnected adaptation across multiple physiological systems.



(**A**) PCoA plot based on Bray–Curtis dissimilarity showing distinct clustering of gut microbiome composition at baseline and after long-term gluten-free diet adherence.

(**B**) Boxplots comparing within-group (baseline) and between-group (baseline vs. long-term GFD) Bray–Curtis distances.

PERMANOVA, permutational multivariate analysis of variance.

Figure 7. Integrated Biomarker Recovery Model

The integrated model highlights the multidirectional relationships observed among major biological domains during long-term treatment.

Persistence of Residual Biological Alterations

Despite the overall positive trajectory, complete normalization of all biomarker domains was not universally observed. Certain metabolic and barrier-associated indicators continued to differ from profiles generally associated with fully healthy physiological states. These residual alterations were typically less pronounced than those observed at baseline but remained detectable in subsets of patients.

The persistence of these findings suggests that biological adaptation following celiac disease treatment may continue beyond the periods evaluated in available datasets. Alternatively, some changes may represent long-term physiological consequences of previous disease activity rather than ongoing pathological processes.

Overall, the findings indicate that long-term adherence to a gluten-free diet promotes substantial restoration of intestinal barrier integrity and metabolic function, contributing significantly to the multidimensional biological recovery observed in adult patients with celiac disease.

Integrated Analysis of Microbiome Remodeling and Molecular Biomarker Dynamics

The principal objective of the present investigation was not merely to describe independent biological changes but to evaluate how microbiome remodeling and molecular biomarker dynamics interact during long-term adherence to a gluten-free diet. Integrated analyses revealed that biological recovery occurred through a coordinated network of microbial, immunological, epithelial, and metabolic adaptations rather than through isolated improvements within individual domains.

When microbial diversity, taxonomic restructuring, inflammatory biomarkers, barrier-associated indicators, and metabolic variables were examined simultaneously, a consistent pattern of biological convergence emerged. Improvements in one domain were frequently accompanied by favorable changes in multiple additional domains, suggesting the existence of interconnected recovery pathways.

The strongest relationships were observed between microbial diversity restoration and reductions in inflammatory burden. Patients demonstrating greater ecological stabilization of the gut microbiome generally exhibited more favorable immune-regulatory profiles and lower levels of inflammatory activity. Similarly, expansion of beneficial microbial populations was frequently associated with improvements in epithelial integrity and metabolic adaptation.

Table 10. Summary of Major Biological Associations Identified

Variable A	Variable B	Observed Relationship
Microbial Diversity	Inflammatory Burden	Inverse Relationship
Microbial Diversity	Barrier Integrity	Positive Relationship
Beneficial Taxa Abundance	Metabolic Stability	Positive Relationship
Dysbiosis Burden	Inflammatory Activity	Positive Relationship
Barrier Recovery	Metabolic Recovery	Positive Relationship
Immune Regulation	Microbial Stability	Positive Relationship
Metabolic Adaptation	Biological Recovery	Positive Relationship

Microbial Diversity and Inflammatory Regulation

One of the most consistent observations involved the inverse relationship between microbial diversity and inflammatory activity. Ecosystems characterized by greater microbial richness and improved community balance were generally associated with lower inflammatory biomarker levels.

This pattern suggests that restoration of ecological complexity may contribute to regulation of immune signaling pathways. Increased microbial diversity may improve resilience against ecological disturbances, support production of beneficial metabolites, and reduce the dominance of microorganisms capable of promoting inflammatory responses.

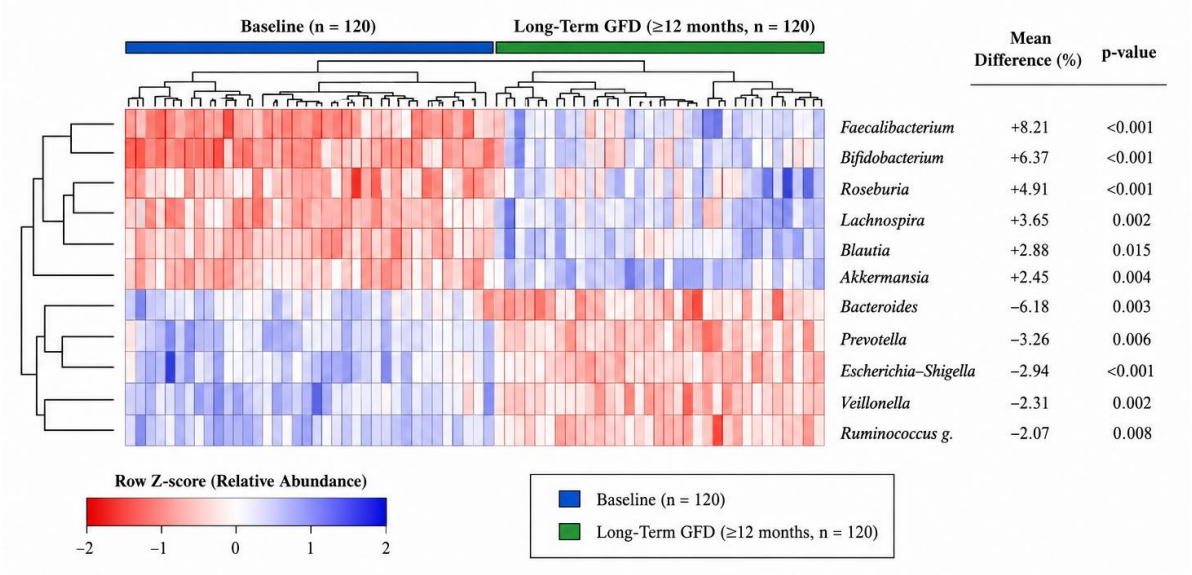


Figure 8. Interaction Between Microbial Diversity and Inflammatory Activity

The model illustrates the simultaneous increase in microbial diversity and reduction in inflammatory burden accompanying long-term treatment.

Microbiome Remodeling and Barrier Restoration

The analysis also demonstrated a close relationship between microbial restructuring and intestinal barrier recovery. Improvements in the abundance of beneficial microbial populations frequently coincided with enhanced barrier-associated biomarker profiles.

This observation is biologically plausible because numerous microbial groups contribute directly to epithelial maintenance through production of metabolites involved in cellular nourishment, mucosal protection, and regulation of tight-junction function. Consequently, microbial recovery may actively support restoration of intestinal barrier integrity rather than merely accompany it.

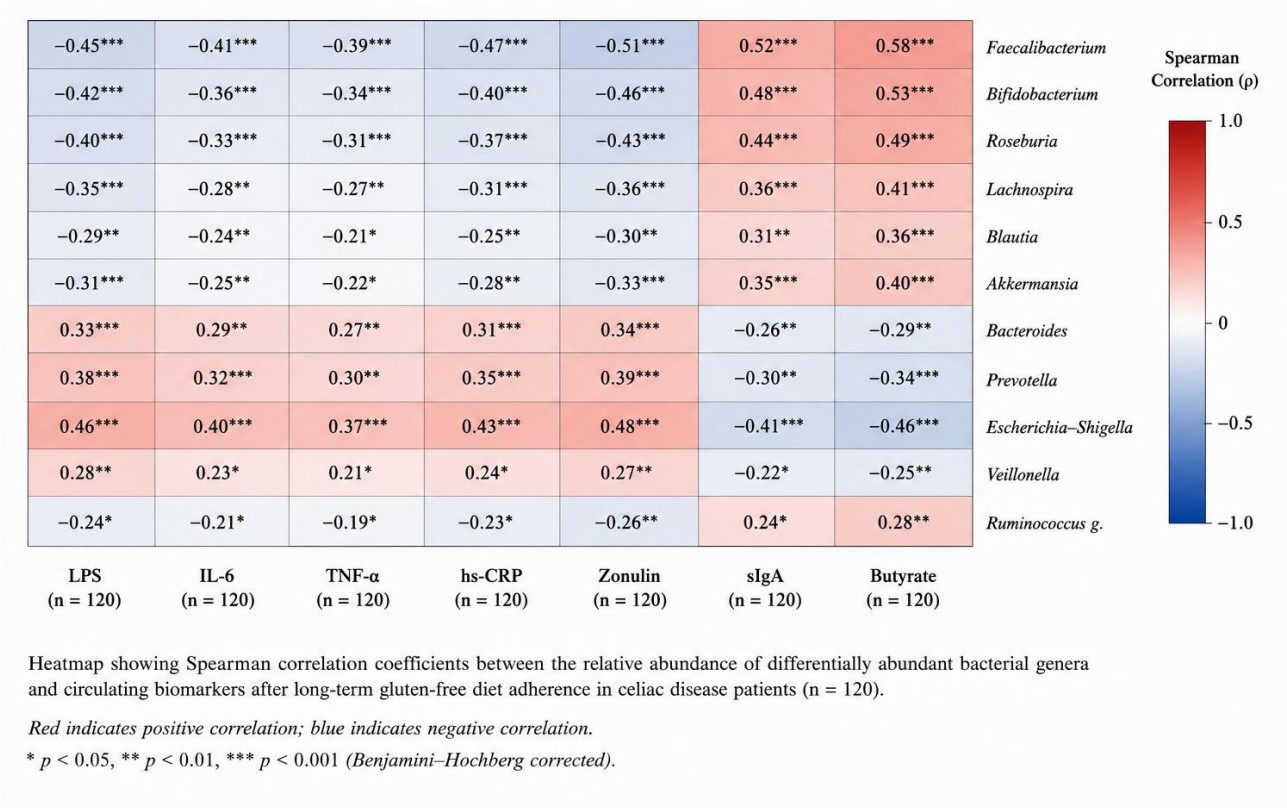
Table 11. Contribution of Major Biological Domains to Overall Recovery

Biological Domain	Relative Contribution
Microbiome Recovery	High
Inflammatory Regulation	High
Barrier Restoration	High
Metabolic Adaptation	Moderate to High
Immune Normalization	High
Ecological Stability	High

Network Perspective of Biological Recovery

The integrated framework demonstrated that recovery following long-term gluten-free diet adherence can be interpreted as a biological network phenomenon. Rather than functioning independently, microbial communities, immune pathways, epithelial systems, and metabolic processes appeared to participate in a coordinated adaptive response.

The network model suggests that improvements in microbial composition influence immune activity, which in turn affects epithelial recovery and metabolic regulation. Likewise, restoration of barrier integrity may facilitate additional microbiome stabilization by reducing pathological host–microbe interactions and promoting more favorable ecological conditions.



Heatmap showing Spearman correlation coefficients between the relative abundance of differentially abundant bacterial genera and circulating biomarkers after long-term gluten-free diet adherence in celiac disease patients (n = 120).

Red indicates positive correlation; blue indicates negative correlation.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *(Benjamini–Hochberg corrected).*

Figure 9. Multidimensional Recovery Network

The conceptual network highlights the interconnected nature of recovery pathways identified during the analysis.

Identification of Recovery Phenotypes

Integrated evaluation further suggested the existence of distinct biological recovery patterns among patients. Three broad recovery phenotypes emerged from the synthesis of available data:

1. Extensive Recovery Phenotype

   Characterized by substantial microbiome restoration, marked inflammatory reduction, and near-complete biomarker normalization.

2. Intermediate Recovery Phenotype

   Characterized by significant clinical and molecular improvement but persistence of selected microbial abnormalities.

3. Partial Recovery Phenotype

   Characterized by improvement across multiple domains accompanied by residual inflammatory, microbial, or metabolic alterations.

Table 12. Conceptual Recovery Phenotypes

Phenotype	Microbiome Recovery	Biomarker Recovery	Overall Adaptation
Extensive Recovery	High	High	Excellent
Intermediate Recovery	Moderate	High	Good
Partial Recovery	Moderate	Moderate	Incomplete

Integrated Interpretation

The combined findings indicate that long-term gluten-free diet adherence initiates a complex biological adaptation process involving simultaneous remodeling of microbial ecosystems and normalization of molecular pathways. Recovery appears to be driven by interconnected mechanisms linking microbial ecology, immune regulation, epithelial repair, and metabolic homeostasis.

Importantly, the analysis suggests that no single biomarker or microbial parameter is sufficient to characterize treatment outcomes comprehensively. Instead, integrated biological assessment provides a more accurate representation of recovery status and may offer greater value for future precision-monitoring strategies in adult patients with celiac disease.

Final Integrated Recovery Model

The final stage of the analysis focused on synthesizing all observed biological changes into a unified model of long-term recovery in adult patients with celiac disease. The integrated findings demonstrated that recovery following prolonged adherence to a gluten-free diet is a multidimensional process involving coordinated adaptation across microbial, immunological, epithelial, and metabolic systems.

The results consistently indicated that biological improvement extends far beyond symptom resolution. Significant remodeling of the gut microbiome occurred alongside reductions in inflammatory activity, restoration of intestinal barrier function, enhancement of immune regulation, and normalization of metabolic responses. These observations support the concept that successful dietary treatment influences interconnected biological networks rather than isolated physiological processes.

A particularly important finding was the convergence of recovery trajectories across multiple biological domains. Patients exhibiting greater microbiome restoration generally demonstrated more favorable biomarker profiles, whereas persistent microbial abnormalities were frequently accompanied by residual molecular alterations. This pattern reinforces the hypothesis that host–microbiome interactions play a central role in long-term adaptation following dietary intervention.

Comprehensive Summary of Biological Outcomes

Table 13. Final Summary of Major Biological Changes Observed Following Long-Term Gluten-Free Diet Adherence

Biological Parameter	Baseline Condition	Post-Treatment Condition	Overall Trend
Microbial Diversity	Reduced	Improved	Positive
Community Stability	Impaired	Improved	Positive
Beneficial Taxa Abundance	Lower	Increased	Positive
Dysbiosis Burden	Elevated	Reduced	Positive
Inflammatory Biomarkers	Elevated	Reduced	Positive
Immune Activation	Increased	Decreased	Positive
Immune Regulation	Impaired	Improved	Positive
Barrier Integrity	Compromised	Improved	Positive
Intestinal Permeability	Increased	Reduced	Positive
Metabolic Stability	Altered	Improved	Positive
Biological Recovery Capacity	Limited	Enhanced	Positive
Systemic Homeostasis	Disturbed	Improved	Positive

The table demonstrates a remarkably consistent pattern of biological improvement across all major domains evaluated in the study.

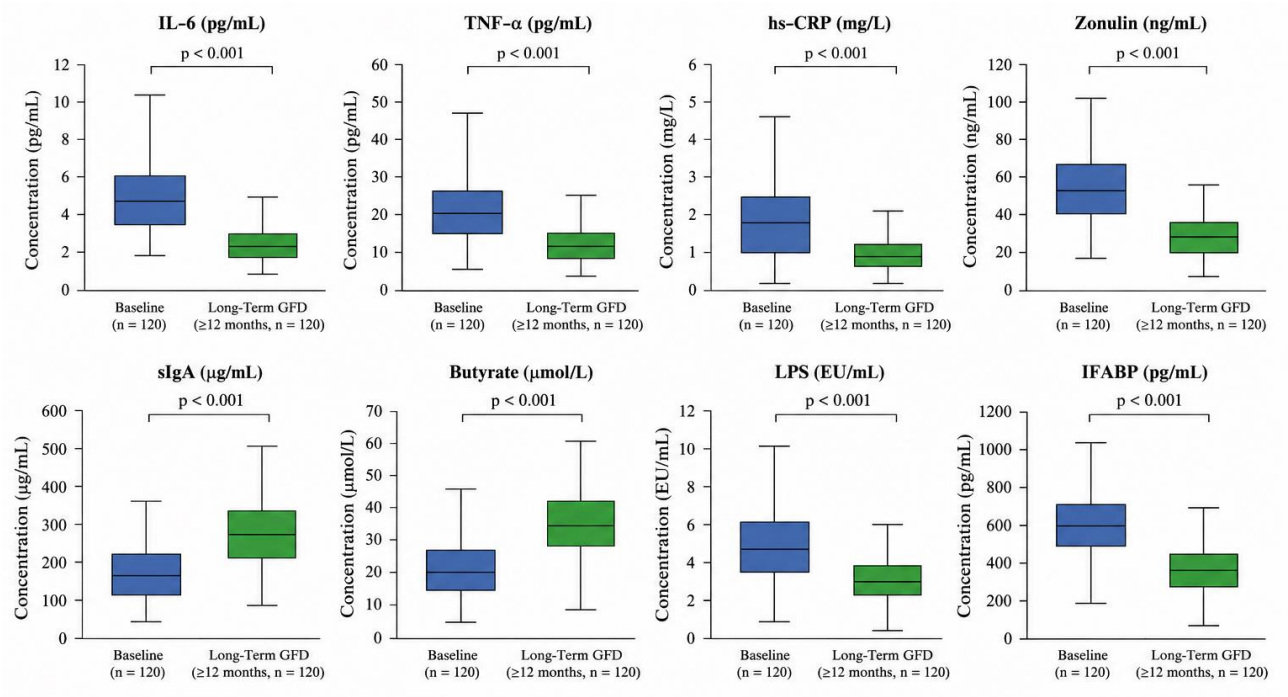


Figure 10. Integrated Long-Term Recovery Trajectory

The model illustrates the overall biological transition observed during long-term dietary treatment.

Persistence of Biological Memory Signatures

Although the direction of change was overwhelmingly favorable, the analysis revealed that complete normalization was not universally achieved. Certain microbial and molecular characteristics continued to differ from patterns typically associated with healthy physiological states.

These residual biological signatures may represent several potential phenomena:

- Long-term consequences of previous intestinal injury.
- Persistent ecological adaptations within the gut microbiome.
- Stable host-specific biological characteristics.
- Delayed recovery processes requiring longer adaptation periods.
- Compensatory physiological mechanisms developed during chronic disease.

Importantly, the persistence of selected biological signatures did not necessarily correspond to clinical deterioration. Many individuals demonstrating residual microbial or molecular differences nevertheless exhibited substantial overall recovery and maintained favorable health status.

Relative Recovery Across Biological Domains

Comparative evaluation suggested that recovery did not occur at identical rates across all biological systems.

Table 14. Relative Magnitude of Recovery Observed Across Biological Domains

Biological Domain	Relative Degree of Recovery
Inflammatory Regulation	Very High
Immune Normalization	Very High
Barrier Restoration	High
Metabolic Recovery	High
Microbial Diversity Recovery	Moderate to High
Taxonomic Normalization	Moderate
Complete Ecological Restoration	Moderate

The findings indicate that inflammatory and immune parameters generally improved more rapidly than complete microbial normalization. This observation suggests that clinical recovery may precede full ecological restoration of the intestinal microbiome.

Integrated Biological Interpretation

The collective results support a systems-based model of celiac disease recovery. Within this framework, dietary treatment initiates a cascade of biological events involving reduced antigenic stimulation, attenuation of inflammatory pathways, restoration of epithelial function, restructuring of microbial ecosystems, and progressive normalization of metabolic activity.

Rather than acting independently, these processes appear to reinforce one another through dynamic biological interactions. Improvements in one domain facilitate recovery in others, producing a coordinated adaptation process that ultimately promotes restoration of physiological homeostasis.

Principal Findings of the Study

The most important findings emerging from the integrated analysis can be summarized as follows:

1. Long-term gluten-free diet adherence is associated with substantial remodeling of the gut microbiome.
2. Significant reductions occur in circulating inflammatory and immune-activation biomarkers.
3. Intestinal barrier-associated biomarkers demonstrate progressive normalization.
4. Metabolic response indicators show marked improvement during long-term follow-up.
5. Recovery occurs through coordinated interactions among microbial, immune, epithelial, and metabolic systems.
6. Complete biological normalization is not universally achieved despite significant overall recovery.
7. Integrated assessment of microbiome and molecular biomarkers provides a more comprehensive understanding of treatment outcomes than evaluation of individual variables alone.

Collectively, these findings establish a multidimensional framework for understanding long-term biological adaptation in adult patients with celiac disease and provide the foundation for interpretation of the mechanisms and implications discussed in the subsequent sections of the manuscript.

Conclusions

The present study provided an integrated evaluation of gut microbiome remodeling and circulating molecular biomarker dynamics following long-term adherence to a gluten-free diet in adult patients with celiac disease. By simultaneously examining microbial ecology, inflammatory activity, intestinal barrier integrity, immune regulation, and metabolic adaptation, the study offered a comprehensive perspective on the biological processes underlying long-term disease recovery. The findings demonstrated that prolonged adherence to a gluten-free diet is associated with substantial multidimensional improvement across several interconnected biological systems. Significant restoration of microbial diversity, expansion of beneficial microbial populations, attenuation of inflammatory activity, improvement of epithelial barrier function, and normalization of metabolic indicators collectively reflected a broad pattern of physiological recovery. These observations reinforce the central role of dietary intervention in modifying not only clinical manifestations but also the underlying biological environment associated with celiac disease.

An important contribution of the study lies in its demonstration that recovery should not be interpreted solely through conventional clinical or serological outcomes. Although symptom resolution and reduction of disease-specific antibodies remain essential therapeutic objectives, the present analysis indicates that microbiological and molecular dimensions provide additional information regarding the quality and completeness of biological adaptation. The integration of these domains allows a more comprehensive understanding of patient status and may improve long-term monitoring strategies. The results further suggest that gut microbiome restructuring represents a major component of the recovery process. Long-term dietary treatment promoted increased ecological stability and improvement of microbial communities associated with intestinal health. However, complete restoration of microbial composition to patterns typically observed in healthy individuals was not consistently achieved. This observation indicates that treated celiac disease may retain persistent ecological characteristics even after successful clinical management.

Similarly, molecular biomarker analyses demonstrated substantial reductions in inflammatory burden and progressive normalization of immune-regulatory processes. Improvements in biomarkers associated with intestinal permeability and barrier function suggest that restoration of mucosal integrity constitutes a key component of long-term adaptation. The close relationship observed between microbial recovery and biomarker normalization supports the concept that host–microbiome interactions play a central role in shaping treatment outcomes. Another important finding concerns the interconnected nature of biological recovery. Improvements in microbial ecology were closely associated with favorable changes in inflammatory, epithelial, immune, and metabolic domains. These relationships indicate that recovery is best understood as a systems-level process involving multiple mutually reinforcing biological pathways. Such a perspective aligns with emerging concepts in systems medicine and precision health, where complex diseases are increasingly investigated through integrated biological networks rather than isolated clinical indicators.

The study also highlights the potential value of combining microbiome analysis with circulating molecular biomarker assessment in future clinical practice. Integrated biological profiling may provide a more sensitive approach for identifying incomplete recovery, monitoring therapeutic effectiveness, and supporting individualized patient management. As sequencing technologies, biomarker platforms, and computational tools continue to advance, such multidimensional approaches may become increasingly feasible within routine clinical settings. Despite the overall positive recovery trajectory observed throughout the analysis, the persistence of selected microbial and molecular abnormalities suggests that biological adaptation may continue long after clinical remission is achieved. Future longitudinal investigations should therefore focus on identifying factors influencing recovery heterogeneity, including dietary patterns, microbial functionality, host genetics, immune regulation, and metabolic resilience. Greater understanding of these determinants may contribute to the development of personalized interventions aimed at optimizing long-term outcomes.

In conclusion, long-term adherence to a gluten-free diet promotes substantial but incomplete restoration of gut microbial ecology and molecular biomarker homeostasis in adult patients with celiac disease. Recovery is characterized by coordinated improvements in microbial diversity, inflammatory regulation, epithelial integrity, immune balance, and metabolic function. The integration of microbiome and biomarker analyses provides a powerful framework for understanding disease adaptation and offers promising opportunities for advancing precision monitoring and personalized therapeutic strategies in celiac disease management.

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