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Review Article

The Effects of Prebiotic Dietary Fibers, Probiotics, and Synbiotics on Gut Permeability and Immunity: A Systematic Review

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What's Known

- The gastrointestinal tract is vital for immunity and disease prevention, enabling nutrient absorption while acting as a barrier against pathogens.
- Gut microbiota, dietary fibers, probiotics, and synbiotics can affect gut barrier integrity and enhance immune function.

What's New

- Prebiotic whole foods or food enriched with prebiotics, probiotics, and synbiotics improve the gut barrier by downregulating zonulin production, modulating inflammation that is involved in the pathophysiology of diseases.
- Prebiotic supplementation increases *Bifidobacteria* abundance and short-chain fatty acids (SCFAs) levels, supporting gut health.

Abstract

Background: Modulation of intestinal barrier, which function through zonulin pathway downregulation, represents a promising therapeutic strategy for chronic diseases. This systematic review aimed to evaluate the effects of prebiotic dietary fibers, probiotics, and synbiotics on intestinal permeability and immunity.

Methods: A systematic literature search of the EMBASE, PubMed, Web of Science, and Scopus electronic databases was conducted from database inception up to May 2024, supplemented by manual reference list searches. Included studies met the following criteria: (a) English language publications; (b) clinical trials; (c) investigated each factor of serum or fecal zonulin levels, serum or fecal calprotectin, glucagon-likepeptide-2 (GLP-2), short chain fatty acids (SCFAs), long chain fatty acids (LCFAs), fecal bile acid (BA), LPS-binding protein (LBP), lipopolysaccharide (LPS), intestinal microbiota composition, or inflammatory factors such as interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP); (d) supplemented prebiotic dietary fibers, probiotics, or symbiotics. Studies were excluded if they contained insufficient data or involved supplementation alongside other interventions. The study quality and risk of bias were assessed using Jadad's Score.

Results: A total of 36 studies were included in this review. Of these, 14 articles (n=580 participants) evaluated the effect of dietary prebiotics, 18 articles (n=1502 participants) evaluated the effect of probiotics, and six articles (n=517 participants) examined the effect of synbiotics on intestinal health and immunity markers. According to the evidence presented in this study, prebiotic whole foods or food enriched with prebiotics, probiotics, and synbiotics might have favorable effects on the serum levels of zonulin as a measure of intestinal permeability. The effects on GLP-2, gut microbiota, and their metabolites (e.g., LCFAs/SCFAs and BA) were contradictory and inconclusive. Some studies indicated increased levels of *Bifidobacteria* and SCFA with prebiotic supplementation or prebiotics-enriched food products. Fecal calprotectin (as an important marker of the local gut inflammation), tumor necrosis factor-α (TNF-α), and hs-CRP were unaffected in most studies.

Conclusion: The lack of consistent replication across studies made it difficult to draw definitive conclusions about the effects of prebiotics, probiotics, and synbiotics on gut-related health and immunity. Therefore, further evidence is required before definitive recommendations can be established.

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Introduction

Zonulin and Intestinal Permeability

The gastrointestinal (GI) tract plays an important role in the body's immunity and disease prevention by facilitating nutrient absorption while maintaining a barrier against pathogen entry.[1] In fact, the intestinal epithelial barrier, through its intercellular tight junctions (TJs), regulates the paracellular passage of ions, molecules, and cells while maintaining the balance between tolerance and immunity to non-self-antigens.[2]

Human zonulin, a ≈47-kDa protein, enhances the intestinal permeability in the small intestine and contributes to intestinal innate immunity.[2-4]

Circulating zonulin in the serum is considered the only physiological marker of intestinal permeability, reversibly regulating permeability through modulation of the intercellular TJs.[3, 5] Human studies using lactulose/mannitol tests validated serum zonulin levels, demonstrating a strong correlation with lactulose/mannitol ratios as an indicator of intestinal permeability.[6] The lactulose/mannitol (La/Ma) test is currently employed to assess intestinal permeability in various gastrointestinal diseases and malnutrition. This test involves orally administering two sugar probes, followed by a 5-hour urinary excretion measurement.[7] High serum levels of zonulin are observed in several autoimmune diseases (e.g., celiac disease and type 1 diabetes), and non-autoimmune diseases (e.g., in type 2 diabetes and obesity).

Zonulin concentration correlates with glucose levels, dyslipidemia, inflammation, and insulin resistance.[8] Through binding to protease-activated receptor 2 (PAR2), zonulin activates protein kinase C alpha (PKC-α), which catalyzes the phosphorylation of target proteins, such as zonula occludens (ZO-1) and myosin 1c, and induces actin polymerization. This process leads to actin-microfilament rearrangement and subsequent displacement of tight junction proteins. The junctional complex reverts to its configuration upon dissociation of zonulin from its receptor.[9] Excessive zonulin secretion and impaired absorption system function contribute to the development of certain inflammatory and chronic diseases. Increased intestinal permeability, "leaky gut," enables harmful substances such as toxins and bacteria to enter the circulation, triggering immune responses that lead to chronic inflammation. This mechanism could be associated with gastrointestinal disorders, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), celiac disease, and even systemic conditions such as rheumatoid arthritis and lupus.[10] The intestinal permeability status serves as an important immune system regulator. The gut plays a crucial role in immune function, and changes in permeability can signal the immune system to respond to potential threats to protect the body, pathogens, and inflammatory compound penetration. However, excessive permeability may trigger immune hyperactivation, potentially contributing to autoimmune pathogenesis. Optimal gut barrier integrity is therefore essential for balanced immune function and disease prevention.[11-13]

Microbiota and the Intestinal Permeability

Recent research has highlighted the intestinal microbiota's role in disease etiology through compositional changes. These symbiotic microorganisms influence intestinal permeability while forming a mechanical barrier on the intestinal mucosa that protects against pathogens. Disruptions in microbial balance favoring opportunistic species may result from lifestyle factors, including antibiotic use, high-fat/low-fiber diets, and weight gain.[14] This change in the balance of the intestinal microbial population and the production of active metabolites influences zonulin secretion, potentially compromising intestinal barrier function. This process can enhance allergens and bacterial toxin penetration, thereby promoting and contributing to the development of allergic, autoimmune, and chronic diseases.[15-18]

Potential Therapeutic Strategies

Therapeutic strategies targeting zonulin pathway downregulation offer promising potential for modulating intestinal barrier function in chronic disease management. For instance, larazotide acetate, a zonulin release inhibitor, demonstrated efficacy in managing autoimmune conditions such as celiac disease.[19] However, additional large-scale clinical trials are required to confirm its safety and therapeutic effectiveness.

Along with routine treatments, prebiotic dietary fibers (e.g., inulin, starch, and fructooligosaccharides, probiotics (live microorganisms), and synbiotics (probiotic-prebiotic combinations) represent promising therapeutic nutrients that modulate intestinal permeability through tight junction protein regulation.[20-22]

Studies indicated that probiotic administration can shift the microbial balance toward beneficial species (e.g., *bifidobacteria and lactobacillus*), potentially reducing zonulin-activating compounds. This dual mechanism may enhance epithelial barrier integrity while attenuating inflammatory processes.[23, 24]

The sole available systematic review and meta-analysis examining probiotics and synbiotics' effects on serum zonulin levels reported favorable outcomes. However, these findings require cautious interpretation due to significant study heterogeneity.[25] Consequently, additional evidence and underlying mechanisms are required before establishing definitive recommendations. The present study included several more recent studies. Furthermore, the probiotics and synbiotics, prebiotic foods were investigated for their effects not only on zonulin levels, but also on other relevant markers, such as calprotectin that is released by neutrophils and monocytes during inflammatory responses, gut microbiota composition, and microbial metabolites involved in the intestinal permeability regulation.

This study aimed to review the therapeutic efficacy of prebiotic dietary fibers, probiotics, and synbiotics on intestinal permeability and immunity. The evaluation was performed by examining some key markers of intestinal permeability, such as serum levels of zonulin, glucagon-likepeptide-2 (GLP-2), and calprotectin. Additionally, we analyzed gut microbiota composition and related metabolites, such as short-chain fatty acids (SCFAs), long-chain fatty acids (LCFAs), fecal bile acids (BA), lipopolysaccharide (LPS), and LPS-binding protein (LBP). The review also assessed relevant inflammatory biomarkers across all age groups to comprehensively determine the effects of these interventions.

Materials and Methods

Search Strategy

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[26] The present study was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethical Code: IR-TBZMED.REC.1402.666).

A comprehensive systematic literature search was conducted using the EMBASE, PubMed, Web of Science, and Scopus electronic databases. c, a manual search of reference lists was performed. The search covered records from the inception of each database up to May 2024. The search strategy incorporated MeSH terms, title/abstract screening, publication type filters, and text terms to identify relevant randomized controlled trials (RCTs), using the following keywords:

"Dietary Fiber" or "Prebiotic" or "Inulin" or "Fructooligosaccharides" or "Starch" or "Chitin glucan" or "CG" or "Probiotics" or "Synbiotic" AND "Gut permeability" or "Zonulin" or "Glucagon-like peptide-2" or "GLP-2" or "Long- and short-chain fatty acids" or "LCFAs" or "SCFAs" or "Lipopolysaccharide binding protein" or "LBP" or "Lipopolysaccharide" or "LPS" or "Bile acids" or "BA", or "Gut microbiota" or "Firmicutes" or "Bacteroidetes" or "Bifidobacteria" or "Lactobacillus" or "High sensitivity C-reactive protein" or "hs-CRP" or "Cytokines" or "Interleukins" or "Calprotectin" AND "Intervention" or "Controlled trial" or "Randomized clinical trial" or "Randomized controlled trial" or "Trial" or "Clinical trial" or "Randomized" or "Random" or "Randomly" or "Placebo" or "RCT".

A detailed search strategy for each database is available in the supplementary materials (Supplementary). We employed broad inclusion criteria encompassing all keywords related to our primary objectives regarding the prebiotics, probiotics, and synbiotics' effect on intestinal permeability and immunity to reduce the risk of missing studies. This systematic review was registered in PROSPERO (code: CRD42023460121).

Eligibility Criteria

This systematic review included studies that met the following criteria: (a) English language publications; (b) clinical trials; (c) investigated at least one of the following biomarkers: serum or fecal zonulin levels, serum or fecal calprotectin, GLP-2, SCFAs/LCFAs, fecal BA, LBP, LPS, intestinal microbiota composition, and inflammatory markers, such as IL-6 and hs-CRP; (d) supplemented prebiotic dietary fibers, probiotics, or synbiotics. The studies with insufficient data, those involving supplementation with other nutrients, or those lacking complete reporting of primary outcomes were excluded. All included studies provided full data on the primary outcomes of interest.

Data Extraction and Quality Assessment of Previous Studies

Three authors independently screened article titles and abstracts based on the inclusion criteria. Studies that failed to meet the predefined criteria were excluded. The full text of the eligible papers was obtained and reviewed for further analysis.

The following information was extracted from eligible studies: first author's name, publication year, study location, the number of study participants, patient characteristics (i.e., age, sex), study design details (i.e., crossover or parallel design), quality assessment scores, intervention specifics (type, dosage, duration), and placebo information, reported side effects, and outcome measures; effects of prebiotic dietary fibers, probiotics and synbiotics on gut permeability and inflammatory markers. Any discrepancies

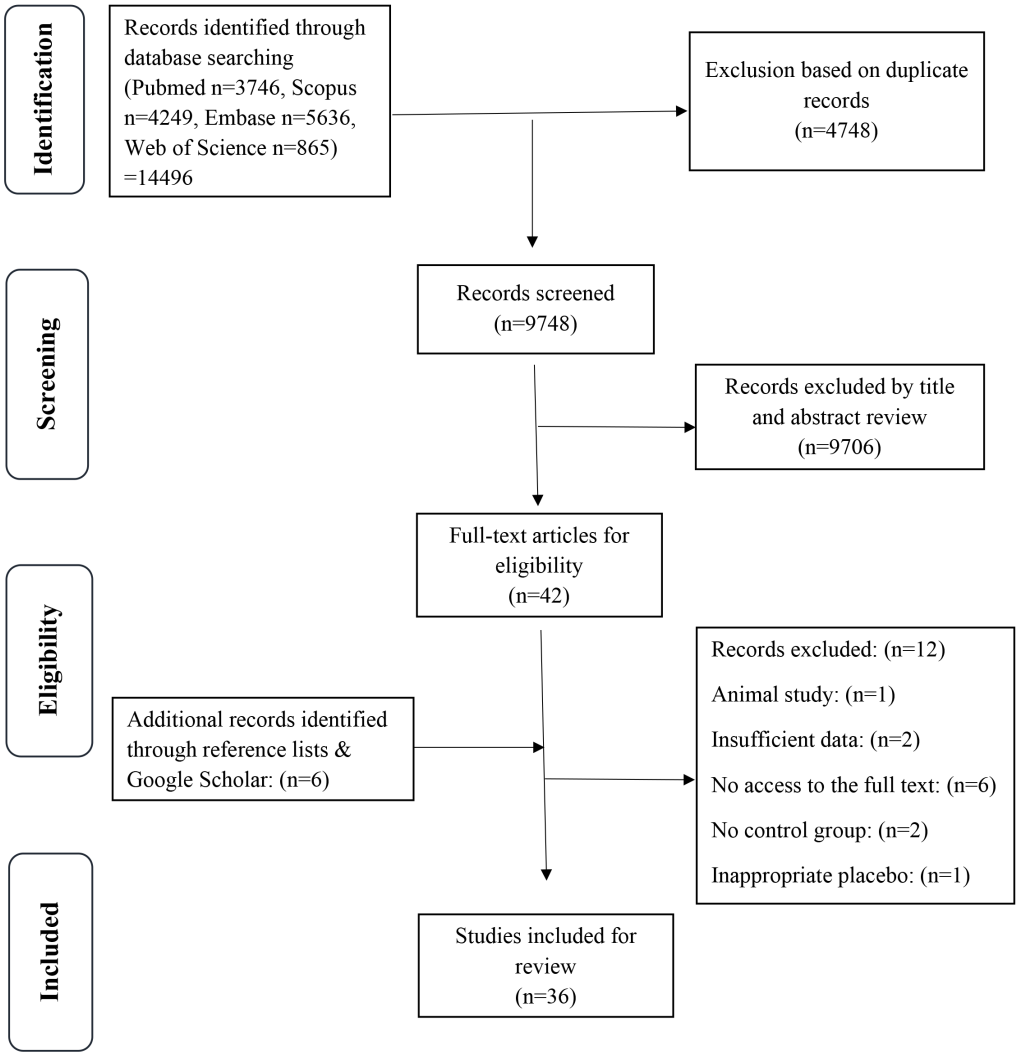

Figure 1: The flow diagram shows the study selection strategies according to the PRISMA guidelines.

regarding study eligibility were resolved through discussion and consensus among all reviewers.

The quality assessment of studies was performed using Jadad's Score, a validated tool that evaluates clinical trial methodology across three domains: (1) randomization procedures, (2) blinding implementation, and (3) reporting of participant withdrawals/dropouts. The scoring system ranged from 0 to 5 points, with trials scoring 3-5 considered methodologically rigorous and those scoring 0-2 representing lower-quality evidence. Any discrepancies regarding study quality assessment were resolved through consensus discussions among all reviewers.

Results

The PRISMA flow diagram summarizes the results of the study selection process for this study (figure 1). Our initial database search, including EMBASE, PubMed, Web of Science, and Scopus electronic databases, identified 14,496 articles. After removing 4,748 duplicates, 9,748 records were screened, and 9,706 articles that did not meet the inclusion criteria were further excluded. Of 42 full-text articles assessed, we excluded 12 studies based on the exclusion criteria.[27-37] Six additional records were identified through the reference lists and Google Scholar.[38-43] Following full eligibility assessment, 36 studies were included. Thirty-one out of 36 studies demonstrated low risk of bias (high quality), and five studies showed high risk of bias (low quality) (tables 1 and 2).

Summary of the Selected Studies

Totally 36 studies underwent qualitative synthesis. The included articles represented participants from 21 countries: the USA, France, Brazil, Iran, Poland, Italy, Belgium, Singapore, Denmark, China, India, South Korea, Austria, Finland, Turkey, the Netherlands, Slovenia, Spain, Pakistan, Malaysia, and the United Arab Emirates. Participants' ages ranged from 10 to 80 years.

Table 1: Jadad score calculation

Item	Score
Randomization present	0/1
An appropriate randomization was utilized.	0/1
Blinding present	0/1
An appropriate blinding method was utilized.	0/1
Appropriate long-term follow-up on all patients	0/1
Maximum possible score	5
Total Score 0-2: Low Quality	5 studies
Total Score 3-5: High Quality	31 studies

Table 2: The quality assessment of the studies

Authors, Year of publication	Randomization present	Appropriate randomization utilized	Blinding present	Appropriate blinding method utilized	Appropriate long-term follow-up on all patients	Quality score	Degree of Trial
Bloomer et al., (2020)[44]	1	0	1	0	1	3	High
Ranaivo et al., (2022)[45]	1	1	1	0	1	4	High
Ramos et al., (2018)[46]	1	1	1	1	1	5	High
Vaghef-Mehrabani et al., (2022)[47]	1	1	1	1	1	5	High
Krawczyk et al., (2018)[48]	1	1	1	0	0	3	High
Drabińska et al., (2020)[49]	1	0	1	0	1	3	High
Russo et al., (2012)[50]	1	0	1	0	0	2	Low
Czerwi´nska-Rogowska et al., (2022)[51]	1	0	0	0	0	1	Low
Riviere et al., (2022)[38]	1	1	0	0	1	3	High
Neyrinck et al., (2021)[52]	1	1	0	0	0	2	Low
Lee et al., (2023)[53]	1	1	1	0	1	4	High
Hiel et al., (2020)[43]	1	1	1	0	1	4	High
Vuholm et al., (2017)[39]	1	1	0	0	1	3	High
Kantah et al., (2017)[40]	1	1	0	0	0	2	Low
Townsend et al., (2018)[41]	1	1	1	1	1	5	High
Lamprecht et al., (2012)[22]	1	1	1	1	1	5	High
Liu et al., (2015)[54]	1	1	1	1	1	5	High
Stenman et al., (2016)[55]	1	1	1	1	1	5	High
de Roos et al., (2017)[56]	1	1	1	0	1	4	High
Liu et al., (2013)[57]	1	1	1	1	1	5	High
Mokkal et al., (2017)[58]	1	1	1	0	1	4	High
Horvath et al., (2016)[21]	1	1	1	1	1	5	High
Horvath et al., (2020)[59]	1	1	1	1	1	5	High
Ghavami et al., (2021)[60]	1	1	1	1	1	5	High
Çakir et al., (2017)[42]	0	0	0	0	1	1	Low
Wilms E. et al., (2016)[61]	1	1	1	1	1	5	High
Petelin et al., (2022)[62]	1	0	1	1	1	4	High
Stadlbauer et al., (2015)[63]	1	1	0	0	1	3	High
Roman et al., (2019)[64]	1	1	1	1	1	5	High
Axelrod et al., (2019)[65]	1	0	1	0	1	3	High
Horvath et al., (2020)[66]	1	0	1	0	1	3	High
Karim et al., (2022)[67]	1	1	1	1	1	5	High
Karim et al., (2022)[68]	1	1	1	1	1	5	High
Ayob et al., (2023)[69]	1	0	1	0	1	3	High
Qaisar et al., (2024)[70]	1	0	1	1	1	4	High
Lennon et al., (2024)[71]	1	1	1	0	0	3	High

Primary Outcomes of Interest

Of the 36 articles included, 14 studies (n=580 participants) evaluated the effect of dietary prebiotics (table 3),[38, 39, 43-53, 62] 18 studies (n=1,502 participants) evaluated the effect of probiotics (table 4),[21, 22, 40, 41, 54-58, 63-71] and six articles (n=517 participants) evaluated the effect of synbiotics (table 5)[40, 42, 55, 59-61] on intestinal health and immunity markers.

Table 3: Summary of the studies that investigated the effects of prebiotic dietary fibers on intestinal permeability and immunity

Authors, Year of publication	Location	Age (years)	Studied Population	Study Design	Intervention/ Dose/Duration	Gut effects	Effects on cytokines	Quality score/ degree of trials	Side effects
Bloomer et al., (2020)[44]	USA	20-65	75 healthy men and women	Double-blind, placebo-controlled, randomized trial	1. Prebiotic: AA at 2 or 4 g daily 2. AL at 2 or 4 g daily 3. Placebo (maltodextrin)/ 8 weeks	No effects on the levels of zonulin	No effects on the levels of IL-6, IL-10, IL-1β, and TNF-α	3/ High	No side effects
Ranaivo et al., (2022)[45]	France	44	15 subjects with cardiometabolic risk (9 men, 6 women)	Double-blind, placebo-controlled, randomized, crossover exploratory trial	1. Prebiotic: 4.5 g of CG 2. Placebo (maltodextrin)/ 3 weeks	A family belonging to the *Actinobacteria* Phylum decreased 3 Bacterial taxa: Erysipelotrichaceae UCG.003, Ruminococcaceae UCG.005, and Eubacterium ventriosum group increased. No effects on zonulin, LBP, BA, LCFAs, and SCFAs levels	No effects on the levels of hs-CRP and Calprotectin	4/High	No side effects
Ramos et al., (2018)[46]	Brazil	18-80	46 chronic kidney disease patients	Double-blind, placebo-controlled, randomized trial	1. Prebiotic: FOS, 12 g/day 2. Placebo (maltodextrin)	No effects on the levels of Zonulin and GLP-2	No effects on the levels of hs-CRP and IL-6	5/High	Abdominal discomfort (one participant)
Vaghef-Mehrabani et al., (2022)[47]	Iran	20-50	45 women with obesity and major depressive disorder (MDD)	Double-blind, placebo-controlled, randomized trial	1. Prebiotic: 10 g/d of inulin 2. Placebo (maltodextrin)/ 8 weeks	No effects on the levels of zonulin and LPS	No impact on the levels of TNF-α, IL-10, MCP-1, TLR-4, and hs-CRP	5/ High	Gastrointestinal complaints (five patients)
Krawczyk et al., (2018)[48]	Poland	48.03±13.13	32 (22 males and 10 females) individuals with NAFLD	randomized controlled intervention trial	1. Diet with 30 -35 g/day dietary fiber (3 portions of vegetables and two portions of fruit)/ 6 months	Zonulin levels decreased	-	3/High	Not reported
Drabińska et al., (2020)[49]	Poland	Not reported	30 children with celiac	A pilot, randomized, placebo-controlled nutritional intervention	1. GFD with prebiotic oligofructose-enriched inulin (10 g per day) 2. Placebo (maltodextrin)/ 12 weeks	Zonulin levels increased. No effects on the levels of GLP-2	No effects on the levels of Calprotectin	3/ High	Not reported
Russo et al., (2012)[50]	Italy	18.8±0.7	20 healthy males	Randomized double-blind crossover design	1. 11% Inulin-enriched pasta or control pasta diet: 100 g/d=11.0 and 1.4 g/d of fructans, respectively/ Two 5-week study periods with a washout period (8 weeks) in between and a 2-week run-in period	Zonulin levels decreased GLP-2 levels increased	-	2/Low	No adverse effects

Czerwi´nska-Rogowska et al., (2022)[51]	Poland	-	59 patients suffering ischemic stroke	Randomized clinical trial	1. The kitchen diet (n=32; 1.2 g fiber in 100 mL) 2. Nutrison Energy® (n=14; 0.02 g fiber in 100 mL) 3. Nutrison Diason Energy HP® (n=13; 1.8 g fiber in 100 mL)/7 days	Zonulin levels decreased. Propionic acid and butyric acid with a kitchen diet increased SCFAs levels and decreased with an industrial diet without fiber.	No effects on the levels of Calprotectin, but hs-CRP levels increased with the kitchen diet.	1/Low	Not reported
Riviere et al., (2022)[38]	USA	50±14	20 males and females with obesity	Controlled-randomized clinical trial	1. Immediate intervention (Frozen GLV) during the first 4 weeks 2. Delayed intervention (Frozen GLV during the last 4 weeks)/ 12-week trial	No effects on the levels of zonulin, LBP, and microbiota Change in serum zonulin was associated with a change in *Proteobacteria* in females and *Bifidobacterium* and *Bacteroidaceae* in males	No effects on the levels of TNF-α, IL-6, and hs-CRP	3/ High	Not reported
Neyrinck et al., (2021)[52]	Belgium	18-65	24 Obese patients	Single-blind, placebo-controlled trial	1. Prebiotic: 16 g/d native inulin 2. Placebo (maltodextrin), coupled with dietary advice to consume inulin-rich versus inulin-poor vegetables for 3 months, in addition to dietary caloric restriction	No effects on the levels of zonulin and SCFAs	Calprotectin levels decreased	2/Low	Not reported
Lee et al., (2023)[53]	Singapore	50-75	15 Healthy middle-aged and older men and women	Randomized controlled crossover trial	1. Control (C) 2. Prebiotic: 20% flour-substituted okara (AOK) 3. Prebiotic: 20% flour-substituted bio-valorized okara (RO) biscuits/ 3 weeks	SCFAs levels increased No effects on the levels of zonulin AOK decreased the levels of Clostridiales RO increased the levels of *Bifidobacteria*	-	4/High	Not reported
Hiel et al., (2020)[43]	Belgium	18 to 65	110 obese patients with at least one obesity-related metabolic disorder	A randomized, single-blinded, multicentric, placebo-controlled trial	1. Preboptic:16 g/d native inulin 2. Placebo (maltodextrin) coupled with dietary advice to consume inulin-rich versus-poor vegetables /3 months	Desulfovibrio and Clostridium levels decreased. *Bifidobacteria* levels increased	-	4/High	Flatulence and bloating, especially at the start of the intervention, which decreased with the duration of the treatment
Vuholm et al., (2017)[39]	Denmark	51.0±9.4	70 healthy adults	A single-blinded randomized controlled trial	Replace all cereal products in their habitual diet with: 1. WGW 2. WGR 3. RW	No effects on the levels of zonulin Fecal butyrate concentration increased by WGR and WGW.	-	3/High	Not reported

Petelin et al., (2022)[62]	Slovenia	NR	27 patients (19 women and 8 men) with metabolic syndrome	Randomized, double-blind comparative trial	Drink 200 mL of either 1. HA or 2. HI tea filter bags containing 1 g of dried plant material every evening, 2 hours after dinner; and a 2-week follow-up phase without any supplementation/4 weeks	Zonulin levels decreased by HI. HA had no effects on the levels of Zonulin. Some generations of *Firmicutes* decreased by both HI and HA. α-diversity decreased by both HI and HA. Proteobacteria decreased by both HI and HA.	IL-6 levels decreased by HI at week 4	4/High	NR

AA: Ambrotose advanced; AL: Ambrotose LIFE; IL-6: Interleukin 6; IL-10: Interleukin-10; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α; CG: Chitin-Glucan; hs-CRP: High sensitivity C-reactive protein; LBP: Lipopolysaccharide binding protein; BA: Bile acids; LCFA: Long-chain fatty acids; SCFA: Short-chain fatty acids; FOS: Fructooligosaccharide; GLP-2: Glucagon-Like Peptide-2; LPS: Lipopolysaccharide; MCP-1: Monocyte chemoattractant protein-1; TLR-4: Toll-like Receptor 4; GFD: Gluten-free diet; WGW: Whole-grain wheat; WGR: Whole-grain rye; RW: Refined wheat; GLV: Green leafy vegetable; HI: *Helichrysum italicum* (Roth) G. Don; HA: Helichrysum arenarium (L.) Moench; NAFLD: Nonalcoholic fatty liver disease; AOK: 20% Flour-substituted okara; RO: 20% flour-substituted bio-valorized okara

Table 4: Summary of the studies that investigated the effects of probiotics on intestinal permeability and immunity

Authors, Year of publication	Location	Age (years)	Studied population	Study design	Intervention/Dose/Duration	Gut effects (intestinal permeability)	Effects on cytokines	Quality score/ degree of trials	Side effects
Kantah et al., (2017)[40]	China, Italy, India, and South Korea	Between 38 and 62	120 individuals	A multicenter randomized study	1. A) given a symbiotic 10 mL t.i.d. for 5 months 2. B) given 1-tab t.i.d of P3T/J (a probiotic mixture) for 5 months 3. C) Given the symbiotic 10 mL t.i.d. for 1 month and then shifted to 1 tablet t.i.d of probiotic mixture for 4 months 4. Control: Prior study supplemented a marine PUFA extract	Zonulin levels decreased by treatment in the B and C groups. *Bacteroidetes/ Firmicutes* ratio normalized by B and C treatments.	-	2/Low	Not reported
Townsend et al., (2018)[41]	USA	20.1±1.5	25 Division I male baseball athletes	Double-blind, placebo-controlled, randomized study	1. Probiotics consisted of 1.2 billion CFU/ capsule 2. Placebo capsule consisted of maltodextrin/ 12 weeks	No effects on the levels of zonulin	TNF-α levels decreased. No effects on the levels of IL-10.	5/High	No adverse effect
Lamprecht et al., (2012)[22]	Austria	30-45	23 trained men	Randomized, double-blinded, placebo-controlled trial	1. Multi-species probiotics (10^{10} CFU/day), 2. OMNi-BiOTiC (n=11) 3. Placebo (n=12) /14 weeks	Zonulin levels decreased.	TNF-α levels decreased. No effects on the levels of IL-6.	5/High	Not reported

Liu et al., (2015)[54]	China	25 and 75 years	117 participants with colorectal liver metastases	a double-center and double-blind randomized clinical trial	1. Probiotic 2 g/day, at a total daily dose of 2.6×10^{14} CFU: Encapsulated admixture of three probiotic bacteria [composed of LP (CGMCC No.1258, cell count ≥10^{11} CFU/g), LA-11 (cell count ≥7.0×10^{10} CFU/g) and BL-88 (cell count ≥5.0×10^{10} CFU/g) every day 2. Placebo encapsulated maltodextrin/6 days preoperatively and 10 days postoperatively	Postoperative zonulin levels decreased	-	5/High	No side effects
Stenman et al., (2016)[55]	Finland	18-65 years old	224 participants with a BMI between 28.0-34.9 and a waist-to-hip ratio of≥0.88 for males and ≥0.83 for females.	Double-blind, randomized, placebo-controlled, multi-center clinical trial	1. Placebo, microcrystalline cellulose, 12 g/d 2. Litesse® Ultra polydextrose (LU), 12 g/d 3. *Bifidobacterium* animalis ssp. *lactis*. 420 (B420), 10^{10} CFU/d in microcrystalline cellulose, 12 g/d 4. LU+B420, 12 g+10^{10} CFU/d, 6 months	Zonulin levels decreased in the B420 and LU+B420 groups. No effects on the levels of LPS	No effects on the levels of hs-CRP, IL-6, and sCD4	5/High	At least one adverse event (Gastrointestinal symptoms) (133 were potentially product-related)
De Roos et al., (2017)[56]	USA	18-70	60 patients (56 women) with migraine	A randomized double-blinded placebo-controlled study	1. Multispecies probiotic (5×10^{9} colony-forming units) 2. Placebo (maize starch and maltodextrin powder) daily/12 weeks	No effects on the levels of zonulin	No effects on the levels of IL-6, IL-10, TNF-α and hs- CRP	4/High	Not reported
Liu et al., (2013)[57]	China	Between 25 and 75 years	150 patients with colorectal cancer who were scheduled to undergo a radical colectomy	A double-center and double-blind randomized clinical trial	1. Probiotics, 2 g/d, at a total daily dose of 2.6×10^{14} CFU. 2. Encapsulated maltodextrin daily/ 6 d preoperative and 10 d postoperative	Zonulin levels decreased	p38 MAPK signaling pathway decreased	5/High	Not reported
Mokkal et al., (2017)[58]	Finland	20-36	200 healthy overweight women	A randomized double-blind placebo-controlled clinical trial	1. One capsule Probiotics (a combination of two bacteria, *Bifidobacterium animalis subsp. Lactic* 420 and *Lactobacillus* rhamnosus HN001; it contained 10^{10} CFU of each in a capsule 2. LC-PUFA (consisted of 1.2 g of n-3 LC-PUFA (79.6% DHA and 9.7% EPA), two capsules consumed per day to give a total daily dose of 2.4 g 3. Probiotics and LC-PUFA 4. Placebo for each supplement (for the probiotics consisted of microcrystalline cellulose and for the n-3 LCPUFA, medium chain fatty acids [capric acid C8 54.6% and caprylic acid C10 40.3%])	No effects on levels of zonulin and LPS	-	4/High	Not reported

Horvath et al., (2016)[21]	Austria	50-64 years	80 patients with liver cirrhosis.	A randomized, double-blind, placebo-controlled study	1. A daily dose of a probiotic powder containing eight different bacterial strains (*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19 and *Lactococcus lactis* W58) 2. Placebo/6 months and were followed up for another 6 months	No effects on the levels of zonulin	No effects on the levels of Calprotectin	5/High	The side effects were mild, and most symptoms were gastrointestinal complaints.
Stadlbauer et al., (2015)[63]	Austria	40-62	28 subjects with metabolic syndrome	Randomized controlled pilot study	1. LcS group: Food supplementation with a milk drink containing LcS (3 bottles a day, 65 mL, containing LcS at a concentration of 10^8/mL, Yakult light1, Yakult Austria, Vienna, Austria) (n=13, LcS group) 2. No intervention (n=15, standard therapy group)/12 weeks	No effects on the levels of zonulin, BA, and *Bacteroidetes*/*Firmicutes* ratio Parabacteroides increased (at the level of the genes)	No effects on the levels of Calprotein	3/High	Not reported
Roman, E et al., (2019)[64]	Spain	60-70	35 patients with cirrhosis	Double-blind placebo-controlled randomized trial	1. Probiotic (probiotic group): 450 billion bacteria twice daily (a mixture of eight strains, namely, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* (*B. breve*) DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737, *Lactobacillus paracasei* (*L. paracasei*) DSM 24733) 2. Placebo (placebo group): contained maltose and silicon dioxide as inactive agents and was formulated as identical in appearance to the active agent/12 weeks	No effects on the levels of zonulin and Microbiota	hs-CRP and TNF-α levels decreased Neutrophil levels increased No effects on the levels of LBP	5/High	No side effects
Axelrod et al., (2019)[65]	USA	18-45 years	7 healthy adults	Randomized, double-blind, placebo-controlled crossover study	4 weeks' intake of 200 mg capsules containing the *Lactobacillus Salivarius* UCC118 (2 9 10_8 CFU/capsule) 2. 4 weeks' intake of placebo (corn starch with magnesium stearate) After 4 weeks of wash-out period before the crossover assessment period (4 weeks)	No effects on fecal levels of zonulin *Verrucomicrobiota* levels decreased *Rosebeburia and Lachnospiraceae* (butyrate-producing) levels increased	No effects on the levels of IL-6	3/High	Not reported

Horvath et al., (2020)[66]	Austria	45-67	58 patients with compensated cirrhosis	Randomized controlled trial	Daily dose of 1. Multispecies probiotics containing 1.5×0^{10} CFU in 6 g of Powder (*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus Lactic* W19 and *Lactococcus lactis* W58 in a matrix of maize starch, maltodextrins, vegetable protein, potassium chloride, magnesium sulfate, enzymes (amylases), and manganese sulfate) 2. Placebo consisted of the matrix without bacteria/6 months	Fecal zonulin levels decreased. The composition of probiotic bacteria, including (*Faecalibacterium prausnitzii, Syntrophococcus sucromutans, Bacteroides vulgatus*, Alistipes shahii, and Prevotella) increased. No effects on microbiota diversity	-	3/High	Not reported
Karim et al., (2022)[67]	Pakistan	63-73	104 patients with COPD	Randomized, double-blind, computer-controlled, multicenter trial	1. Vivomix 112 billion live bacteria (*Streptococcus thermophilus DSM 24731, bifidobacteria (B. longum DSM 24736, B. breve DSM*, 24732, DSM *24737), lactobacilli (DSM 24735, DSM 24730, DSM 24733, L. delbrueckii subsp. bulgaricus DSM 24734*) along with maltose, anti-caking agent: silicon dioxide 1 capsule a day 2. Placebo (inactive agents in similar capsules) /16 weeks	Zonulin levels decreased	hs-CRP levels decreased	5/High	Not reported
Karim et al., (2022)[68]	Pakistan	58-73	92 CHF patients	Randomized controlled trial	1. Vivomix 112 billion live bacteria (*Streptococcus thermophilus DSM 24731), bifidobacteria (B. longum DSM 24736, B. breve DSM*, 24732, DSM *24737), lactobacilli (DSM 24735, DSM 24730, DSM 24733, L. delbrueckii subsp. bulgaricus DSM 24734*) along with maltose, anti-caking agent: silicon dioxide, 1 capsule a day 2. Placebo (inactive agents in similar capsules) /12 weeks	Zonulin levels decreased	-	5/High	Not reported

Ayob et al., (2023)[69]	Malaysia,	40-68	40 Patients with non-alcoholic fatty liver disease	Randomized, double-blind, placebo-controlled trial	1. Probiotics Each sachet (3 g) consists of a total of 30 billion CFU with six probiotic strains (*Lactobacillus acidophilus* BCMC® 12130 (107 mg), *Lactobacillus casei sub-spp.* BCMC® 12313 (107 mg), *Lactobacillus lactis* BCMC® 12451 (107 mg), *Bifidobacterium bifidum* BCMC® 02290 (107 mg), *Bifidobacterium infantis* BCMC® 02129 (107 mg) and *Bifidobacterium longum* BCMC® 02120 (107 mg) 2. The placebo group received an identical sachet without probiotic strains/6 months	Zonulin levels increased in both groups. Unclassified-proteobacteria, unclassified-*streptococcus,* and unclassified-*stenotrophomonas* decreased.	TNF-α levels decreased IL-6 levels increased	3/High	Not reported
Qaisar et al., (2024)[70]	United Arab Emirates	71.4±3.9	123 older adults with age-related muscle decline	Randomized, controlled-double blinded study	1. Probiotic: one capsule of Vivomix, 112 included bifidobacteria (*B. longum DSM* 24736, *B. breve DSM* 24732, DSM 24737), *Streptococcus thermophilus DSM* 24731, and *lactobacilli* (*DSM* 24735, *DSM* 24730, *DSM* 24733, *L. delbrueckii subsp. bulgaricus DSM* 24734) 2. Placebo/ 16 weeks	Zonulin levels decreased	No effects on levels of hs-CRP	4/High	Not reported
Lennon et al., (2024)[71]	USA	18-50	16 runners	Randomized double-blinded crossover clinical trial	1. Probiotic: daily supplementation with a probiotic cocktail containing *P.acidilatici* and *L.plantarum* *2. Placebo/* 4 weeks	No effects on the levels of zonulin, microbiota, SCFA, and LBP	No effects on the levels of IL-1β, IL-6, IL-10, MCP-1, TNF-α, hs-CRP, and Calprotectin	3/High	Not reported

CFU: Colony-forming unit; TNF-α: Tumor necrosis factor-α; IL-10: Interleukin-10; IL-6: Interleukin-6; LPS: Lipopolysaccharide; sCD4: Stearoyl-CoA desaturase-4; p38 MAPK: p38 mitogen-activated protein kinase; LC-PUFA: Long-chain polyunsaturated fatty acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; LcS: *Lactobacillus casei Shirota*; t.i.d: Three times a day; COPD: Chronic obstructive pulmonary disease; CHF: Congestive heart failure; SCFA: Short-chain fatty acids; LBP: Lipopolysaccharide-binding protein

Table 5: Summary of the studies that investigated the effects of synbiotics on intestinal permeability and immunity

Authors, Year of publication	Location	Age (years)	Population Studied	Study design	Intervention/ Dose/Duration	Gut effects (intestinal permeability)	Effects on cytokines	Quality score/ degree of trials	Side effects
Kantah et al., (2017)[40]	China, Italy, India, and South Korea	Between 38 and 62	120 individuals	A multicenter randomized study	1. A) given a symbiotic 10 mL t.i.d. for 5 months 2. B) given 1 tablet t.i.d of P3T/J (a probiotic mixture) for 5 months 3. C) given the symbiotic 10 mL t.i.d. for 1 month and then shifted to 1 tab t.i.d of probiotic mixture for 4 months 4. Control: Prior study supplemented a marine PUFA extract	Zonulin levels decreased by treatment B and C, *Bacteroidetes*/ *Firmicutes* ratio, normalized by B and C treatment	-	2/Low	Not reported
Horvath et al., (2020)[59]	Austria	54-65	26 diabesity patients	A randomized, double-blind, placebo-controlled pilot study	1. A daily dose of a multispecies probiotic and a prebiotic (Each dose contains a total of approximately 1.5×10^{10} CFU of a blend containing *B. bifidum W23*, *B. lactis W51*, *B. lactis W52, L. acidophilus W37*, *L. casei W56*, *L. brevis W63*, *L. salivarius W24*, *Lc. lactis W58* and *Lc. lactis W19* in 6 g of a matrix (maize starch, maltodextrins, vegetable protein, potassium chloride, magnesium sulfate, amylases, and manganese sulfate) 2. Placebo (The matrix without bacteria was used as a placebo)/6 months	Zonulin and LPS levels No effects on alpha or beta diversity of the microbiome between groups or time points	-	5/High	Flatulence and diarrhea in one person
Ghavami et al., (2021)[60]	Iran	20-50	69 patients with migraine	A multi-center, randomized, double-blind controlled trial	1. Synbiotic (10^9 CFU of 12 types of probiotics (Each capsule: 500 mg contains 10^9 CFU of 12 types of probiotics including *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus gasseri, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium bifdum,* and *Streptococcus thermophilus,* and FOS as prebiotics) 2. placebo (starch) twice per day/12 weeks	Zonulin levels decreased	hs-CRP levels decreased.	5/High	No adverse effects were reported following synbiotic supplementation.

Stenman et al., (2016)[55]	Finland	18-65 years old	224 participants with a BMI between 28.0-34.9 and a waist-to-hip ratio of ≥0.88 for males and ≥0.83 for females	Double-blind, randomized, placebo-controlled, multi-center clinical trial	1. Placebo, microcrystalline cellulose, 12 g/d 2. Litesse® Ultra polydextrose (LU), 12 g/d 3. *Bifidobacterium* animalis ssp. lactis 420 (B420), 10^{10} CFU/d in microcrystalline cellulose, 12 g/d 4. LU+B420, 12 g+10^{10} CFU/d/6 months	Zonulin levels decreased in the B420 and LU+B420 groups. No effects on the levels of LPS	No effects on the levels of hs-CRP, IL-6, and sCD4	5/High	At least one adverse event (Gastrointestinal symptoms) (133 were potentially product-related).
Çakir et al., (2017)[42]	Turkey	10-12.5 years old	28 children with NAFLD and 30 healthy controls	A longitudinal study of 4 months	1. A capsule/day of synbiotics (Maflor plus capsules®) contained 7×10^{9} CFU active probiotics (*Bifidobacterium lactis*, *Lactobacillus acidophilus, and Lactobacillus casei*) and 100 mg chicory inulin. In addition to the medical treatment, patients were prescribed a low-calorie diet (approximately 10%-20% low-calorie according to their age with 50%-60% carbohydrates, 20%-30% fat: two-thirds saturated and one-third unsaturated, and 10%-20% protein) and a moderate exercise program (aerobic exercise 30-45 min/d at least 3 times a week) 2. Healthy controls/ 4 months.	No effects on the levels of zonulin	TNF-α and hs-CRP levels decreased. No effects on the levels of Calprotectin	1/Low	Not reported
Wilms E. et al., (2016)[61]	Netherlands	Between 18 and 65 years	20 healthy adults	A double-blind, controlled, randomized, parallel-design study	1. Synbiotic (1.5×10^{10} CFU Ecologic® 825 comprising *Bifidobacterium bifidum* (W23), *B. lactis* (W51), *B. lactis* (W52), *Lactobacillus acidophilus* (W22), *L. casei* (W56), *L. paracasei* (W20), *L. plantarum* (W62), *L. salivarius* (W24) and *Lactococcus lactis* (W19)+10 g fructo-oligosaccharides (FOS P6) per day) 2. Control supplements /2 weeks	No effects on the levels of zonulin	No effects on the levels of TNF-α, IL-1β, IL-6, IL-8, and MCP-1	5/High	Not reported

PUFA: Polyunsaturated fatty acid; LPS: Lipopolysaccharide; CFU: Colony-forming unit; hs-CRP: High sensitivity C-reactive protein; FOS: Fructooligosaccharide; Il-6: Interleukin-6; sCD4: Stearoyl-CoA desaturase-4; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-8: Interleukin 8; MCP-1: Monocyte chemoattractant protein-1; BMI: Body mass index; t.i.d.: Three times a day

It should be pointed out that two studies[40, 55] were included in both the probiotic and symbiotic groups. These studies collectively assessed intestinal health and immunity markers, including serum or fecal zonulin levels, serum or fecal calprotectin, GLP-2, SCFAs/LCFAs, fecal BA, LPS, and LBP levels, intestinal microbiota composition, and inflammatory factors.

Serum or Fecal Zonulin and Glucagon-like Peptide-2 (GLP-2)

Fourteen studies examined dietary prebiotics, 18 investigated probiotics, and six studies investigated synbiotics for their effects on serum zonulin levels. Most prebiotic studies showed insignificant effects on the serum or fecal levels of zonulin.[38, 39, 44-47, 52, 53, 62] In contrast, reduced serum levels of zonulin were reported in studies by Krawczyk and others,[48] Russo and others,[50] Czerwi´nska-Rogowska and others,[51] and in Petelin and others.[62] However, Drabińska and others observed increased serum levels of zonulin.[49] Moreover, in most studies that investigated the effects of probiotics on zonulin levels, decreased levels of serum[40, 54, 55, 57, 67, 68, 70] or fecal zonulin[22, 66] were observed. In contrast, no significant changes were reported in the serum levels of zonulin in some other studies.[21, 41, 56, 58, 63-65, 71] Ayob and others reported increased zonulin levels in both intervention and placebo groups.[69] Furthermore, several studies investigating the effects of synbiotics observed a significant decrease in zonulin levels,[21, 40, 55, 60] while others reported no significant changes.[42, 61] Regarding GLP-2, studies examining prebiotic interventions found no significant alterations in its levels.[46, 49] However, Russo and colleagues reported an increase in the levels of GLP-2 following the consumption of inulin-enriched pasta.[50]

Gut Microbiota and Bacterial Metabolites

Few studies have investigated the effects of prebiotics, probiotics, and synbiotics on the gut microbiota changes associated with gut permeability. Ranaivo and others found that chitin-glucan (CG) supplementation decreased one *Actinobacteria phylum* family and increased three bacterial taxa: *Erysipelotrichaceae* UCG.003, *Ruminococcaceae* UCG.005, and *Eubacterium ventriosum* group.[45] Several studies reported increased *Bifidobacterium* abundance,[43, 52, 53] alongside decreased levels of *Clostridale*,[43,53] and *Desulfovibrio*.[43] Serum zonulin levels were found to correlate with Proteobacteria in females and with Bifidobacterium and Bacteroidaceae in males, indicating gender-specific associations.[38] Additionally, Petelin and others also reported reductions in certain *Firmicutes* and *Proteobacteria* by a slight reduction in α-diversity.[62] However, Vuholm and others reported no significant changes in levels of gut microbiota.[39] Concerning the probiotic effects on the gut microbiota, Axelrod and others reported a decline in the levels of *Verrucomicrobia*, alongside an increase in the levels of butyrate-producing bacteria, including *Roseburia* and *Lachnospiraceae,* following probiotic supplementation.[65] Horvath and others showed an increase in the levels of some specific probiotic bacteria taxa, including *Faecalibacterium prausnitzii*, *Syntrophococcus sucromutans*, *Bacteroides vulgatus*, *Alistipes shahii,* and *Prevotella*. However, microbiota diversity remained unchanged.[66] Stadlbauer and others reported a rise in the *Parabacteroides* at the genetic level with no significant changes in the *Bacteroidetes/Firmicutes* ratio.[63] Ayob and others showed decreases in the levels of *unclassified-Proteobacteria*, *Streptococcus,* and *Stenotrophomonas*.[69] However, other studies found no significant changes in the intestinal microbiota composition or diversity following probiotic supplementation.[64, 71] Furthermore, Kantah and others observed normalization of the *Bacteroidetes/Firmicutes* ratio with both probiotic alone and combined probiotic and synbiotic administration.[40] Moreover, Horvath and others reported no significant differences in α- and β-diversity of the gut microbiome following synbiotic intake.[59] Several studies demonstrated unchanged levels of bacterial metabolites, such as SCFAs/LCFAs and BA.[45, 52, 71] Nevertheless, Czerwi´nska-Rogowska and others[51] and Lee others[53] observed an increase in SCFA production, while Stadlbauer and others found no alterations in BA levels.[63]

Inflammatory Markers

Fecal calprotectin, a marker of the local gut inflammation, showed no significant changes with prebiotics intake in some studies,[45, 49, 51] while Neyrinck and others reported decreased levels.[52] In addition, Petelin and others showed a reduction in the serum levels of IL-6 following drinking *Helichrysum italicum* tea, though this reduction was insignificant in the long term.[62] Several studies demonstrated decreased TNF-α[41, 42, 64, 69] and hs-CRP levels[42, 60, 64, 67] with probiotics or synbiotics supplementation. However, insignificant alteration was shown in the levels of calprotectin by intake of probiotics[42, 63, 71] or symbiotic,[42] and other inflammatory markers with prebiotic,[38, 44, 46, 47] probiotics[22, 55, 56, 65, 70, 71] or synbiotics supplementation.[55, 61] The levels of LPS[47, 55, 58] or LPB[38, 45, 64, 71] had no change in most

studies except for Horvath and others' trials that reported decreased LPS levels after 6 months of synbiotic intervention.[59]

Discussion

According to the evidence presented in this study, prebiotic whole foods or food enriched with prebiotics, probiotics, and synbiotics might have favorable effects on the serum levels of zonulin as a measure of intestinal permeability. The effects on GLP-2, gut microbiota, and their metabolites, such as LCFAs, SCFAs, and BA, were contradictory and inconclusive. Some studies have indicated increases in *Bifidobacterium* levels and SCFA levels with the intake of prebiotic supplements or food products enriched with prebiotics. Fecal calprotectin, an important marker of local gut inflammation, as well as TNF-α and hs-CRP, were not affected in most studies. The gut epithelium is an important protective barrier that separates internal organs from the potentially harmful environment of the gut lumen.[31] An increasing body of evidence demonstrated a connection between intestinal permeability and various disease conditions, such as autoimmune, liver, and neurological diseases, diabetes, and irritable bowel syndrome (IBS).[48] The gut permeability is regulated by multiple protein adhesive complexes, such as TJs, underlying adherens junctions (AJ), and desmosomes. The intestinal barrier, as a constantly changing structure, is influenced by interactions with both internal and external factors, such as cytokines, growth factors, and bacteria.[38] In humans, due to the challenges of assessing *in vivo* effects, indirect biological markers, such as serum or fecal zonulin and GLP-2, were proposed for evaluating intestinal epithelial barrier integrity.[48]

Zonulin is a protein released by the enterocytes that reduces the tightness of intercellular junctions in response to various factors,[46] facilitating the increased entrance of bacteria and their components, such as LPS, a structural component of gram-negative bacteria, into the bloodstream. Consequently, LBP serves as a marker for bacterial translocation. As the bacteria and bacterial metabolites enter the bloodstream, they trigger systemic inflammatory responses, elevating pro-inflammatory cytokines, including IL-6, TNF-α, and hs-CRP. This cascade promotes further zonulin release, exacerbating TJ disruption and intestinal permeability. The resulting cycle of bacterial translocation and chronic inflammation has been implicated in the abovementioned diseases, where elevated levels are consistently observed.[38] As zonulin plays a crucial role in initiating systemic inflammation, identifying the triggers of this pathway is essential for understanding chronic inflammation and related diseases. One possible mechanism by which zonulin contributes to the breakdown of TJs and the development of intestinal permeability is through a reversible signaling pathway. Zonulin triggers phosphorylation of the zonulin occluden-1 complex (ZO-1) and myosin 1C, leading to ZO-1 displacement. This disruption of the TJs connection results in heightened intercellular permeability.[38] Furthermore, the collaborative effects of gut-trophic factors such as GLP-2 play a role in a role in maintaining the integrity of the intestinal barrier by promoting cellular growth, development, and maturation.[46] The regulators and mechanisms controlling zonulin production in humans remain poorly understood. It is hypothesized that physiologically, zonulin may facilitate the removal of harmful bacteria and other harmful molecules from the small intestine by increasing the influx of water into the gastrointestinal tract. Consequently, the gut microbiome has emerged as a key focus for understanding factors that influence both zonulin secretion and TJs permeability.[38, 48]

The secretion of zonulin into serum is believed to be regulated by multiple factors, including dietary components.[48] The positive impacts of dietary fibers (DF) are well-established, with current recommendations advising a 25-30 g/day intake.[28] Certain vegetables contain high levels of dietary fiber that may act as prebiotics, which are selectively metabolized by gut microbiota to confer health benefits.[43]

Furthermore, through mechanisms that are not fully understood, experimental studies documented a protective impact of prebiotics on the barrier of the intestinal epithelium. One proposed pathway involves prebiotic-mediated modulation of gut microbiota composition and activity, which in turn influences bacterial co-metabolite production, such as SCFAs,[46] LCFAs, BA, and endotoxemia; thereby affecting intestinal permeability.[45] Significantly, SCFAs-including acetate, propionate, and butyrate-generated through the microbial fermentation of carbohydrates and fiber, directly influences gut hormone secretion by the enteroendocrine L cells. This modulation exerts positive effects on both metabolic function and intestinal epithelial integrity.[72-75] There are many *in vitro* and animal studies that indicated the beneficial effects of butyrate on metabolic diseases through gut health and immune modulation.[76] Additional bacterial co-metabolites, such as BAs, generated via

microbial and host enzymatic activity, similarly regulate gut endocrine function, metabolism, energy balance, and inflammation.[77]

Fructans, one of the few well-established substances, have been extensively studied for their beneficial health effects. These fructose polymers function as carbohydrate storage in plants, occurring naturally in chicory roots, artichokes, onions, bananas, and other sources. This group encompasses fructooligosaccharide (FOS), oligofructose, and inulin, which vary in degree of polymerization.[46] Several studies have investigated the effects of dietary prebiotics on gut health and immunity by assessing: gut permeability via measuring markers such as serum or fecal zonulin,[38, 39, 43-53, 62] gut microbiota composition,[38, 43, 45, 53] and their metabolites including SCFAs,[45, 51, 52] LCFAs, BA,[45] LPS,[77] LBP,[38, 45] GLP-2 factor,[46, 49, 50] and inflammatory markers, such as hs-CRP, TNF-α, IL-6 and calprotectin.[38, 44-47, 49, 51, 52, 62] However, the results of these studies were contradictory.

Most studies investigating single prebiotic supplements found no significant effects on serum[38, 39, 44-47, 53] or fecal[38, 52] zonulin levels. These studies utilized various dietary prebiotics, including Ambrotose products (Advanced or LIFE; a promising prebiotic that is a blend of glyconutrients),[44] Chitin-glucan (CG),[45] FOS,[46] inulin,[47] and native inulin.[52] Bloomer and others examined the effects of eight-week supplementation with 2 or 4 g of the traditional and novel Ambrotose formulations on immunity, gut health, and psychological well-being in 75 healthy adults.[44] In another study, Ranaivo and others investigated the impacts of 4.5 g/day CG supplementation for 8 weeks on the cardiometabolic profiles and the gut microbiota composition in 15 individuals with cardiometabolic risk factors.[45] Ramos and others assessed the influence of three months supplementation of 12 g/day prebiotic fructooligosaccharide (FOS) on the serum markers of the intestinal permeability (zonulin), gut-trophic factor (GLP-2), and inflammation (hs-CRP and IL-6) in 46 non-diabetic chronic kidney disease patients.[46] Vaghef-Mehrabani and others studied the effects of short-term prebiotic intake (10 g/day inulin for 8 weeks) on gut permeability, inflammatory biomarkers, and also depressive symptoms in 45 women with obesity and depression.[47] Neyrinck and others investigated the influences of three-month supplementation with 16 g/d native inulin versus maltodextrin on the fecal microbial-derived metabolites and markers of gut integrity and inflammation in the obese patients.[52] The results of these studies were either consistent[38, 39, 53] or contradictory[48-51] to the results from other studies that investigated different prebiotic-enriched foods with prebiotics and their effects on zonulin markers. Several studies indicated that diets with low intake of vegetables and whole grains as the main sources of fiber (e.g., the western diet) were associated with increased zonulin production and increased intestinal permeability.[78, 79] Thus, enhancing the quality of diet could reduce the zonulin expression and enhance gut health.

A previous study demonstrated reductions in zonulin levels following prebiotic interventions. Krawczyk and others observed decreased zonulin in 32 non-alcoholic fatty liver disease (NAFLD) patients (22 men, 10 women) consuming a high-fiber diet (30-35 g/day from three vegetable and two fruit portions) for 6 months.[48] Similarly, Petelin and others reported decreased levels of zonulin after 4 weeks of *Helichrysum italicum* (Roth) G. Don (HI) tea consumption in patients with traits of metabolic syndrome.[62] Russo and others reported decreased levels of serum zonulin with intake of inulin-enriched pasta (containing 11.0 g fructans per 100 g pasta/day) compared to a control pasta diet (1.4 g of fructans/100 g pasta/day) in a crossover study of 20 healthy men, with two 5-week intervention periods separated by an 8-week washout.[50] Czerwi´nska-Rogowska and others also demonstrated reduced levels of zonulin in 59 patients suffering from ischemic stroke who received a fiber-enriched kitchen diet (n=32; 1.2 g fiber in 100 mL) versus placebo.[51] Drabinska and others observed significant differences in the serum levels of zonulin in the intervention group, compared to the baseline after 12 weeks of supplementation of a gluten-free diet (GFD) plus prebiotic oligofructose-enriched inulin (10 g/day) in 30 children with celiac disease.[49] It seems that while individual nutrients were found to impact zonulin levels in various observational and mouse studies, human dietary interventions have struggled to consistently replicate these findings. Certain studies indicated that metabolic factors such as body mass index (BMI), waist circumference, and age might have stronger associations with the serum levels of zonulin changes than dietary modifications alone.[8, 80-82] Several methodological challenges complicate the interpretation of existing prebiotic studies and their effects on zonulin levels. These include: (1) heterogeneity in study designs examining different prebiotics, (2) variability in participant characteristics and underlying health conditions, (3) inconsistent assessment of intestinal permeability disorders, (4) absence of standardized cut-off values for

permeability markers such as zonulin, (5) diverse measurement techniques, (6) varying baseline biomarker values, and (7) inadequate dietary monitoring regarding fiber intake and potential nutrient synergies. These limitations collectively obscure potential relationships between prebiotic interventions and serum zonulin levels. Consequently, there remains a critical need for higher-quality studies featuring larger sample sizes, longer intervention durations, and optimized dosing regimens to establish definitive conclusions.

It is well-established that the gut microbiome and its metabolites play a role in intestinal health and permeability. Zonulin, in particular, has been associated with alterations across bacterial phylogenetic levels, dysbiosis, and small intestinal bacterial overgrowth.[38] Therefore, prebiotics, the substrates that are selectively utilized by host microorganisms, might be a promising intervention for enhancing gut barrier function.[61, 83] However, the findings of the clinical trials regarding prebiotic efficacy remain inconsistent.[84-86] Ranaivo and others found that a 3-week supplement with 4.5 g CG in individuals with cardiometabolic risk improved the postprandial metabolism and modified the gut microbiota composition by changing the relative abundance of specific gut bacterial taxa. The CG decreased a family belonging to the *Actinobacteria phylum* and increased three bacterial taxa: *Erysipelotrichaceae* UCG.003, *Ruminococcaceae* UCG.005, and *Eubacterium ventriosum group*. Notably, β-glucans (BG), a CG component, independently influenced gut microbiota. However, this intervention did not significantly alter fecal metabolite concentrations (SCFAs, BA, and LCFAs).[45] Studies in healthy subjects revealed that CG increased the relative abundance of the butyrate-producing bacteria *Roseburia spp*., and concurrently increased SCFAs production, including butyric acid.[87] These findings indicated that the generation of metabolites might vary based on the metabolic status of subjects or their gut microbiota traits. In obese patients, Neyrinck and others found a substantial increase in the *Bifidobacterium* after 3 months of 16 g/day native inulin supplementation versus maltodextrin. While this prebiotic intervention did not significantly modify the fecal SCFAs content, it increased the fecal rumenic acid, a conjugated linoleic acid (cis-9, trans-11 CLA), with the immunomodulatory properties that showed a strong correlation with *Bifidobacterium* expansion.[52]

There are conflicting findings concerning the dietary prebiotic food effects on the gut barrier, with a focus on the gut microbiota. Riviere and others reported no significant changes in the serum and fecal zonulin or overall fecal microbiota. However, a change in the serum zonulin was associated with changes in the *Proteobacteria* in females and *Bifidobacteria* and *Bacteroidaceae* in males.[38] Petelin and others showed that the consumption of both *Helichrysum italicum* (Roth) G. Don (HI) and *Helichrysum arenarium* (L.) Moench (HA) infusions reduced zonulin levels and some genera belonging to *Firmicutes*, confirming the modulatory effect of both infusions on the intestinal microbiota composition. These interventions also caused a slight but significant reduction in the α-diversity and decreased *Proteobacteria* abundance. Given that *Proteobacteria* overrepresentation was linked to inflammatory conditions and obesity, the observed zonulin reduction and attenuated inflammation might result from their diminished abundance.[62]

Several studies have evaluated the effects of fiber-rich Okara, containing both soluble (42-67% dry weight) and insoluble fiber (1-15% dry weight), on gut health. While serum zonulin showed no significant differences between the interventions, notable microbial changes were observed: 20% flour-substituted Okara reduced the Clostridiales, while bio-valorized Okara at the same substitution rate increased the *Bifidobacterium* abundance. In addition, Okara consumption improved serum levels of zonulin without affecting fecal SCFAs concentration.[53] These findings were inconsistent with animal studies demonstrating increased fecal SCFAs following Okara treatment.[88, 89] This discrepancy might stem from physiological differences between species; humans exhibit longer intestinal transit times than animal models, potentially allowing for greater SCFAs absorption and consequently reduced excretion of SCFAs.[90] A study by Vuholm and others,[39] showed that regular consumption of whole-grain wheat (WGW) and whole-grain rye (WGR) that are rich in total fiber and fermentable fiber (e.g., arabinoxylans, β-glucans, and fructans) had no effect on the gut microbiota composition and the serum levels of zonulin.[91] The failure to replicate the results of the limited studies that reported effects of whole grains on human gut bacterial abundance—and their narrow focus on specific bacterial genera and species (*Bifidobacterium*,[92] *Lactobacillus*,[45, 92] and *Clostridium leptum*[93])—made these findings difficult to interpret. Moreover, there were uncertainties regarding whether studies were adequately powered to detect microbiota changes.[94] However, consistent with the previous studies,[95-97] the fecal SCFA,

such as butyrate concentration, were affected in the healthy overweight adults,[39] while other studies reported no such effects.[92, 95, 98] These inconsistencies highlighted the need for larger, more comprehensive investigations in this field. Elevated fecal SCFA concentrations, particularly butyrate, are considered beneficial due to their protective health effects. Butyrate plays a crucial regulatory role in colonocyte growth and differentiation, which has been associated with reduced risks of colorectal cancer and inflammatory bowel disease.[99, 100] The whole-grain products are particularly valuable as they contain significantly higher amounts of dietary fibers[101] and resistant starch[102] that is fermented in the colon and produces SCFAs, with notable increases in fecal butyrate concentrations. The production of SCFAs is influenced by several factors: (1) gut transit time, (2) substrate type, and (3) microbiota composition.[100] Shortened GI transit time results in increased SCFAs and butyrate concentration.[103] This relationship underscored the importance of whole grains in optimizing colonic fermentation and SCFAs production.[104]

Studies investigating the effect of prebiotics on the inflammatory markers related to intestinal permeability have yielded mixed results. Bloomer and others reported that 8-week supplementation with 2-4 g of Ambrotose products (Advanced or LIFE) was safe and reduced the subclinical cellular stress, as evidenced by decreased monocyte counts while remaining within normal physiological ranges, with no change in total white blood cell counts. This finding is particularly relevant since elevated monocytes typically indicate chronic infection; they have also been associated with reduced insulin sensitivity and have recently emerged as an independent cardiovascular risk factor. Consequently, a lower overall monocyte percentage might be linked to long-term health improvement. It was reported that monocyte numbers were elevated in all Ambrotose groups, compared to the placebo group at the initial measurement, and showed a significant decrease during the 8-week intervention. Despite the reduction in monocytes due to treatment, the body's ability to respond to the LPS challenge remained unaffected, as no changes in cytokines IL-6, IL-1β, and TNF-α response were noted with stimulation via LPS. However, an increase was observed in plasma IL-10 by intake of 4 g of Ambrotose LIFE.[44] This cytokine is crucial for maintaining intestinal health, as evidenced by the development of cellular challenges in the intestines of an IL-10-deficient mouse model,[105] similar to observations in humans with polymorphisms linked to IL-10.[45] The rise in plasma IL-10 by a dietary intervention suggested potential microbiome-mediated effects,[44] as gut-resident bacteria were demonstrated to have beneficial effects through IL-10 stimulation.[106] These findings suggested that Ambrotose might exert its prebiotic effects by promoting beneficial gut microbiota proliferation. This mechanism could be linked to its acemannan content, as studies indicated that Aloe vera-derived acemannan positively influenced microbial communities,[107, 108] indicating potential prebiotic properties. These results indicated that the Ambrotose supplement is probably a modulator of the immune system rather than a direct immune stimulator.

Supporting this notion, Ranaivo and others found no significant effects of 3-week 4.5 g CG supplementation on fecal calprotectin (a marker of localized gut inflammation) or plasma LBP levels.[45] Similarly, Ramos and others reported no significant changes in the serum levels of hs-CRP and IL-6 after 3 months of 12 g/day FOS supplementation in 46 non-diabetic chronic kidney disease patients,[46] contrasting with results from healthy volunteers,[50] and animal studies.[109,110] These discrepancies might stem from patients' clinically stable conditions and/or structural differences among prebiotic compounds that could affect their functional outcomes.

Major depressive disorder (MDD) is increasingly understood as a condition characterized by both inflammatory processes and gut microbiota dysbiosis, a pattern similarly observed in obesity, which results in endotoxemia and inflammatory status, eventually exacerbating depressive symptoms. This microbial imbalance can contribute to endotoxemia and chronic inflammation, potentially worsening depressive symptoms. Several studies have assessed the effects of prebiotic supplementation (inulin) on psychological outcomes and various biomarkers, including gut permeability (serum zonulin), endotoxemia (LPS), inflammation (TNF-α), monocyte chemoattractant protein-1 (MCP-1), toll-like receptor-4 (TLR-4) and (hs-CRP), and brain-derived neurotrophic factor (BDNF) in females with obesity and depression following calorie-restricted diets.[47, 50, 111, 112] Vaghef-Mehrabani and others showed that short-term intake of prebiotic supplementation (10 g/d of inulin for 8 weeks) had no significant beneficial effects on gut permeability, inflammatory biomarkers, or depressive symptoms in their study of 45 women with obesity and depression.[47] These results were inconsistent with the results of studies reported that prebiotics modulated the gut microbiota composition, and the serum levels of zonulin, LPS, and inflammatory cytokines

following supplementation with various types of prebiotics (e.g., inulin, oligofructose-enriched inulin, resistant dextrin).[50, 111, 112] The discrepancy in results might stem from differences in study populations, intervention durations, or specific prebiotic formulations used across studies.

The observed lack of changes in gut permeability markers, including zonulin, might be attributed to the differences in baseline values of this biomarker. However, Neyrinck and others reported reduced fecal calprotectin following inulin-type fructans treatment,[52] a potentially beneficial effect for patients with comorbidities such as obesity-related diverticulosis.[113] In contrast, several studies found no significant changes in calprotectin levels after specific dietary interventions, including a gluten-free diet (GFD) supplemented with oligofructose-enriched inulin (10 g/day),[32] and a kitchen diet (1.2 g fiber in 100 mL).[51] Similarly, Riviere and others found no changes in the serum levels of LBP or inflammatory markers, such as TNF-α, IL-6, and hs-CRP following 12 weeks of high green leafy vegetable consumption in individuals with a BMI greater than 30 Kg/m^2.[38] Notably, Petelin and others found that daily intake of *Helichrysum italicum* (Roth) G. Don (HI) infusion for 4 weeks significantly decreased circulating levels of zonulin, and pro-inflammatory cytokines, including IL-1β, IL-6, and MCP-1. On the other hand, infusion of Helichrysum arenarium (L.) Moench (HA) had no effect on the serum levels of zonulin, and the effects on the inflammatory markers were less prominent after the 4-week intervention than *Helichrysum italicum* (Roth) G. Don (HI). However, two weeks after both interventions, IL-1β was significantly decreased only in the HA group. The differences between *Helichrysum italicum* (Roth) G. Don (HI) and Helichrysum arenarium (L.) Moench (HA) effects were probably related to their distinct chemical profile. Biological processes related to hemostasis, wound healing, cytoskeletal rearrangement, and epithelial development were proposed as the main pathways affected by *Helichrysum italicum* (Roth) G. Don (HI) and pointed to the activity of *Helichrysum italicum* (Roth) G. Don (HI) in regulating the intestinal epithelial barrier and thereby protecting underlying tissues external stressors. Therefore, the observed reduction in inflammation could also be mediated through multiple mechanisms: *Helichrysum italicum* (Roth) G. Don (HI) to maintain the intestinal epithelial barrier integrity, as supported by decreased serum zonulin levels in this study; (2) modulation of gut microbiota composition; and (3) the anti-inflammatory effects of dietary components. Specifically, diets rich in fiber and polyphenol-containing plant foods demonstrated potent antioxidant, anti-inflammatory, and immunomodulatory properties that might contribute to these effects.[62]

The effect of prebiotic compounds on the levels of GLP-2, an essential regulator of the intestinal barrier function, has yielded mixed results across studies. Russo and others showed that the consumption of inulin-rich pasta could significantly decrease the serum levels of zonulin and increase the GLP-2 levels in healthy young subjects compared to the control pasta.[50] However, other clinical studies failed to observe significant effects of prebiotic interventions on GLP-2 levels.[46, 49] These discrepancies might reflect differences in study populations, prebiotic formulations, or intervention durations.

Probiotics (live microorganisms) and synbiotic supplements, which contain probiotic strains with prebiotics, such as inulin, starch, and fructooligosaccharides (FOS), represent promising nutraceuticals for modulating intestinal permeability through the expression of TJs.[21] While numerous studies have investigated the effects of probiotics on serum and fecal zonulin levels, the findings remained inconsistent and inconclusive.[21, 22] This variability might stem from differences in probiotic strains, dosages, treatment durations, or study populations across investigations.

Some studies suggested that certain probiotic strains[22, 40, 54, 55, 57, 66-68, 70] and also synbiotic supplements[40, 55, 60] might help regulate the serum levels of zonulin and improve the intestinal barrier function, potentially reducing the risk of leaky gut syndrome and other gastrointestinal issues. However, current findings remain inconsistent, as other studies reported no significant effects of probiotics,[21, 41, 56, 58, 63-65, 71] or synbiotics[42, 61] on the serum or fecal zonulin levels. These discrepancies highlighted the need for further research to elucidate the precise mechanisms underlying probiotic-zonulin interactions and establish strain-specific effects.

A recent systematic review with meta-analysis demonstrated that probiotic/synbiotic supplementation significantly reduced serum zonulin levels compared to the placebo. However, considerable heterogeneity existed among the included studies.[25] When the analysis was separately performed for probiotics and synbiotics, a significant reduction was observed in those that received probiotics only. These results were consistent with the findings of *in vitro* studies suggesting that the combination of probiotics with prebiotics might change intestinal permeability simultaneously.[114, 115] In addition, the results were insignificant when the analysis

was confined to high-quality studies, blinded trials, studies with longer duration, and those that recruited healthy subjects younger than 45 years old. However, when they examined the association between study duration and the effect of probiotics/synbiotics on the serum levels of zonulin, they found that studies with longer durations of intervention had lower serum levels of zonulin. This duration-dependent effect suggested that sustained microbial modulation might be necessary to achieve significant improvements in intestinal barrier function.

Some mechanisms have been proposed to explain probiotic-mediated improvements in intestinal barrier function. Probiotics could outperform specific lactic acid bacteria in activating the TLR2 signaling pathway.[116] TLR2 is found in the cell membranes of the intestinal epithelium, where its activated form triggers resistance in the epithelial cells.[117, 118] Additionally, the beneficial impact of probiotics on intestinal permeability might be partially attributed to their ability to inhibit p38 Mitogen-activated protein kinases (MAPK), a Ser/Thr kinase associated with the increase of various inflammatory marker expression.[77] Supporting these mechanisms, multiple clinical studies reported probiotic-associated reductions in TNF-α[41, 42, 64, 69] and hs-CRP.[42, 60, 64, 67] However, some studies reported insignificant changes in the levels of fecal calprotectin[63, 71] and the levels of other inflammatory markers with intake of neither probiotics[21, 22, 55, 56, 63, 65, 70, 71] nor synbiotics.[42, 55, 61] Furthermore, Stenman and others[55] and Mokkal and others[58] showed no alteration in the levels of LPS. Likewise, Roman and others[64] and Lennon and others[71] showed no alteration in the levels of LBP. However, Horvath and others showed decreased levels of LPS after 6 months of intervention with a multispecies synbiotic supplementation.[59]

Few studies have investigated the simultaneous effects of probiotics or synbiotics on gut microbiota and intestinal permeability. Kantah and others found that intake of probiotics and synbiotics normalized the *Bacteroidetes*/*Firmicutes* ratios,[40] while Stadlbauer and others found no significant alteration of this ratio with *Lactobacillus casei* Shirota (LcS) supplementation on the *Bacteroidetes*/*Firmicutes* ratios in the metabolic syndrome (MetS) patients. However, MetS patients exhibited significantly different baseline *Bacteroidetes/Firmicutes* ratios, markers of the gut barrier disruption, and inflammatory profiles compared to healthy controls. While supplementation with LcS increased the abundance of *Parabacteroides* (*phylum Bacteroidetes*), it indicated no effect on other markers such as zonulin, calprotectin, and bile acids. Importantly, a compositional shift from *Firmicutes* towards *Bacteroidetes* could represent a beneficial modulation in metabolic disorders.[63] Several studies have examined probiotic-induced microbiota changes and their relationship to intestinal permeability, with varying results. Axelrod and others assessed the efficacy of UCC118, a characterized probiotic strain, on exercise-induced GI permeability in healthy individuals and found decreased levels of *Verrucomicrobia* and increased levels of butyrate-producing microbiota, such as *Roseburia* and *Lachnospiraceae,* which enhance protection against exercise-induced hyperpermeability. However, no notable changes were observed in markers of inflammation or tight junction regulation.[65] Horvath and others also found that multispecies probiotic supplementation modified specific bacterial taxa, including *Faecalibacterium prausnitzii*, *Syntrophococcus sucromutans*, *Bacteroides vulgatus*, *Alistipes shahii,* and *Prevotella* species. Moreover, patients with an increase in *Syntrophococcus sucromutans* and/or *Prevotella spp.* showed a significant decrease in zonulin levels compared to placebo-treated patients. However, no significant changes in the microbiota diversity were observed.[66] These findings were consistent with their subsequent study, which detected significant changes in α- or β-diversity between treatment groups or time points.[59] Several studies reported insignificant changes in gut microbiota composition or diversity following probiotic supplementation.[64, 71] Differences in population, probiotic, and synbiotics products, and length of supplementation, made it difficult to compare the results between studies. Therefore, more powerful clinical trials with longer follow-ups are needed to draw definitive conclusions about the effects of probiotics/synbiotics on gut permeability-related markers (e.g., zonulin), gut microbiota composition and associated metabolites (including SCFAs, LCFAs, and BAs), systemic inflammatory markers (such as LPS, LBP, and cytokines), and gut-specific inflammatory markers (e.g., calprotectin).

This study was the first systematic review that investigated the effects of prebiotics, probiotics, and synbiotics on gut health, based on clinical trials. Despite extensive investigation, the underlying molecular mechanisms of prebiotics, probiotics, and synbiotics on the gut permeability, gut microbiota, and inflammation that affect chronic disease pathogenesis are still unclear. These knowledge gaps highlighted

the need for more *in vivo, in vitro,* and clinical studies. A key limitation of the present study was the inability to perform meta-analysis, primarily due to significant heterogeneity across studies, particularly regarding supplement types and participant health statuses. Future studies should aim for more homogeneous studies to facilitate quantitative synthesis. In addition, the determination of appropriate formulations and effective doses requires careful evaluation in subsequent trials to establish evidence-based recommendations.

Conclusion

The present study indicated that prebiotic whole foods or prebiotic-enriched foods, in contrast to isolated prebiotic supplements, might have beneficial effects on gut permeability markers, such as zonulin and GLP-2. Several studies indicated that oral probiotics and synbiotics supplements could favorably influence serum zonulin levels. Certain investigations demonstrated that prebiotic supplementation or consumption of prebiotic-enriched foods leads to increased *Bifidobacterium* abundance and elevated SCFAs production.

Regarding inflammatory markers: (1) Fecal calprotectin (an important marker of local gut inflammation) remained stable in most studies, with only limited reports of significant changes; (2) TNF-α and hs-CRP levels were generally unaffected, though occasional reductions were noted; and (3) some studies observed increased plasma IL-10 levels following prebiotic fiber consumption, a crucial cytokine for maintaining the intestinal health. Except for one trial that reported decreased levels of LPS after 6 months of supplement with synbiotics, the levels of LPS and LPB did not change in the studies. The inconsistent findings across studies complicate definitive conclusions about the effects of these interventions on gut health and immunity. Several factors likely contribute to this variability, including differences in subjects' metabolic status and baseline gut microbiota composition, variations in supplement dosages and formulations, and heterogeneity in study durations. These limitations underscored the need for additional high-quality research before establishing evidence-based recommendations.

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Authors' Contribution

L.MN: Conceptualization, study design, data gathering, and drafting; A.BK: Conceptualization, study design, data interpretation, and reviewing the manuscript; Sh.AM: Conceptualization, study design, and drafting; L.H: Conceptualization, study design, data interpretation, and reviewing the manuscript; F.S: Conceptualization, study design, data gathering, and drafting; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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