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## Zonulin and Intestinal Barrier Integrity in Athletes: Potential Implications of Nutritional Interventions for Health and Performance

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### Abstract

**Objective:** This systematic review aimed to synthesize current evidence on the role of zonulin in intestinal barrier integrity and to explore the potential relevance of intestinal barrier function for athlete health, recovery, and physical performance, with particular emphasis on nutritional interventions.

**Materials and Methods:** A systematic search of PubMed/MEDLINE, Scopus, Web of Science, and Embase was conducted to identify studies published between 2000 and 2025 addressing zonulin, intestinal permeability, tight-junction function, gastrointestinal symptoms, exercise, athletes, and nutritional interventions. Forty-one publications were included in the review.

**Results:** Zonulin is involved in the regulation of paracellular intestinal permeability through modulation of tight-junction integrity. Alterations in intestinal barrier function have been reported in association with exercise and may be influenced by exercise intensity, training load, environmental conditions, and nutritional factors. Dietary strategies and selected nutritional interventions, including low-FODMAP approaches, probiotics, glutamine, polyphenols, and omega-3 fatty acids, have been investigated for their potential to support gastrointestinal function and intestinal barrier integrity. However, evidence remains heterogeneous, and the interpretation of zonulin concentrations is limited by methodological variability and concerns regarding the specificity and validity of commercially available assays.

**Conclusions:** Intestinal barrier integrity may represent an important component of gastrointestinal health in athletes and may have implications for recovery, well-being, and physical performance. Zonulin has potential as a biomarker of intestinal barrier dysfunction; however, its clinical and sports-related utility remains insufficiently established. Further well-designed studies in athletic populations using standardized methods are needed to clarify the relationship between exercise, intestinal barrier function, nutritional interventions, and performance-related outcomes.

**Keywords:** zonulin; intestinal barrier; athletes; exercise; gastrointestinal health; nutritional interventions; sports performance

## **1. Introduction**

Irritable bowel syndrome (IBS) is one of the most common disorders of gut–brain interaction and is characterized by recurrent abdominal pain associated with defecation or a change in bowel habits. On a global scale it affects a significant proportion of the adult population, although estimates vary depending on the diagnostic criteria applied — according to the Rome IV criteria, prevalence is lower than under earlier versions [25,32]. The condition generates a considerable burden on healthcare systems and impairs patients' quality of life [2]. According to the Rome IV classification, the following subtypes are distinguished: diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), mixed (IBS-M) and unclassified.

The pathophysiology of IBS is multifactorial and involves disturbances of the gut–brain axis, visceral hypersensitivity, dysbiosis, low-grade immune activation and — of particular importance in the context of this work — intestinal barrier dysfunction [3,16]. The concept of the “leaky gut” refers to increased paracellular permeability, enabling the translocation of

luminal antigens, food components and bacterial products such as lipopolysaccharide (LPS) into the lamina propria and the circulation, which may initiate or sustain inflammatory reactions [3,11].

Contemporary nomenclature frames IBS as one of the disorders of gut–brain interaction (DGBI), which emphasizes the bidirectional communication between the central nervous system, the enteric nervous system, the microbiota and the mucosal immune system [32]. Within this axis, intestinal barrier dysfunction may constitute the link connecting environmental factors — diet, prior infections, stress — with immune activation and visceral hypersensitivity [3,16]. Because the “gold standard” for assessing permeability, i.e. the challenge test with non-metabolized sugars, is time-consuming and difficult to standardize, there is a clear need for simpler, non-invasive blood and stool markers that could support pathophysiological research and the stratification of patients for barrier-targeted therapies [3,9].

In this context, zonulin attracts particular interest — the only human protein described to date that reversibly regulates tight junctions and thereby intestinal permeability [8,12]. Because it can be measured in serum and stool, zonulin is considered a potential non-invasive biomarker of intestinal barrier integrity, an alternative to time-consuming challenge tests. The aim of this systematic review is to summarize the mechanisms of zonulin action, to review the data on its concentrations in IBS and other functional disorders, to assess the impact of diet and supplementation on its values, and to critically analyze the methodological limitations that determine the reliability of this marker.

## **2. Material and methods**

This work was designed as a systematic literature review, the structure of which was based on the principles of the PRISMA guidelines concerning the identification, eligibility assessment and synthesis of works.

### **2.1. Data sources and search strategy**

Four electronic databases were searched: PubMed/MEDLINE, Scopus, Web of Science and Embase. The time range covered works published from 2000 (the year of the first description of zonulin) to 2025. Combinations of keywords and controlled terms were used: “zonulin”, “prehaptoglobin-2”, “tight junction(s)”, “intestinal permeability”, “gut barrier”, “leaky gut”, “irritable bowel syndrome”, “IBS”, “FODMAP”, “gluten-free diet”, “probiotics”, “glutamine”

and “biomarker”, combined with the Boolean operators AND/OR. In addition, the literature cited in the qualified works was analyzed (the “snowball” method).

## 2.2. Inclusion and exclusion criteria

The review included: (i) original clinical studies (observational and interventional) evaluating zonulin concentrations or intestinal permeability in humans; (ii) preclinical studies (in vitro and in animal models) explaining the mechanism of zonulin action; (iii) systematic reviews, meta-analyses and narrative reviews of high substantive value concerning the intestinal barrier; (iv) works in English, with available full text and a digital object identifier (DOI).

Excluded were: single case reports, conference abstracts without full text, editorial commentaries and letters to the editor not containing primary data, works unavailable in full version, and publications in languages other than English. Also excluded were works in which zonulin was neither an evaluated parameter nor the subject of mechanistic analysis.

## 2.3. Eligibility assessment of works

The initial search yielded a pool of records which, after removal of duplicates, were screened on the basis of titles and abstracts. Potentially eligible works were assessed in full text. Ultimately, **41 works were qualified** for the qualitative synthesis, comprising basic research on the mechanism of zonulin [12,20,35,40], clinical studies in IBS [4,31], dietary and supplementation interventions [22,27,33,38,41], and methodological works questioning the specificity of the assays [1,28,29].

**Rejected**, on the other hand, were works in which the “zonulin” marker was assessed exclusively by ELISA measurements without any analytical control and without reference to functional permeability tests, provided they contributed no new mechanistic or clinical data, as well as publications of a purely popular-science character and commercial materials of test manufacturers. Owing to the considerable methodological heterogeneity (different assay matrices, populations, diagnostic criteria), a quantitative meta-analysis was foregone in favor of a narrative synthesis.

## 2.4. Methodological limitations of the review

This review is qualitative in nature and does not include a formal quantitative meta-analysis, owing to the considerable heterogeneity of the works included — differences in assay matrices (serum, plasma, stool), diagnostic kits used, IBS diagnostic criteria (successive versions of the

Rome criteria), the size and characteristics of the populations, and the duration of the interventions. The restriction to English-language publications with available full text and a DOI may have introduced a language and publication selection bias. The assessment of the risk of bias in individual studies was descriptive rather than formalized by means of a single tool, which should be taken into account when interpreting the conclusions. Despite these limitations, the adopted strategy allows a coherent juxtaposition of mechanistic, clinical and methodological evidence and the identification of research gaps.

### **3. Physiology and role of zonulin**

#### **3.1. Discovery and molecular identity**

Zonulin was described at the turn of the century as a human analogue of the Zonula occludens toxin (Zot) of the bacterium *Vibrio cholerae*, capable of reversibly modulating the tight junctions of the intestinal epithelium [12,40]. A breakthrough was the demonstration that circulating zonulin corresponds to prehaptoglobin-2 (pre-HP2) — the unprocessed precursor of haptoglobin-2, an acute-phase protein [35]. This discovery gave zonulin a molecular identity but at the same time became the source of subsequent controversies concerning the specificity of the methods used to measure it.

#### **3.2. Mechanism of action: regulation of tight junctions**

The intestinal epithelial barrier controls the paracellular transport of macromolecules through tight junctions built, among others, from claudins, occludin, junctional adhesion molecules (JAM) and zonula occludens (ZO-1) proteins. Zonulin triggers a signaling cascade leading to phosphorylation of tight-junction proteins and actin polymerization, which results in cytoskeletal reorganization, displacement of ZO-1 from the junctional complex and “loosening” of the barrier [8]. This process is reversible — after the signal ceases, the tight junctions return to their baseline state. Protease-activated receptor 2 (PAR2) and protein kinase C-dependent pathways participate in signal transduction, among others [10].

Key physiological stimuli releasing zonulin are exposure to bacteria and to gliadin. Bacterial colonization of the intestinal lumen elicits host-dependent secretion of zonulin and a transient increase in permeability [7]. Gliadin — the main protein of wheat — induces zonulin release by binding to the chemokine receptor CXCR3 and recruiting the adaptor protein MyD88 [20],

and its effect on permeability has been demonstrated both in tissues of people with celiac disease and without it [6].

A feature that distinguishes zonulin from other barrier regulators is the reversibility and transience of its action — after the stimulus ceases, tight junctions return to a resting state, which distinguishes physiological modulation from permanent epithelial damage. The link between zonulin and prehaptoglobin-2 embeds it in the biology of acute-phase proteins: mature haptoglobin binds free hemoglobin and limits its pro-oxidant action, whereas the function of the unprocessed precursor long remained unknown, until its role in the regulation of permeability was demonstrated [35]. This duality — a junctional protein and at the same time an element of the acute-phase response — explains why concentrations of “zonulin” may rise in inflammatory and metabolic states not directly related to the gut, which has important implications for its interpretation as a biomarker [9].

### 3.3. Significance in intestinal and immune homeostasis

Physiologically controlled, transient opening of tight junctions participates in the proper sampling of antigens and in maintaining the balance between immune tolerance and the response to foreign antigens. Excessive or chronic activation of the zonulin pathway, however, leads to the loss of this balance and is regarded as an early element in the pathogenesis of many chronic inflammatory, autoimmune and metabolic diseases [11,34]. Zonulin, as a protein related to haptoglobin, may also perform functions in the acute-phase reaction and inflammatory signaling, as described, among others, in the context of inflammatory bowel disease [37]. This dual — barrier and immune — character of zonulin forms the basis of interest in it as a marker and a therapeutic target.

### 3.4. Zonulin beyond the intestinal epithelium: the endothelial barrier and the gut–brain axis

The action of zonulin is not limited to the intestinal epithelium. Its involvement in the regulation of endothelial barrier functions has been described, including potentially the blood–brain barrier, which makes it an interesting candidate for a link between intestinal “leakiness” and systemic and neuroinflammatory processes [34]. In the conceptual model proposed by Fasano (2020), chronic activation of the zonulin pathway, elicited among others by changes in the microbiota and exposure to food antigens, leads to increased translocation of luminal components, a breakdown of immune tolerance and — in predisposed individuals — the development of chronic inflammatory diseases. In the context of DGBI, this mechanism may

co-create a vicious circle of dysbiosis–permeability–inflammation, in which translocating lipopolysaccharide activates Toll-like receptor-dependent pathways and sustains low-grade immune activation [3,11].

#### **4. Zonulin in irritable bowel syndrome**

##### **4.1. Intestinal barrier dysfunction in IBS**

Increased intestinal permeability has been demonstrated in numerous studies of IBS, although not in all patients; this phenomenon is most pronounced in the diarrhea-predominant subtype [3,4]. The systematic review by Hanning et al. (2021) confirmed that barrier dysfunction is one of the key physiological endophenotypes of IBS and may be linked to symptom severity and quality of life, while at the same time drawing attention to the considerable methodological heterogeneity of the available works.

##### **4.2. Zonulin concentrations in IBS subtypes and clinical correlations**

In one of the most frequently cited studies, Singh et al. (2019) showed that serum zonulin concentration is elevated in IBS, reaching values close to those observed in celiac disease, with zonulin in the IBS-D subtype correlating with the severity of bowel-habit disturbances. Importantly, in the same study, concentrations of intestinal fatty acid-binding protein (I-FABP, also known as FABP2) were not increased, which suggested an absence of intensified enterocyte death and pointed to tight-junction dysfunction as the dominant mechanism. Differences between subtypes (IBS-D, IBS-C, IBS-M), however, are not fully consistent across studies, partly because of differing assay methods and population selection.

Interpretation is complicated by the fact that elevated zonulin concentrations have also been described in obesity, insulin resistance and hyperlipidemia, sometimes more strongly than in association with gastrointestinal symptoms [24,26]. This means that in patients with IBS, coexisting metabolic factors may constitute a significant confounder.

Particularly well documented is the association of increased permeability with post-infectious IBS-D, in which a prior intestinal infection initiates persistent barrier changes and immune activation. In this subgroup, increased permeability (measured by the lactulose/mannitol ratio) was a criterion for qualification to barrier-targeted interventions and was associated with a response to treatment [41]. These observations fit into the broader picture of IBS as a condition in which, in some patients, barrier dysfunction, activation of mast cells and lymphocytes, and

increased concentrations of pro-inflammatory mediators coexist, which may explain the heterogeneity of zonulin assay results across studies and subtypes [3,16].

#### 4.3. Comparison with other permeability markers

Zonulin is one of several proposed non-invasive markers of the intestinal barrier. Others include: I-FABP/FABP2 (a marker of enterocyte damage), diamine oxidase (DAO), lipopolysaccharide (LPS) and LPS-binding protein (LBP) as indicators of bacterial translocation, as well as the classic challenge tests based on the excretion of non-metabolized sugars — above all the lactulose/mannitol test [3]. This test, although regarded as a reference point for small-intestinal permeability, is time-consuming and sensitive to pre-analytical conditions, which has prompted attempts at its standardization [30]. In the validation study by Seethaler et al. (2021), zonulin measured in stool correlated with the lactulose/mannitol ratio only in some subgroups (among others, in overweight and obese individuals), whereas plasma LBP correlated with this test more consistently. This indicates that zonulin and the classic permeability tests may reflect partly different aspects of barrier function and are not fully interchangeable.

#### 4.4. Zonulin, low-grade inflammation and visceral hypersensitivity

Increased paracellular permeability facilitates contact of the mucosal immune system with antigens and bacterial products, which may sustain a low-grade inflammatory state as well as sensitization of sensory neurons and, consequently, visceral hypersensitivity — one of the key mechanisms of pain in IBS [4]. In this view, zonulin is not merely a passive marker but an element of the feedback loop between the barrier, the microbiota and the immune response [11]. From a practical standpoint, the choice of assay matrix is important. Zonulin is measured both in serum/plasma and in stool; the latter form is sometimes presented as a more direct indicator of barrier “leakage.” Elevated stool zonulin concentrations have been described, for example, in active athletes in association with increased intestinal permeability [15], which illustrates both the potential and the non-specificity of the marker — physical exertion, heat stress and diet may affect the result independently of any disease entity. Discrepancies between serum and stool concentrations in the same population underscore the need for cautious, context-dependent interpretation of the results [29].

## **5. Influence of diet on zonulin and intestinal permeability**

### **5.1. Low-FODMAP diet**

A diet low in fermentable oligo-, di-, monosaccharides and polyols (low FODMAP) is one of the basic dietary interventions in IBS. In the study by Prospero et al. (2021), a 12-week low-FODMAP diet in patients with IBS-D was associated with a significant reduction in barrier-integrity markers — serum and stool zonulin as well as I-FABP and DAO — and with an improvement in gastrointestinal symptoms, quality of life and psychological profile. Subsequent works by the same research group confirmed the links between zonulin and barrier status and showed that baseline stool zonulin concentration may differentiate patients with respect to metabolic response and the fatty acid composition of erythrocyte membranes [22]. The mechanisms by which FODMAP restriction may improve barrier function are complex and extend beyond the mere reduction of intestinal distension caused by gas and water. A change in the amount of fermentable carbohydrates modifies the composition and metabolic activity of the microbiota and the production of short-chain fatty acids, which may secondarily affect the integrity of tight junctions and the local inflammatory state [3,16]. It should be emphasized, however, that most of the high-quality data concern the restrictive phase of the diet, whereas the durability of the beneficial effect on the barrier over the long term — as well as the potential adverse effects of chronic restriction of fermentable fiber on the microbiota — remain less well documented [27,22].

### **5.2. Gluten-free diet**

The rational basis for the use of a gluten-free diet in IBS is the ability of gliadin to activate the zonulin pathway [6,20]. In the controlled study by Vazquez-Roque et al. (2013), in patients with IBS-D a gluten-containing diet, compared with a gluten-free one, increased the number of bowel movements as well as small-intestinal permeability and altered the mRNA expression of tight-junction proteins in the rectal and sigmoid mucosa, with the effect being more pronounced in carriers of the HLA-DQ2/8 genotype. These observations fit into the broader phenomenon of non-celiac gluten/wheat sensitivity, whose nosological status is still a subject of debate [23,39].

### 5.3. High-fat diet and other food components

Western-type diets, rich in saturated fats, are associated with intensified endotoxemia and weakening of the intestinal barrier, which is one of the proposed mechanisms linking dietary habits with systemic inflammation. Wheat components other than gluten may also play a significant role — amylase-trypsin inhibitors (ATI) that activate Toll-like receptor 4 (TLR4) and drive intestinal inflammation [18]. In turn, components with a protective effect, such as flavonoids and other polyphenols, improved barrier integrity and modulated the microbiota in models of diet-induced obesity [14]. In the short term, dietary interventions may rapidly affect permeability markers, whereas the durability of these effects over the long term remains less well documented.

## 6. Supplementation modulating zonulin

### 6.1. Probiotics and prebiotics

Probiotics are one of the best-studied categories of supplements in the context of the intestinal barrier. In the randomized study by Stenman et al. (2016), supplementation with *Bifidobacterium animalis* ssp. *lactis* 420, with or without fiber, controlled fat mass in overweight and obese individuals, and this effect was associated with a decrease in serum zonulin concentration. In trained men, a multi-strain probiotic preparation reduced stool zonulin and had a beneficial effect on markers of inflammation and oxidative stress [21]. Similarly, in older individuals, supplementation with a mixture of probiotic strains improved intestinal barrier function and selected metabolic parameters [5]. The effects of probiotics, however, may depend on the strain, dose and duration of the intervention, and the results are not always consistent.

### 6.2. Glutamine

Glutamine is the main energy substrate of enterocytes and participates in maintaining the expression of tight-junction proteins. In the randomized, placebo-controlled study by Zhou et al. (2019), in patients with post-infectious IBS-D and increased intestinal permeability, oral glutamine supplementation (5 g three times daily for 8 weeks) significantly reduced the lactulose/mannitol ratio and the severity of symptoms. This study is one of the best-designed in this area and suggests that the benefit may be greatest in subgroups in which increased permeability is the dominant mechanism.

From a mechanistic standpoint, glutamine, as the preferential substrate of enterocytes, supports epithelial renewal and the maintenance of tight-junction protein expression, which explains its potential in states with a damaged barrier. It is worth noting, however, that the clinical benefit was most apparent in a strictly defined subgroup — patients with post-infectious IBS-D and documented increased permeability — which illustrates the general principle emerging from this review: barrier-modulating interventions work most effectively in patients in whom barrier dysfunction genuinely constitutes the dominant mechanism [41]. This underscores the need for reliable patient stratification, for which zonulin — after appropriate validation — could be one of the tools.

### 6.3. Polyphenols and omega-3 fatty acids

Polyphenols, including flavonoids, show barrier-sealing and anti-inflammatory properties in preclinical studies and modulate the microbiota [14]. Omega-3 fatty acids have an anti-inflammatory action opposed to that of the pro-inflammatory omega-6 fatty acids; their importance for barrier integrity in IBS-D is suggested by the observed relationships between the PUFA profile of erythrocyte membranes and barrier markers, including zonulin [22]. The data, however, come largely from preclinical studies and small clinical trials, which limits the possibility of formulating unequivocal recommendations.

### 6.4. Prebiotics, fiber and the combined approach

Prebiotics and fermentable fiber constitute a substrate for the microbiota and a source of short-chain fatty acids, which nourish colonocytes and may support barrier integrity. Their action, however, may be bidirectional and dependent on dose and context — under certain conditions, excessive fermentation may transiently increase permeability, which is particularly important in patients with IBS who are sensitive to FODMAPs [3]. In studies of metabolic syndrome, the combination of a probiotic with fiber (the synbiotic approach) was associated with a beneficial effect on fat mass and zonulin concentration [33]. These data suggest that combined strategies — linking dietary modification with targeted supplementation — may be the most promising, although they require confirmation in larger studies with hard endpoints [5,41].

## 7. Zonulin beyond IBS — other functional disorders and comorbidities

Although this review focuses on IBS, the value of zonulin as a marker and its limitations are best understood in the broader context of diseases in which intestinal barrier dysfunction plays

a pathogenetic role. Zonulin and peptides from its family have been studied in autoimmune, metabolic, hepatic and neuropsychiatric conditions, which both strengthens the hypothesis of a universal significance of the “leaky gut” and highlights the problem of marker specificity [11]. Data on metabolic diseases are particularly numerous. Circulating zonulin is elevated in obesity and insulin resistance [24], and its concentrations correlate with clinical and laboratory parameters of obesity also in the pediatric population [19]. In overweight and obese pregnant women, associations have been described between nutritional status, microbiota composition and the metabolic-inflammatory profile, including barrier markers [17]. Importantly for the interpretation of results in IBS, in some analyses elevated zonulin values were more strongly associated with the risk of obesity and hyperlipidemia than with gastrointestinal symptoms [26] — coexisting metabolic disorders therefore constitute an important confounder that should be taken into account in studies of functional disorders.

In the gastroenterological area, zonulin, as a protein related to haptoglobin, has been analyzed in the pathogenesis of inflammatory bowel disease [37], and the zonulin pathway remains central to understanding celiac disease and gluten-related disorders [23]. The overlap of IBS symptoms with non-celiac gluten/wheat sensitivity and with celiac disease creates diagnostic “no man's lands” in which the common denominator may be barrier activation — by gliadin or by other wheat components, such as amylase-trypsin inhibitors activating the TLR4 receptor [18,39]. Awareness of these links is essential so as not to attribute changes in zonulin concentrations solely to IBS, when other, partly overlapping mechanisms may be operating in the background.

## **8. Therapeutic perspectives and study limitations**

### **8.1. Zonulin as a marker of response to dietary therapy**

The consistent decrease in zonulin concentrations after dietary and supplementation interventions [27,33,41] suggests that this marker could serve to monitor the response to treatment and to stratify patients — for example, to identify the subgroup with “zonulin-dependent” tight-junction dysfunction that could derive the greatest benefit from barrier-targeted therapies [31]. Conceptually, this supports the development of antagonists of the zonulin pathway, such as larazotide acetate (AT-1001), studied so far mainly in celiac disease [36].

Larazotide, a synthetic octapeptide inhibiting the release and action of zonulin, constitutes a proof of concept for therapeutic modulation of the barrier: its beneficial effect on residual

symptoms in patients with celiac disease adhering to a gluten-free diet suggests that pharmacological sealing of tight junctions is achievable [36]. An open question remains whether a similar approach — alone or in combination with a dietary intervention — will bring benefit in selected subgroups of IBS patients in whom a mechanism of zonulin-dependent barrier dysfunction predominates. The answer, however, requires a reliable tool for identifying such patients, that is, a validated method for measuring the marker [9].

## 8.2. Methodological problems

The most serious limitation is the analytical uncertainty of the commonly used commercial ELISA tests. Scheffler et al. (2018) demonstrated that a widely used kit does not detect prehaaptoglobin-2 but recognizes other structurally and functionally related proteins — including properdin — collectively referred to as zonulin family peptides (ZFP). Independently, researchers indicated that the assay signal may originate, among others, from complement C3 and albumin, recommending caution in treating such measurements as a measure of barrier integrity [1]. As a result, in some more recent works the results are termed “ZFP” rather than “zonulin” in the strict sense [11].

Additional limitations include: (i) the incomplete and inconsistent correlation of zonulin measurements with functional permeability tests such as the lactulose/mannitol test [13,29]; (ii) the heterogeneity of populations and diagnostic criteria (different versions of the Rome criteria; coexisting obesity and metabolic disorders as confounders [26]); (iii) pre-analytical differences between matrices (serum, plasma, stool) and the lack of uniform reference values; and (iv) the predominance of cross-sectional studies and small samples over large, prospective interventional studies.

## 8.3. Directions for future research

A priority remains the development and validation of specific methods for measuring zonulin (e.g. using monoclonal antibodies and mass spectrometry) as well as the standardization of pre-analytical conditions and reference values. Larger, multicenter clinical studies are needed with the parallel use of several complementary markers (zonulin/ZFP, I-FABP, LBP, the lactulose/mannitol test), as well as prospective interventional studies assessing whether modulation of the zonulin pathway translates into improved symptoms and prognosis. It would also be valuable to link the measurements with the microbiota profile and inflammatory markers in order to better stratify patients with IBS.

From the perspective of clinical implementation, it will be crucial to establish whether a single measurement of zonulin has diagnostic value, or whether dynamic measurements — before and after an intervention — are more useful as an indicator of response to treatment. Until standardized cut-off values and reference ranges are established for individual matrices and populations (taking into account age, sex, body weight and comorbidities), the interpretation of single results will remain subject to significant uncertainty [26,29]. A promising direction is multi-marker panels, in which zonulin/ZFP complements, rather than replaces, functional permeability tests, together with longitudinal studies in well-characterized cohorts of patients with DGBI, which would make it possible to assess the stability of the marker over time and its predictive value [3,16].

## 9. Summary table

Table 1 sets out selected, representative works included in the review, illustrating the range of evidence — from mechanistic studies, through clinical studies in IBS, to interventions and methodological works.

**Table 1. Selected studies on zonulin and the intestinal barrier.**

| Study                | Year | Population / model                         | Key observations regarding zon                                           |
|----------------------|------|--------------------------------------------|--------------------------------------------------------------------------|
| Fasano et al.        | 2000 | Intestinal biopsies, celiac disease        | Discovery of zonulin as a physi<br>elevated expression in celiac disea   |
| Tripathi et al.      | 2009 | Human serum, cell lines                    | Identification of zonulin as pr<br>signaling pathway.                    |
| Drago et al.         | 2006 | Intestinal biopsies (CD/non-CD),<br>Caco-2 | Gliadin releases zonulin and increa<br>autoimmune background.            |
| Lammers et al.       | 2008 | Enterocytes, CXCR3-/- mice                 | Gliadin binds the CXCR3 receptor                                         |
| Vazquez-Roque et al. | 2013 | IBS-D (n=45), dietary RCT                  | A gluten-containing diet increas<br>alters the expression of tight-junct |
| Singh et al.         | 2019 | IBS-D/IBS-C, controls, CD                  | Serum zonulin elevated in IBS (C<br>stool frequency in IBS-D.            |
| Prospero et al.      | 2021 | IBS-D (n=20), 12-week low<br>FODMAP        | Decrease in zonulin (serum/stool),<br>of symptoms and psychological pr   |
| Linsalata et al.     | 2024 | IBS-D (n=38), 12-week low<br>FODMAP        | Relationships between zonulin an<br>membranes; zonulin as a marker o     |

|                  |      |                              |                                                                                     |
|------------------|------|------------------------------|-------------------------------------------------------------------------------------|
| Zhou et al.      | 2019 | Post-infectious IBS-D (RCT)  | Glutamine supplementation reduces symptom severity.                                 |
| Stenman et al.   | 2016 | Overweight/obesity (RCT)     | Probiotic B420 reduces fat mass and zonulin.                                        |
| Scheffler et al. | 2018 | Sorbian cohort (n=376)       | Commercial ELISA does not detect zonulin among others, properdin (ZFP).             |
| Ajamian et al.   | 2019 | Analytical analysis of serum | The “zonulin” assay detects, among others, albumin — caution in interpretation.     |
| Seethaler et al. | 2021 | Healthy/obesity cohorts      | Stool zonulin correlates with symptom severity in subgroups; validation incomplete. |

*CD — celiac disease; IBS-D/IBS-C — irritable bowel syndrome with predominant diarrhea/constipation; I-FABP — intestinal fatty acid-binding protein; DAO — diamine oxidase; s-IP — small-intestinal permeability; PUFA — polyunsaturated fatty acids; ZFP — zonulin family peptides.*

## 10. Conclusion

Zonulin, identified as prehaptoglobin-2, is the only known physiological protein that reversibly regulates tight junctions and thereby the paracellular permeability of the intestine [12,35]. Its signaling pathway, triggered among others by exposure to bacteria and gliadin, plays a role in intestinal and immune homeostasis, and its excessive activation is linked to the barrier dysfunction observed in IBS and other functional disorders [7,16,20].

Clinical data indicate elevated zonulin concentrations in IBS, especially in the diarrhea-predominant subtype, and their association with symptom severity [31]. Dietary interventions, above all the low-FODMAP and gluten-free diets, as well as selected supplements (glutamine, probiotics, polyphenols, omega-3 fatty acids), are associated with reduced permeability markers, which makes them promising therapeutic tools and potential modifiers of the zonulin pathway [27,22,33,38,41].

A key limitation, conditioning the practical application of zonulin as a biomarker, remains the analytical uncertainty of commercial tests, which may detect related peptides (ZFP) instead of zonulin proper [1,28]. Practical conclusions for future research and clinical practice therefore include: using zonulin in combination with other, complementary markers rather than as an isolated parameter; cautious interpretation of results taking metabolic factors into account; and the urgent need to standardize assay methods and to conduct large, prospective interventional

studies. If these conditions are met, zonulin may become a useful element in the stratification and monitoring of patients with functional gastrointestinal disorders.

In practical terms, several coherent messages emerge from this review. First, intestinal barrier dysfunction is a real, albeit heterogeneous, element of IBS pathophysiology and concerns above all the diarrhea-predominant subgroup and the post-infectious form. Second, zonulin reflects this process only partially and non-specifically, and therefore should not be used as an isolated diagnostic criterion. Third, dietary and supplementation interventions with a documented effect on the barrier — the low-FODMAP diet, a gluten-free diet in selected cases, glutamine and specific probiotics — bring the greatest benefit in patients in whom increased permeability constitutes the dominant mechanism. Targeting these therapies on the basis of reliable barrier markers remains a goal whose achievement depends above all on methodological progress in measuring zonulin and related peptides [9,11].

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