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Fecal Zonulin as a Potential Biomarker of Intestinal Barrier Dysfunction in Obesity: Molecular Mechanisms, Clinical Validation, and Methodological Challenges - Review of Literature

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Abstract

Introduction: Intestinal permeability is a tightly regulated physiological process that enables the transport of nutrients and ions while preventing the passage of harmful luminal antigens. The term "leaky gut" is often misused in pseudoscientific contexts, despite the existence of well-established physiological mechanisms regulating epithelial barrier function. In obesity, chronic low-grade inflammation and gut dysbiosis stimulate the secretion of zonulin, a physiological modulator of tight junctions (TJs). Increased zonulin levels promote pathological disruption of the leak pathway, contributing to systemic low-grade inflammation. Assessment of intestinal barrier integrity may have clinical value in evaluating metabolic risk and monitoring subclinical inflammation.

Methods: A literature search was conducted using the PubMed/MEDLINE and Google Scholar databases. Publications addressing zonulin, intestinal permeability, obesity, fecal biomarkers, epithelial transport mechanisms, ELISA assay specificity, and clinical correlations were reviewed.

Results: Higher fecal zonulin concentrations were consistently associated with increased body mass index (BMI). Clinical studies demonstrated a direct relationship between impaired intestinal barrier integrity and systemic low-grade inflammation. Weight loss improved intestinal epithelial integrity and was accompanied by favorable changes in gut microbiota composition.

Conclusions: Increased intestinal permeability in obesity is a pathological consequence of tight junction dysfunction. Fecal zonulin is a validated, non-invasive biomarker of intestinal barrier impairment and may be useful for monitoring the effectiveness of metabolic interventions. However, its routine clinical application remains limited due to the need for further standardization and validation.

Keywords: zonulin, intestinal permeability, obesity, tight junctions, fecal zonulin, gut microbiota, gastrointestinal.

1. Introduction

1.1 Obesity has become one of the most significant global health challenges of the twenty-first century and is a major risk factor for metabolic disorders, including insulin resistance, type 2

diabetes mellitus, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease. Current research on obesity pathogenesis extends beyond the concept of energy imbalance and increasingly focuses on chronic low-grade systemic inflammation. Intestinal barrier integrity has emerged as a key link between environmental factors, particularly a high-fat diet, and activation of the host immune system. (8,17)

The intestinal barrier serves as a critical interface separating the external environment, rich in dietary antigens and microorganisms, from the host's internal milieu. Its structural and functional integrity depends on a single layer of intestinal epithelial cells connected by specialized barrier complexes. (7,14,15)

The principal structures regulating selective paracellular permeability are the tight junctions (TJs), which are located at the apical region of adjacent enterocytes. These multiprotein complexes control the passage of water, ions, and small solutes while preventing the translocation of pathogens and other potentially harmful luminal components. (2,3,4,13)

At the molecular level, tight junctions (TJs) do not represent a static, impermeable seal but rather a highly dynamic and adaptable protein structure. They consist of transmembrane proteins, including occludin, junctional adhesion molecules (JAMs), and a diverse family of claudins, which interact with intracellular adaptor proteins from the zonula occludens family (ZO-1, ZO-2, and ZO-3) to connect directly with the actin cytoskeleton. (7,14,15,19)

The regulation of paracellular transport occurs through two major physiological pathways: the highly selective pore pathway, which controls the passage of small ions and water, and the more flexible leak pathway, regulated among others by myosin light chain kinase (MLCK). The leak pathway enables passive transport of larger macromolecules and antigens across the intestinal epithelium. (19,20)

A clinically relevant modulator of intestinal permeability is zonulin, a protein that initiates a signaling cascade leading to reversible disorganization of ZO-1 and occludin complexes. Elevated zonulin concentrations in serum or feces correlate with the degree of intestinal barrier impairment and increased antigen translocation into the lamina propria. (1,3,5,6,15)

A key pathophysiological link between increased intestinal permeability ("leaky gut") and the metabolic phenotype of obesity is the phenomenon of metabolic endotoxemia. Disruption of the leak pathway facilitates the translocation of lipopolysaccharide (LPS), a component of

Gram-negative bacterial cell walls, from the intestinal lumen into the portal and systemic circulation. (8,13,18)

1.2 Contemporary gastroenterology and molecular immunology distinguish three major pathways of transport through tight junctions (TJs):

1. **Pore pathway:** A highly selective pathway primarily regulated by claudins, allowing the passage of small ions and water.
2. **Leak pathway:** A less selective pathway regulated by occludin and ZO-1, activated among others by tumor necrosis factor alpha (TNF- α) and myosin light chain kinase (MLCK). It enables the transport of larger macromolecules across the intestinal epithelium.
3. **Unrestricted pathway:** A pathway resulting from enterocyte apoptosis or mechanical damage (e.g., ulceration), leading to complete disruption of the epithelial barrier. (15,16)

Circulating LPS, acting as a potent pathogen-associated molecular pattern (PAMP), binds to Toll-like receptor 4 (TLR4) expressed on immune cells, adipocytes, and macrophages within adipose tissue and the liver. This activation induces an NF- κ B-dependent inflammatory cascade, directly contributing to peripheral tissue insulin resistance, adipocyte metabolic dysfunction, and progression of hepatic steatosis toward metabolic dysfunction-associated steatohepatitis (MASH). (1,3,8,11,13,18,24,25,26)

Despite strong evidence linking intestinal barrier dysfunction with obesity, implementation of intestinal permeability assessment into routine clinical practice remains limited by significant methodological challenges. The current "gold standard", based on oral administration of probe molecules (e.g., the urinary lactulose/mannitol excretion ratio test), is time-consuming, burdensome for patients, and applicable only in prospective studies. Therefore, considerable efforts have been directed toward identifying alternative surrogate biomarkers that can be easily and reproducibly measured from readily available biological samples.

A major breakthrough in understanding TJ regulation was the discovery of zonulin, a physiological modulator of epithelial intercellular permeability. Zonulin, a precursor of haptoglobin-2, initiates a signaling cascade leading to increased intestinal permeability through the disassembly of occludin and claudin complexes. Pioneering studies in this field, including those conducted by Alessio Fasano and colleagues, demonstrated that excessive zonulin

activation is closely associated with the pathogenesis of inflammatory and autoimmune disorders. (1,5,7,15,16,25)

In the context of obesity, increased serum zonulin concentrations are increasingly interpreted as a potential biomarker of intestinal barrier dysfunction ("leaky gut"). Increased intestinal permeability facilitates the translocation of bacterial endotoxins into the portal and systemic circulation, initiating metabolic endotoxemia and amplifying inflammatory processes in adipose tissue and the liver.

Particular interest has been focused on the assessment of fecal zonulin concentrations. An important step in validating this approach was the study by Seethaler et al. (2021), in which the authors evaluated the clinical relevance of various plasma and fecal biomarkers in comparison with the classical lactulose/mannitol test in healthy individuals and patients with obesity. The study provided important insights: while plasma zonulin demonstrated inconsistent correlations with the reference method, potentially due to limited specificity of commercially available ELISA assays, fecal zonulin concentrations showed a significant association with the lactulose/mannitol ratio, particularly among individuals with overweight and obesity. Furthermore, fecal zonulin levels were directly correlated with body mass index (BMI). These findings suggest that fecal zonulin represents a promising, non-invasive biomarker of increased intestinal permeability, particularly applicable to patients with obesity-related metabolic disorders. (1,3,5,7,9,14,21,27)

This review aims to integrate and summarize current knowledge regarding fecal zonulin measurement as a potential biomarker of intestinal permeability in obesity. The article discusses molecular mechanisms underlying zonulin pathway activation in adipose tissue and the intestine, evaluates its diagnostic value compared with other available barrier biomarkers, and identifies methodological limitations and future perspectives regarding the use of fecal zonulin as a tool for monitoring the effectiveness of personalized dietary and therapeutic interventions.

2. Methodology

2.1. Search strategy and databases

A literature review was conducted through a systematic search of electronic medical and biomedical databases, including PubMed/MEDLINE, Scopus, and Google Scholar. The search

strategy included combinations of the following keywords in English and Polish: *zonulin*, *intestinal permeability*, *obesity*, *tight junctions*, *fecal zonulin*, *metabolic endotoxemia*, *przepuszczalność jelitowa*, *zonulina w kale*, and *otyłość*.

2.2. Inclusion and exclusion criteria

To maintain strict thematic relevance, the selection of publications was based on predefined eligibility criteria.

Inclusion criteria:

- Original and review articles describing the structure, molecular biology, and pathophysiological mechanisms of zonulin activity on tight junction complexes.
- Clinical and validation studies assessing zonulin concentrations (in serum or feces) as direct or surrogate markers of intestinal barrier integrity.
- Studies involving participants with overweight, obesity, or associated metabolic disorders, including insulin resistance and metabolic syndrome.
- Landmark historical studies essential for understanding the discovery of the zonulin pathway and the evolution of knowledge regarding the gut–circulatory system axis.
- Studies evaluating metabolic correlations between intestinal barrier markers and obesity-related parameters, including weight reduction, inflammatory markers (TNF- α , IL-6), insulin resistance (HOMA-IR), and hepatic steatosis.
- Publications without strict time restrictions, with particular emphasis on studies describing the contemporary model of intestinal transport pathways (*pore*, *leak*, and *unrestricted pathways*) and the most recent scientific evidence.
- Original research studies, including prospective and retrospective observational studies, case-control studies, randomized controlled trials (RCTs), and peer-reviewed preclinical studies (*in vivo* and *in vitro*).

Exclusion criteria:

- Studies assessing other intestinal permeability markers (e.g., occludin, claudins, LBP, calprotectin), unless they directly addressed correlations with zonulin concentrations or provided relevant methodological background for zonulin assessment.

- Clinical studies focused exclusively on other diseases (e.g., inflammatory bowel diseases or food allergies), unless they represented fundamental model studies for understanding zonulin secretion mechanisms.
- Studies involving patients undergoing active antibiotic therapy, chemotherapy, or regular high-dose non-steroidal anti-inflammatory drug (NSAID) treatment during sample collection, as these factors may significantly alter tight junction function independently of obesity.
- Publications in which "leaky gut" was presented only as a speculative hypothesis without biochemical or molecular evidence, such as measurements of zonulin, LPS, tight junction proteins, or permeability assays using tracer molecules.

2.4. Selection and structuring of collected material

Based on the predefined eligibility criteria, a complementary database of publications was selected for detailed analysis and subsequently divided into major thematic areas.

Molecular and physiological basis of paracellular pathways: To understand the mechanisms underlying intestinal barrier disruption, fundamental studies by Fasano et al. (2008) and Horowitz et al. (2023), describing the regulation of paracellular transport, were analyzed. Structural studies on the zonula occludens toxin by Di Pierro et al. (2001), as well as experimental studies investigating factors stimulating zonulin secretion, including exposure to microorganisms and gliadin, were included. A key contribution to this section was provided by pioneering studies by Turner et al. (2009), which enabled precise molecular classification of epithelial transport pathways, including the highly selective pore pathway, the occludin- and ZO-1-dependent leak pathway regulated by myosin light chain kinase (MLCK), and the pathological unrestricted pathway. (1,3,6,7,19,20)

Historical background and biomarker evolution:

Landmark studies identifying zonulin expression in pathological conditions associated with autoimmunity were included. These publications enabled the reconstruction of the methodological progression from the identification of zonulin involvement in celiac disease and type 1 diabetes mellitus to the implementation of this biomarker in metabolic disorders. (1,3,4,5,15,18)

Clinical validation in obesity and metabolic disorders: The primary focus of clinical evaluation consisted of contemporary cohort studies, particularly those conducted by Seethaler et al. (2021). These publications provided direct evidence for assessing and comparing the diagnostic reliability of zonulin (and zonulin-like proteins) measured in plasma and feces among patients with obesity, using the classical lactulose/mannitol test as a reference method. This evidence base was expanded by the large cohort study of Ohlsson et al. (2017), which investigated associations between elevated zonulin concentrations, waist circumference, dyslipidemia, and obesity risk. Additionally, the interventional study by Gummesson et al. (2011) provided clinical evidence demonstrating a direct relationship between intestinal permeability, body weight, insulin resistance (HOMA-IR), and hepatic steatosis during weight reduction in humans.

The analysis was further supplemented by the comprehensive review of Pereira SS et al. (2014) and the systematic review by Camilleri (2019), which summarized the effects of dietary, pharmacological, and surgical interventions (bariatric surgery) on the gut microbiome, obesity severity, and tight junction integrity in humans. Recent prospective data from Ciurea et al. (2025) were also included, evaluating fecal zonulin concentrations as a non-invasive marker of pathological changes within the gut–liver axis, particularly in patients with obesity and metabolic syndrome presenting with the MASLD phenotype. (9,21,22,24,25)

Critical methodological assessment of immunoenzymatic assays: Due to the importance of analytical reliability for clinical interpretation, a separate section was dedicated to evaluating the specificity of commercially available ELISA assays. Fundamental validation studies by Scheffler et al. (2018) were included. Using advanced proteomic analyses and mass spectrometry, these studies demonstrated that commonly used laboratory assays do not show absolute specificity for pre-haptoglobin-2, revealing significant cross-reactivity with properdin and other acute-phase proteins induced by metabolic low-grade inflammation. These findings enabled a critical assessment of the diagnostic value of zonulin as an isolated biomarker within the framework of evidence-based medicine (EBM). (1,3,9,17,27)

2.5. AI.

AI was utilized for two specific purposes in this research. Text analysis of clinical reasoning narratives to identify linguistic patterns associated with specific logical fallacies. Assistance in refining the academic English language of the manuscript, ensuring clarity, consistency, and

adherence to scientific writing standards. AI were used for additional linguistic refinement of the research manuscript, ensuring proper English grammar, style, and clarity in the presentation of results. It is important to emphasize that all AI tools were used strictly as assistive instruments under human supervision. The final interpretation of results, classification of errors, and conclusions were determined by human experts in clinical medicine and formal logic. The AI tools served primarily to enhance efficiency in data processing, pattern recognition, and linguistic refinement, rather than replacing human judgment in the analytical process

3. Results

3.1. Discovery of the zonulin pathway and molecular structure of zonulin

Fasano et al. (2000) and Di Pierro et al. (2001) identified zonulin as the only known human endogenous modulator of tight junctions (TJs), demonstrating that it represents a structural precursor of haptoglobin-2 (pre-HP2). Functional studies investigating the structure of the zonula occludens toxin (Zot) secreted by *Vibrio cholerae* (Di Pierro et al., 2001) enabled precise identification of its receptor-binding domain. It was demonstrated that both the bacterial toxin and human zonulin contain a specific amino acid motif enabling interaction with receptors located on the surface of enterocytes (PAR2/EGFR), representing the initial trigger for intestinal barrier disruption. (2,5)

3.2. Stimuli triggering zonulin secretion and intracellular signaling cascade

El Asmar et al. (2002) and Clemente et al. (2003) provided key insights into the dynamics of zonulin release in response to external stimuli. El Asmar et al. (2002) demonstrated in *in vitro* and *ex vivo* models that extensive exposure to bacteria, including both pathogenic and commensal species, induces rapid zonulin secretion. This mechanism appears to represent a physiological defense response, in which the organism temporarily increases intestinal permeability to promote fluid secretion into the intestinal lumen and facilitate microbial clearance.

Clemente et al. (2003) further elucidated the biochemical cascade underlying this process. They demonstrated that stimulation of enterocytes, in their model induced by gliadin exposure, activates intracellular protein kinase C (PKC) and phospholipase C within minutes, leading to

actin polymerization and redistribution of barrier proteins, including ZO-1 and occludin, away from tight junction complexes. (3,6,15)

3.3. Systemic correlations and pathophysiological background

Collin et al. (1989), Fasano (2011), and Horowitz et al. (2023) contributed important evidence regarding the systemic implications of intestinal barrier dysfunction. The study by Collin et al. (1989) provided early epidemiological evidence that impaired barrier function, initially investigated in the context of celiac disease and type 1 diabetes mellitus, was associated with severe systemic disorders, stimulating the search for common pathogenic mechanisms.

The comprehensive review by Fasano (2011), together with the contemporary analysis by Horowitz et al. (2023), integrated these findings into a modern pathogenic model. According to this concept, chronic activation of the zonulin pathway leads to impaired intestinal barrier function, facilitating continuous paracellular antigen translocation into the portal circulation.

In obesity, a key finding is that chronic exposure to dysbiotic intestinal microbiota, characteristic of metabolic disorders, continuously stimulates this pathway. This promotes metabolic endotoxemia through increased lipopolysaccharide (LPS) translocation into the circulation and contributes to systemic low-grade inflammation. (1,4,7,23)

3.4. Clinical validation of zonulin in obesity: plasma versus fecal measurements

Seethaler et al. compared two commercially available diagnostic approaches (plasma and fecal zonulin measurements) with an objective intestinal permeability assessment method, the lactulose/mannitol ratio (lac/man ratio).

The main findings of the study included:

- Lack of correlation in plasma: Plasma zonulin concentrations did not correlate with lactulose/mannitol test results. This may be explained by obesity-associated inflammation, which stimulates hepatic production of haptoglobin, an acute-phase protein. Commercial ELISA assays demonstrate cross-reactivity and may incorrectly detect circulating haptoglobin as zonulin, leading to inaccurate results.

- Significant correlation in feces: Fecal zonulin concentrations showed a significant positive correlation with the lac/man ratio exclusively among individuals with overweight and obesity.
- Association with BMI: A strong direct correlation was demonstrated between higher fecal zonulin concentrations and increased body mass index (BMI).

Clinical studies involving patients with obesity demonstrated a direct association between intestinal barrier dysfunction, insulin resistance, and non-alcoholic fatty liver disease. Importantly, from a therapeutic perspective, weight reduction was associated with significant improvement in intestinal epithelial integrity. (9,12,22,26)

The impact of obesity on intestinal barrier function is multifactorial, and modulation of intestinal permeability may be achieved through various dietary, pharmacological, and surgical interventions. These interventions are directly associated with changes in gut microbiota composition, highlighting the importance of the microbiome–intestinal barrier axis in metabolic health. (10)

3.5. Methodological challenges and specificity of zonulin measurements in clinical practice

Understanding the limitations and discrepancies observed in zonulin research requires a detailed analysis of its biological identity and the specificity of currently available laboratory methods. As demonstrated in pioneering studies by Fasano, zonulin was identified as a precursor of haptoglobin-2 (pre-HP2), a protein with a molecular weight of approximately 47 kDa and the only currently known physiological modulator of tight junctions (TJs). Zonulin exerts its effects by binding to a specific receptor complex on the surface of enterocytes, including protease-activated receptor 2 (PAR2) and the epidermal growth factor receptor (EGFR). This interaction initiates an intracellular signaling cascade involving phospholipase C and protein kinase C (PKC), resulting in actin polymerization and contraction of the perijunctional actomyosin ring, which physically disrupts tight junction integrity. (1,2,5,9)

A major methodological concern currently highlighted by researchers is the question of what is actually measured by commercially available enzyme-linked immunosorbent assays (ELISA). Most commonly used diagnostic kits, including those produced by Immundiagnostik AG and ScheBo Biotech, were developed using polyclonal antibodies directed against

synthetic peptides mimicking the amino acid sequence of *Vibrio cholerae* zonula occludens toxin (Zot) or pre-HP2. Structural biology studies and mass spectrometry analyses have demonstrated that these antibodies exhibit significant cross-reactivity with multiple structurally related proteins. Consequently, these assays do not exclusively measure biologically active pre-HP2 (zonulin), but rather detect a broader group of so-called zonulin-related proteins, including mature haptoglobin, properdin, and selected components of the complement system.

This limited antigenic specificity has fundamental implications for the choice of biological material used for zonulin assessment, particularly in patients with obesity. In the study by Seethaler et al. (2021), the reliability and stability of plasma and fecal zonulin measurements were critically evaluated and compared with the lactulose/mannitol permeability test (lac/man ratio), considered a reference method for intestinal barrier assessment. The authors found no significant correlation between plasma zonulin concentrations and urinary sugar excretion ratios. This finding may be explained by the fact that obesity is characterized by chronic low-grade systemic inflammation, during which hepatic synthesis of haptoglobin, an acute-phase protein, is markedly increased. High circulating concentrations of haptoglobin may interfere with plasma ELISA measurements, resulting in misidentification of this protein as zonulin and limiting the ability to accurately assess intestinal barrier function. (9,27)

In contrast, Seethaler et al. (2021) obtained more promising results when analyzing zonulin-reactive proteins (*zonulin-like proteins*) directly in fecal samples. Unlike plasma measurements, fecal biomarker concentrations demonstrated a statistically significant correlation with the lac/man ratio, with the strongest and most specific association observed among individuals with overweight and obesity. Furthermore, a strong positive correlation was identified between fecal zonulin concentrations and body mass index (BMI).

The use of fecal samples provides important methodological advantages: it reflects local intestinal secretion of zonulin induced by direct exposure of enterocytes to stimulating factors, such as obesity-associated dysbiotic microbiota and dietary factors. Additionally, fecal samples are not influenced by the high concentrations of hepatic acute-phase proteins present in the circulation, thereby reducing the impact of ELISA cross-reactivity. (9,27,28)

In summary, current evidence indicates that although ELISA-based zonulin measurements have limitations regarding molecular specificity, as they detect a broader group of zonulin-related proteins, their application to fecal samples in patients with obesity may overcome

important systemic confounding factors. Measurement of fecal zonulin-like proteins, unlike serum concentrations, represents a promising, reproducible, and fully non-invasive surrogate biomarker that may reflect metabolically induced impairment of tight junction integrity in this patient population. (9,26,27,28)

4. Discussion

4.1. From molecular mechanisms to clinical biomarkers: the role of intestinal permeability in obesity

A major challenge in understanding the role of intestinal barrier dysfunction in obesity is the precise mapping of the cascade of events, from the initial stimulus, through intracellular structural regulation, to a clinically measurable biomarker. Analysis of studies by El Asmar et al. (2002), Horowitz et al. (2023), and Seethaler et al. (2021) enables integration of these three levels into a coherent pathophysiological model. (6,7,9)

From bacterial stimulation to intestinal barrier disruption

The pioneering experimental study by El Asmar et al. (2002) provided the first direct evidence that exposure to pathogens and bacterial antigens induces rapid zonulin release by enterocytes. The authors demonstrated that this process was independent of bacterial species, as both pathogenic and non-pathogenic strains stimulated zonulin secretion. This suggests the existence of a universal host defense mechanism aimed at facilitating microbial clearance through controlled enhancement of paracellular fluid transport. (6)

In obesity, this mechanism acquires a pathological and chronic character. Patients with obesity exhibit qualitative and quantitative alterations in gut microbiota composition (dysbiosis), including a reduction in butyrate-producing bacteria and an increased proportion of Gram-negative microorganisms. Persistent exposure of enterocytes to lipopolysaccharide (LPS) and other bacterial ligands becomes a continuous stimulus activating the zonulin pathway.

The complexity of tight junction (TJ) regulation should also be emphasized. Modulation of proteins such as occludin, claudins, and ZO-1 by zonulin is not an all-or-none process ("open/closed"), but rather involves subtle kinetic changes. In obesity, continuous microbiota-

driven zonulin secretion may prevent complete restoration and stabilization of TJ complexes, resulting in chronic intestinal barrier dysfunction. (6,7,25,26)

Transition from molecular models to clinical application

The translation of molecular findings into clinical practice remains limited by methodological challenges. Previous literature has reported inconsistent results regarding zonulin concentrations in individuals with obesity, which Seethaler et al. attributed primarily to analytical limitations of plasma ELISA assays, particularly cross-reactivity with haptoglobin and properdin. Because obesity is characterized by chronic low-grade systemic inflammation, plasma zonulin measurements may reflect hepatic inflammatory activity and acute-phase protein synthesis rather than true intestinal barrier integrity.

A solution supported by methodological validation has been the shift toward fecal biomarker analysis. Seethaler et al. demonstrated that fecal zonulin concentrations correlate with the reference lactulose/mannitol permeability test specifically in individuals with overweight and obesity. The direct association between fecal zonulin levels and BMI further supports the concept that this marker reflects local intestinal production associated with the severity of metabolic disease.

Therefore, in patients with obesity, fecal zonulin may represent more than an auxiliary biomarker and could become an important indicator of intestinal barrier dysfunction and metabolic endotoxemia at its origin. (6,7,9,27,28)

4.2. Limitations and future perspectives

Despite promising findings, implementation of fecal zonulin measurement into routine clinical practice requires further standardization. Important limitations include substantial interindividual variability in daily zonulin excretion, the influence of short-term dietary factors, and the absence of universally accepted reference ranges for populations with metabolic syndrome.

Nevertheless, integrated evidence from the analyzed studies suggests that monitoring fecal zonulin concentrations in patients with obesity may provide new opportunities for personalized medicine. It may allow evaluation of whether interventions such as targeted dietary therapy,

prebiotic supplementation, or bariatric surgery effectively reduce inflammation at its intestinal source.

Results obtained over the years using commercially available ELISA assays also require reconsideration of terminology. This parameter should not be interpreted as an absolute concentration of pure zonulin (pre-HP2), but rather as a composite measurement of zonulin-related proteins, including mainly properdin and other immunologically related molecules. Such measurements may reflect systemic immune and metabolic activation rather than exclusively intestinal barrier function. (9,22,25,26,27)

Disclosure

Author Contributions

Conceptualization: AU, MP, RP, KK, EP

Methodology: MP, AU, ZW, JW, KK

Investigation: MP, AU, PW, JW

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