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Connecting the Dots: Zonulin, Microbiota-Gut-Brain Axis and Diabetes

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ABSTRACT:

Pathophysiology of T2DM study reports advocate that gut microbiota (GM) plays a significant role in its development. Interaction between the GM and the CNS is a complex bidirectional, interdependent link, known as the "gut-brain axis," via the nervous, neuroendocrine, and immune systems. Since the microbiota also ply an important role in the gut brain axis modulation it can be considered as Gut microbiota brain axis (GMBA). This can happen through a leaky gut and disturbed BBB where the TJ morphology gets disrupted due to the altered “gatekeeper” molecule Zonulin levels. it is said to be of enteric epithelial cells and endothelial cells of BBB.

Increased serum zonulin levels are accompanied by a leaky intestinal barrier, dysbiosis and inflammation. Regardless of the significant task of the brain, it is difficult to trace the prime role in glucose metabolism and homeostasis. It is observed that along with appetite control and intestinal glucose uptake, it would be probable to regulate glucose homeostasis by maintaining normal intestinal microbiota. GM also regulate brain activity via various signalling molecules passing through BBB. Thus, it appears that the GM is a potential new therapeutic target for the effective treatment of metabolic diseases.

1. INTRODUCTION

Diabetes mellitus, commonly referred to as sugar, is a common endocrinological problem characterized by continuous elevation of blood glucose level in the body. A small glimpse into diabetes shows its existence since ancient times. The word diabetes mellitus is a combination of two words, diabetes is taken from Greek meaning siphon-to pass through & mellitus from Latin, meaning sweet [1]. 537 million adults are living with diabetes and this number is said to rise to 643 million by 2045 [2]. Maintaining normal glucose levels is crucial in delaying or potentially preventing diabetes-related health complications such as cardiovascular issues, renal problems, and nerve damage. Effective management and control are achievable through pharmacological therapy, external insulin, or allogeneic islet transplantation. However, they have certain limitations & cannot cure diabetes permanently [1]. Understanding a simple aspect affecting the disease can also be helpful in preventing or delaying the disease and its complications.

The American Diabetes Association [ADA] classifies diabetes into several categories based on genetic, metabolomic and other factors that influences the disease pathophysiology [3,4].

1. Type 1 diabetes: autoimmune beta cell destruction leading to insulin deficiency
2. Type 2 diabetes: non-autoimmune progressive loss of beta cell insulin secretion
3. Specific types due to other causes- neonatal diabetes, maturity-onset diabetes of the young [MODY]
4. Gestational diabetes

Diabetes mellitus not only affects glucose homeostasis, but also increases the risk of macrovascular and microvascular complications leading to a wide spectrum of cardiovascular, renal and neuronal complications. These complications, driven by chronic hyperglycemia and associated metabolic disturbances, often lead to multiple long-term conditions that significantly impact quality of life and healthcare outcomes [5].

Emerging evidence highlights the systemic nature of diabetes, where metabolic dysregulation extends beyond traditional target organs to include the gastrointestinal [GI] tract. Diabetes can impact every part of the GI tract

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from oesophagus to rectum. Altered gut function, including gastroparesis, enteropathy, and large bowel dysfunction, has been identified as frequent yet unrecognized complications in individuals with diabetes. These conditions manifest as symptoms like diarrhea, constipation, abdominal discomfort, nausea, heartburn, etc., and are linked to changes in gut motility, microbiota composition, and mucosal permeability. Together, they represent a significant source of morbidity, compounding the overall disease burden [6] [7].

As the gut becomes increasingly involved in diabetes-related complications, recent studies have highlighted the role of Zonulin, a key regulator of intestinal permeability, which also said to have an important role in the pathogenesis of diabetes [8,9]. Increased levels of zonulin enhanced gut permeability, commonly known as 'leaky gut", allowing pathogens and harmful substances to pass through the gut barrier [10]. This disruption is known to be associated with gut microbiota, due to altered composition and diversity. Changes in the bacterial composition of the gut are referred to as dysbiosis [11]. Dysbiosis have also been observed before the onset of conditions like diabetes & Parkinson's disease, with growing evidence supporting its role in the pathogenesis of various immune-mediated, metabolic, and neurological disease [12]. Hence it could be considered as an effect as well as after-effect of the condition.

Any disruption in the host microbiota crosstalk can act as a trigger or reinforce disease development. A wide range of metabolites mediate the interaction between the host and its gut microbiome. The three most extensively studied metabolites are 1. short chain fatty acid [SCFAs], produced by bacterial fermentation of fibers; 2. bile acids, synthesized in the liver and modified by the gut microbiota before impacting the host again; and 3. tryptophan [Trp] metabolites [13]. Hence, the gut microbiome has also gained significant attention as a therapeutic target for metabolic, neurological and psychiatric disorders [14] and are all the said to be the consequences of DM also. A key mediator in this complex interplay is zonulin, a protein that regulates intestinal permeability and plays a vital role in gut barrier function. Altered levels of zonulin are one of the important disrupters of gut microbiota distribution, further exacerbating metabolic and inflammatory processes.

In this article, we review the most recent insights into the role of zonulin and gut microbiota with a focus on its impact on both physiology and diseases, particularly diabetes. Furthermore, we explore the role of zonulin in modulating intestinal permeability, its impact on gut microbiota composition, and the implications for diabetes, where disruptions in and gut barrier function contribute to disease progression.

ZONULIN IN REGULATION OF INTESTINAL PERMEABILITY

In 2000s Alessio Fasano and his team at the university of Maryland School of Medicine, discovered zonulin, a human protein analogous to the zonula occludens toxin [Zot] secreted by cholera pathogen *Vibrio cholerae*. While much of the research on zonulin has focused on intestinal diseases, its significance has now been recognised in almost all organs including brain, heart, lungs, kidneys, liver and skin [15–20].

INSIGHT INTO STRUCTURE AND GENERAL CHARACTERISTIC FEATURES:

Zonulin, or haptoglobin 2 precursor or pre-haptoglobin 2 [HP2], a dimeric paracrine glycoprotein, synthesized in enterocytes and hepatocytes, that reversibly regulates tight junctions linking the cell wall of the intestinal cells and refrains intestinal permeability. Its molecular weight is about 47 kDa & is made up of-

- A chain: cell surface receptor binding part [N-terminal receptor binding motif]
- B chain: involved in the regulation of gut permeability [C-terminal domain is thought to be involved in rearranging the cytoskeletal elements that are connected to the intercellular tight junctions [TJs]] and are connected by disulfide bridges [21].

The intestinal epithelium serves as a largest surface between the external and internal environment of the host body, acting as a barrier against pathogens and selectively allowing the nutrient components, water and electrolytes. In the gut, tight junctions [TJs] are multiprotein complexes that seal the spaces between adjacent enterocytes to maintain the barrier function of the intestinal epithelium. These TJs act as dynamic doors, responding to physiological or pathological stimuli such as inflammation or imbalances in TJ components, which leads to either close or open the intercellular space [20].

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To date, only zonulin is the human protein known to reversibly regulate intestinal permeability by altering the TJs. Indeed, zonulin acts as a gate keeper/ watchdog.

Dysbiosis and exposure to gliadin are some reasons to trigger the release of zonulin. Gliadin binds to the chemokine receptor type 3 [CXCR3], on enterocytes and monocytes, signalling the cells to increase zonulin production. Once secreted into the lumen of intestine, it activates the epidermal growth factor receptor [EGFR] via protease activated receptor 2 [PAR2]. Upon activation it triggers an intracellular cascade of biochemical reactions, leading to the phosphorylation of zona occluden [ZO] proteins and myosin, along with actin polymerisation. As a result, detachment of ZO proteins from the TJ complex takes place and impairing the structural features of tight junctions [22]. As the zonulin level increases and it weakens the TJs, the subsequent cellular entry of metabolites and toxins triggers the local inflammation. These are known to be associated with many chronic inflammatory diseases including type 1 diabetes, type 2 diabetes, multiple sclerosis, HIV, Glioma., [10]. It has been observed that zonulin regulates paracellular permeability via activation of protein kinase C [PKC]- dependent rearrangement of actin microfilaments and the disruption of the Zot-1protein structure. Role of the zonulin pathway in regulating intestinal permeability has been further validated by using an antagonist larazotide acetate [AT 1001], during phase 2 clinical studies showed beneficial effects in patients with celiac disease [23].

FACTORS AFFECTING ZONULIN EXPRESSION AND ACTIVITY

Zonulin expression and activity are modulated by a variety of factors, ranging from dietary components to genetic including environmental influences. These factors collectively impact intestinal permeability and contribute to the pathogenesis of various diseases, such as metabolic and diabetes, celiac diseases, and inflammatory disorders [10]. Understanding these factors can help us in identifying potential therapeutic targets to restore gut barrier integrity.

TABLE 1: KEY FACTORS AFFECTING ZONULIN'S EXPRESSION AND THEIR IMPLICATIONS

Category	Factors	Impact on zonulin	implications
Dietary components	Gliadin [Gluten]	Strong inducer of zonulin release	Impairs gut barrier, linked to celiac disease & inflammation [23,24]
	High fat diet	Upregulates zonulin expression	Contributes to leaky gut & metabolic disorder [25]
	Excessive fructose	Elevates zonulin levels	Associated with increase increased intestinal permeability and systemic inflammation [26]
Microbial factor	Pathogenic bacteria [*E. coli*, *V. cholera*, *S. typhimurium* .,]	Stimulate zonulin release to disrupt TJ's	Increases gut permeability and infection related complications [27]
	Dysbiosis	Alters zonulin regulation	Associated with leaky gut [22]
Inflammatory mediators	Cytokines [TNF alpha]	Elevates zonulin expression	Enhances intestinal permeability in chronic inflammat

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			ory state [28]
	Autoimmune conditions	Triggers zonulin release	Dysfunction of tight junction in autoimmune disease in celiac disease and type 1 diabetes [29]
Metabolic dysregulation	Hyperglycaemia	Upregulates zonulin level	Exacerbate gut barrier dysfunction in diabetes [30]
	Obesity	Associated with elevated zonulin	Linked with low grade inflammation and metabolic syndrome [31]
Environmental factors	Toxins and pollutants	Increases zonulin expression	Disrupts gut barrier integrity [10]
Hormonal regulation	Cortisol	Fluctuates gut permeability	Influences gut permeability during stress [32]
	Insulin	Increases gut barrier permeability	Contributes gut barrier dysfunction in diabetes [33]

ZONULIN IN DIABETES

A plethora of studies have highlighted the dysregulation of zonulin and its significant association with diabetes, particularly with respect to its role in modulating intestinal permeability, which is believed to contribute to both the onset and progression of the disease.

Numerous studies involving both animal and clinical trials have shown that increased intestinal permeability often anticipate the development of T1D [34]. Notably, the oral administration of the zonulin blocker AT1001 in these rats rectified the gut barrier defect and reduced the incidence of diabetes, suggesting that zonulin-dependent gut barrier modulation plays a mechanistic role in T1D pathogenesis [35]. One more study revealed that in men with T1D, during severe exercise, levels of muscle damage markers and zonulin were elevated [36]. A study in mice, focused on the interplay between the gut barrier, microbiome, and immune system revealed that susceptibility to T1D is due to disrupted gut barrier integrity where dysbiosis played a key role [35]. BioBreeding diabetes-prone rats, which spontaneously develop T1D, showed elevated intestinal permeability at least a month before the onset of glucose intolerance [37]. Moreover, human studies have further reinforced the involvement of zonulin in T1D, reported that nearly 50% of T1D patients have elevated serum zonulin levels. However, some of these showed changes even during the pre-diabetic phase. Interestingly, about 25% of healthy first-degree relatives of T1D patients also exhibited increase in serum zonulin levels [34].

A study by Jayashree *et al.,* shows an association between elevated circulatory lipopolysaccharide [LPS], it's activity, and zonulin levels in Asian Indian T2DM patients, suggesting that these could serve as novel biomarkers of proinflammation and poor glycaemic control [38]. One more study on Brazilian T2D patients with renal complications identified serum zonulin levels as a marker of impaired intestinal permeability in comparison with that of normal renal functions [18]. This observation also highlights the role of zonulin in diabetic kidney disease. A study by E*rdem et al.,* found that in T2D patients with proliferative diabetic retinopathy, zonulin levels were significantly higher when compared to those with non-proliferative diabetic retinopathy and those without diabetic retinopathy [39]. This suggests that setting intestinal permeability maybe crucial in

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therapeutic approach towards diabetic retinopathy. Other study conducted by *K Mokkala et al.,* found a correlation between serum zonulin levels in early pregnancy and gestational diabetes mellitus, proposing zonulin could be a potential marker for gestational diabetes mellitus [40].

Together these findings indicate that zonulin and intestinal permeability plays a significant role in diabetes and its complications.

ZONULIN AND MICROBIOTA GUT BRAIN AXIS

The microbiota-gut-brain-axis [MGBA] refers to the bidirectional communication between the gut microbiota and the brain. This crosstalk between the gut microbiota and the brain occurs through several interconnected systems including the immune system, the vagus nerve, enteric nervous system, neuroendocrine system, and the circulatory system [41]. Although the concept of microbiota-gut-brain-axis is not new, the significance of the MGBA has been highlighted by numerous human studies showing a strong association between alterations in gut microbiota composition and neurological disorders. These include conditions such as Parkinson's disease [42], Alzheimer's disease [43], autism [44], anxiety & depression [45]. These findings highlight the vital role of gut microbiota in affecting the brain health and neurodegenerative conditions. Moreover, the role of microbiota-gut-brain-axis has significant impact even in metabolic disorder like obesity [46], diabetes [41], NAFLD [47].

An adult human body comprises of 100 trillion bacteria, with around 80% residing in the gut [48]. Having considered as one of the important regulators of gut-brain-axis, GM plays an integral role in the maintenance of gut's structural and functional barriers [48] through TJ modifications.[29]. This makes GM a crucial performer in understanding the complex interaction of gut-brain-axis in diabetes-related complications.

Dysbiosis is known to be associated with increased intestinal zonulin levels, impaired gut permeability. Similar to that of gliadin, a minute exposure to bacterial overgrowth triggers zonulin pathway to serves as a defensive mechanism that expels the microorganisms by contribuing to innate immune response of the host against dysbiosis, and heightened levels of inflammatory mediators [10].

Consequently, gut derived microbial fragments, toxins, and inflammatory markers can spread to distant organs, including central nervous system, this spread leads to the increased permeability of the blood brain barrier [BBB], neuroinflammation, and associated behavioural changes [49]. Together these finding suggest that dysbiosis and

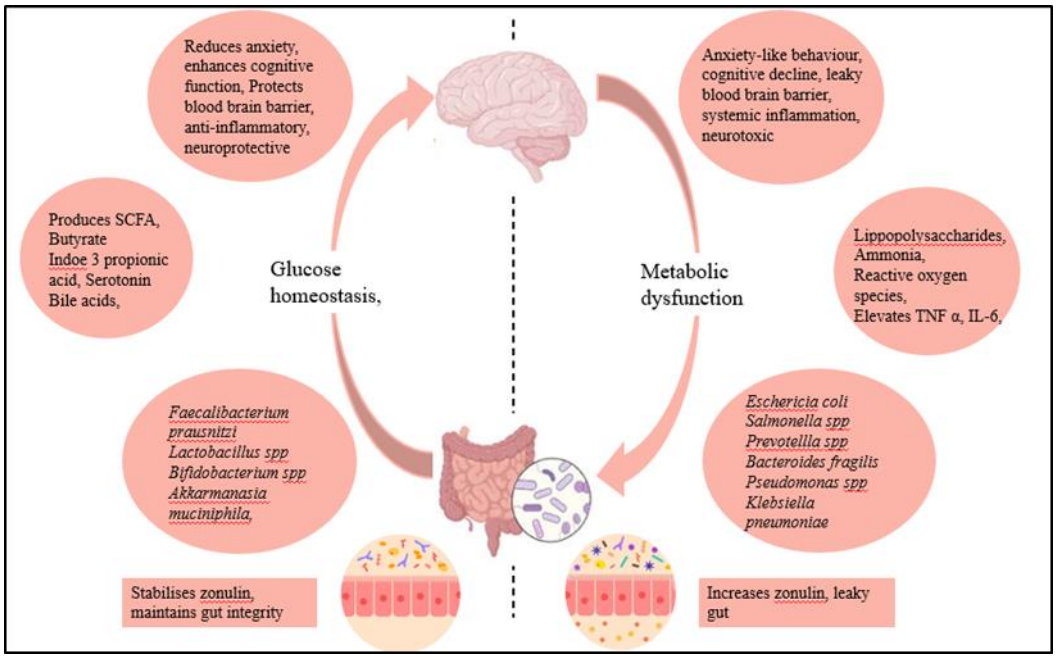

Figure 1 Microbiota-Gut Brain Axis and Glucose homeostasis

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the zonulin pathway may play a crucial role in diseases related microbiota gut brain axis [MGBA].

Nonetheless, the connection between gut microbiota and the regulation of zonulin release is undeniable. Number of studies are focusing on the impact of different microbial species on zonulin levels in both descriptive and interventional human studies. Briefly, several gram-negative bacterial strains, such as *E. coli, Prevotella, Pseudomonas,* and *Salmonella spp.,* promote the release of intestinal zonulin, while other strains, primarily gram-positive, like *Bifidobacterium* and *Lactobacillus spp.* involves their ability to break down gluten peptides by hydrolysing enzymes, thereby prevents gliadin-induced cytotoxic responses in intestinal epithelial cells. Interestingly, recent studies suggest an additional pathway, as heat-killed *Bifidobacterium* and *Lactobacillus spp.,* which lack active enzymes and still

exhibits beneficial effects on epithelial barrier function [50,51].

Zonulin figures not only the permeability of the intestinal barrier but also the BBB. Simillar to that of intestinal ECs, in BBB endothelial cells, pericytes, and astrocytes separates CNS from the blood vessels and performs the primary role in the maintenance of BBB integrity and protects the CNS [52]. The leaky BBB enables the invasion of microbiota and immune cells Under normal conditions CNS is devoid of microbiota and immune cells and a “leaky BBB” allows these to disturb the natural neuronal environment [53]. This may be a cause or consequence of an impaired gut immune barrier [GIB]and is one of the checkpoints/ first line of defence against microbial invasion and food antigens, which alters the properties of zonulin [52]. Hence the microbiota–gut–brain axis, a defective GIB also impacts brain pathogenesis. It is also evident that, under long run impaired claudin-5 assembly due to zonulin is another cause of leaky BBB.

ALTERATIONS IN GUT MICROBIOTA COMPOSITION AND THEIR INFLUENCE ON THE GUT-BRAIN AXIS IN DIABETES

Microbiota gut brain axis is a complex network of communication between the gut microbiota, the enteric nervous system, and the central nervous system [CNS]. This bidirectional signalling pathway has gained significant attention in recent years, especially concerning its role in various metabolic disorders, including diabetes. Due to the interplay between the gut microbiome and the host homeostasis, the gut microbiome is thought to play a vital role in obesity and metabolic dysfunction [54]. The intestinal microbiota influences key processes such as host metabolism, appetite regulation, body weight, glucose and lipid balance. Alteration in its composition, or gut dysbiosis, has been consistently associated with obesity and diabetes in both human and animal studies [55].

Gut dysbiosis also can prompt imbalance in the cellular oxidant: antioxidant status through excessive production of ROS and could be another source for inflammation through disrupted gut barrier integrity for activation of the immunological processes and altered metabolic pathways. Gut dysbiosis through inflammation also, inflammation-induced ROS can also intensify gut dysbiosis. Experimental studies in mice have shown that host ROS production alters the gut microbiota species diversity and gut microbiome composition. It has been observed that ROS induced dysbiosis and inflammation can be mitigated through probiotics and enhance the beneficial bacteria [56]

Changes in the composition of the gut microbiota in patients with DM have been studied in recent years. The results have shown that the Firmicutes/Bacteroidetes ratio is higher in patients with diabetes as compared with healthy individuals [57]. Therapeutic approaches like probiotics, prebiotics, antidiabetic drugs, or dietary interventions have shown potential in modulating the growth or restoration of the beneficial bacteria while reducing harmful ones. Such changes have been attributed to beneficial outcomes, including weight reduction, reduced inflammation, and improved glucose tolerance/ regulation. Hence, targeting the gut microbiota is emerging as one of the promising possibilities for diabetes management and therapy [58].

There are evidence suggesting a correlation between total bacteria and serum zonulin levels, indicating that gut microbiota may elevate zonulin levels, leading to abnormal gut permeability to endotoxins and subsequent micro-inflammation in obesity [59]. Additionally, recent findings showed that serum zonulin levels correlate with total calorie, protein, carbohydrate, sodium, and vitamin B12 intake in obese women. *Ruminococcaceae* and *Faecalibacterium* were more abundant in the low-

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zonulin group, implying that butyrate-producing gut bacteria like Faecalibacterium may reduce gut permeability by lowering zonulin levels and mitigates the inflammation [60].

CONCLUSION

The bidirectional communication between the gut microbiota and brain plays an important role in the host's optimal metabolic and neurological. Alterations in gut microbiota composition in diabetes disrupt the gut-brain axis by impairing gut barrier function and promoting systemic and neuroinflammation. This highlights the role of gut integrity and immune modulation in diabetes pathogenesis. Conversely, eubiosis through targeted interventions such as probiotics, dietary modifications, or zonulin inhibitors offers potential therapeutic approach to modulating the gut-brain axis and improving both metabolic and neurological outcomes in diabetes.

Future research should focus on unraveling the complex mechanisms underlying microbiota-host interactions, enabling the development of precision therapies that restore microbial balance, improve glucose homeostasis, and enhance overall well-being in individuals with diabetes.

Refrences

1. Mudaliar, S., 2023. The evolution of diabetes treatment through the ages: from starvation diets to insulin, incretins, SGLT2-inhibitors and beyond. *Journal of the Indian Institute of Science*. *103*(1), 123-133.
2. Magliano, D.J., Boyko, E.J. and Atlas, I.D., 2021. What is diabetes? In *IDF DIABETES ATLAS [Internet]*. *10th edition*. International Diabetes Federation.
3. Diagnosis and classification of diabetes: standards of care in diabetes—2024." *Diabetes Care* 47, no. Supplement_1 (2024): S20-S42.
4. American Diabetes Association, 2013. Diagnosis and classification of diabetes mellitus. *Diabetes care*, *36*(Supplement_1), S67-S74.
5. Gregg, E. W., Pratt, A., Owens, A., Barron, E., Dunbar-Rees, R., Slade, E. T., Hafezparast, N., Bakhai, C., Chappell, P., Cornelius, V., Johnston, D. G., Mathews, J., Pickles, J., Bragan Turner, E., Wainman, G., Roberts, K., Khunti, K., & Valabhji, J., 2024. The burden of diabetes-associated multiple long-term conditions on years of life spent and lost. *Nature medicine*, *30*(10), 2830–2837.
6. Verne, G. N., & Sninsky, C. A., 1998. Diabetes and the gastrointestinal tract. *Gastroenterology clinics of North America*, *27*(4), 861–vii.
7. Maisey A., 2016. A Practical Approach to Gastrointestinal Complications of Diabetes. *Diabetes therapy: research, treatment and education of diabetes and related disorders*, *7*(3), 379–386.
8. Wood Heickman, L. K., DeBoer, M. D., & Fasano, A., 2020. Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity. *Diabetes/metabolism research and reviews*, *36*(5), e3309.
9. Mønsted, M. Ø., Falck, N. D., Pedersen, K., Buschard, K., Holm, L. J., & Haupt-Jorgensen, M., 2021. Intestinal permeability in type 1 diabetes: An updated comprehensive overview. *Journal of autoimmunity*, *122*, 102674.
10. Fasano A., 2020. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000Research*, *9*, F1000 Faculty Rev-69.
11. Bidell, M. R., Hobbs, A. L. V., & Lodise, T. P. 2022. Gut microbiome health and dysbiosis: A clinical primer. *Pharmacotherapy*, *42*(11), 849–857.
12. Hrncir T., 2022. Gut Microbiota Dysbiosis: Triggers, Consequences, Diagnostic and Therapeutic Options. *Microorganisms*, *10*(3), 578.
13. Agus, A., Planchais, J., & Sokol, H. 2018. Gut Microbiota Regulation of Tryptophan Metabolismin Health and Disease. *Cell host & microbe*, *23*(6), 716–724.
14. Roth, W., Zadeh, K., Vekariya, R., Ge, Y., & Mohamadzadeh, M., 2021. Tryptophan Metabolism and Gut-Brain Homeostasis. *International journal of molecular sciences*, *22*(6), 2973.
15. Koksal, B. T., Zengin, H. Y., & Ozbek, O. Y., 2022. Assessment of Mucosa-Associated Epithelial Chemokine, Thymus-Expressed Chemokine, Periostin and Zonulin Levels in Infants With Atopic Dermatitis. *Indian journal of dermatology*, *67*(3), 312.
16. Kim, N. Y., Shin, E., Byeon, S. J., Hong, S. J., Kang, S. H., Lee, T., Kim, T. B., & Choi, J. H. 2023. Serum Zonulin Is a Biomarker for Severe Asthma. *Allergy, asthma & immunology research*, *15*(4), 526–535.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(1), 113-122 | ISSN:2251-6727

17. Covino, M., Gallo, A., Macerola, N., Pero, E., Ibba, F., Camilli, S., Riccardi, L., Sarlo, F., De Ninno, G., Baroni, S., Landi, F., & Montalto, M. 2023. Role of Intestinal Inflammation and Permeability in Patients with Acute Heart Failure. *Medicina (Kaunas, Lithuania)*, *60*(1), 8.
18. Carpes, L. S., Nicoletto, B. B., Canani, L. H., Rheinhemer, J., Crispim, D., & Souza, G. C. 2021. Could serum zonulin be an intestinal permeability marker in diabetes kidney disease?. *PloS one*, *16*(6), e0253501.
19. Voulgaris, T. A., Karagiannakis, D., Hadziyannis, E., Manolakopoulos, S., Karamanolis, G. P., Papatheodoridis, G., & Vlachogiannakos, J. 2021. Serum zonulin levels in patients with liver cirrhosis: Prognostic implications. *World journal of hepatology*, *13*(10), 1394–1404.
20. Boschetti, E., Caio, G., Cervellati, C., Costanzini, A., Rosta, V., Caputo, F., De Giorgio, R., & Zuliani, G. 2023. Serum zonulin levels are increased in Alzheimer's disease but not in vascular dementia. *Aging clinical and experimental research*, *35*(9), 1835–1843.
21. Fasano A. 2011. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiological reviews*, *91*(1), 151–175.
22. Macura, B., Kiecka, A., & Szczepanik, M. 2024. Intestinal permeability disturbances: causes, diseases and therapy. *Clinical and experimental medicine*, *24*(1), 232.
23. Veres-Székely, A., Szász, C., Pap, D., Szebeni, B., Bokrossy, P., & Vannay, Á. 2023. Zonulin as a Potential Therapeutic Target in Microbiota-Gut-Brain Axis Disorders: Encouraging Results and Emerging Questions. *International journal of molecular sciences*, *24*(8), 7548.
24. Clemente, M. G., De Virgiliis, S., Kang, J. S., Macatagney, R., Musu, M. P., Di Pierro, M. R., Drago, S., Congia, M., & Fasano, A. 2003. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. *Gut*, *52*(2), 218–223.
25. Ahmadi, S., Wang, S., Nagpal, R., Wang, B., Jain, S., Razazan, A., Mishra, S. P., Zhu, X., Wang, Z., Kavanagh, K., & Yadav, H. (2020). A human-origin probiotic cocktail ameliorates aging-related leaky gut and inflammation via modulating the microbiota/taurine/tight junction axis. *JCI insight*, *5*(9), e132055.
26. Guo, P., Wang, H., Ji, L., Song, P., & Ma, X. (2021). Impacts of Fructose on Intestinal Barrier Function, Inflammation and Microbiota in a Piglet Model. *Nutrients*, *13*(10), 3515.
27. Serek, P., & Oleksy-Wawrzyniak, M. 2021. The Effect of Bacterial Infections, Probiotics and Zonulin on Intestinal Barrier Integrity. *International journal of molecular sciences*, *22*(21), 11359.
28. Sturgeon, C., & Fasano, A. 2016. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue barriers*, *4*(4), e1251384.
29. Fasano A. 2012. Zonulin, regulation of tight junctions, and autoimmune diseases. *Annals of the New York Academy of Sciences*, *1258*(1), 25–33.
30. Yuan, J. H., Xie, Q. S., Chen, G. C., Huang, C. L., Yu, T., Chen, Q. K., & Li, J. Y. 2021. Impaired intestinal barrier function in type 2 diabetic patients measured by serum LPS, Zonulin, and IFABP. *Journal of diabetes and its complications*, *35*(2), 107766.
31. Ohlsson, B., Orho-Melander, M., & Nilsson, P. M. (2017). Higher Levels of Serum Zonulin May Rather Be Associated with Increased Risk of Obesity and Hyperlipidemia, Than with Gastrointestinal Symptoms or Disease Manifestations. *International journal of molecular sciences*, *18*(3), 582.
32. Linninge, C., Jönsson, P., Bolinsson, H., Önning, G., Eriksson, J., Johansson, G., & Ahrné, S. 2018. Effects of acute stress provocation on cortisol levels, zonulin and inflammatory markers in low- and high-stressed men. *Biological psychology*, *138*, 48–55.
33. Wood Heickman, L. K., DeBoer, M. D., & Fasano, A. 2020. Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity. *Diabetes/metabolism research and reviews*, *36*(5), e3309.
34. Sapone, A., de Magistris, L., Pietzak, M., Clemente, M. G., Tripathi, A., Cucca, F., Lampis, R., Kryszak, D., Cartenì, M., Generoso, M., Iafusco, D., Prisco, F., Laghi, F., Riegler, G., Carratu, R., Counts, D., & Fasano, A. 2006. Zonulin upregulation is associated with increased gut permeability in subjects with type

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www.jchr.org

JCHR (2025) 15(1), 113-122 | ISSN:2251-6727

1 diabetes and their relatives. *Diabetes*, *55*(5), 1443–1449.

35. Watts, T., Berti, I., Sapone, A., Gerarduzzi, T., Not, T., Zielke, R., & Fasano, A. 2005. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(8), 2916–2921.
36. Matejko, B., Tota, Ł., Morawska-Tota, M., Pałka, T., Malecki, M. T., & Klupa, T. 2023. Assessment of selected muscle damage markers and zonulin concentration after maximum-intensity exercise in men with type 1 diabetes treated with a personal insulin pump. *Acta diabetologica*, *60*(12), 1675–1683.
37. Meddings, J. B., Jarand, J., Urbanski, S. J., Hardin, J., & Gall, D. G. 1999. Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat. *The American journal of physiology*, *276*(4), G951–G957.
38. Jayashree, B., Bibin, Y. S., Prabhu, D., Shanthirani, C. S., Gokulakrishnan, K., Lakshmi, B. S., Mohan, V., & Balasubramanyam, M. 2014. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Molecular and cellular biochemistry*, *388*(1-2), 203–210.
39. Erdem, B., Kaya, Y., Kıran, T. R., & Yılmaz, S. 2023. An Association Between the Intestinal Permeability Biomarker Zonulin and the Development of Diabetic Retinopathy in Type II Diabetes Mellitus. *Turkish journal of ophthalmology*, *53*(2), 91–96.
40. Mokkala, K., Tertti, K., Rönnemaa, T., Vahlberg, T., & Laitinen, K. 2017. Evaluation of serum zonulin for use as an early predictor for gestational diabetes. *Nutrition & diabetes*, *7*(3), e253.
41. Moysidou, C. M., & Owens, R. M. 2021. Advances in modelling the human microbiome-gut-brain axis in vitro. *Biochemical Society transactions*, *49*(1), 187–201.
42. Tan, A. H., Lim, S. Y., & Lang, A. E. 2022. The microbiome-gut-brain axis in Parkinson disease - from basic research to the clinic. *Nature reviews. Neurology*, *18*(8), 476–495.
43. Doifode, T., Giridharan, V. V., Generoso, J. S., Bhatti, G., Collodel, A., Schulz, P. E., Forlenza, O. V., & Barichello, T. 2021. The impact of the microbiota-gut-brain axis on Alzheimer's disease pathophysiology. *Pharmacological research*, *164*, 105314
44. Morton, J. T., Jin, D. M., Mills, R. H., Shao, Y., Rahman, G., McDonald, D., Zhu, Q., Balaban, M., Jiang, Y., Cantrell, K., Gonzalez, A., Carmel, J., Frankiensztajn, L. M., Martin-Brevet, S., Berding, K., Needham, B. D., Zurita, M. F., David, M., Averina, O. V., Kovtun, A. S., … Taroncher-Oldenburg, G. 2023. Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. *Nature neuroscience*, *26*(7), 1208–1217.
45. Kumar, A., Pramanik, J., Goyal, N., Chauhan, D., Sivamaruthi, B. S., Prajapati, B. G., & Chaiyasut, C. 2023. Gut Microbiota in Anxiety and Depression: Unveiling the Relationships and Management Options. *Pharmaceuticals (Basel, Switzerland)*, *16*(4), 565.
46. Loos, R. J. F., & Yeo, G. S. H. (2022). The genetics of obesity: from discovery to biology. *Nature reviews. Genetics*, *23*(2), 120–133.
47. Song, Q., & Zhang, X. (2022). The Role of Gut-Liver Axis in Gut Microbiome Dysbiosis Associated NAFLD and NAFLD-HCC. *Biomedicines*, *10*(3), 524.
48. Bhalla, D., Dinesh, S., Sharma, S., & Sathisha, G. J. (2024). Gut-Brain Axis Modulation of Metabolic Disorders: Exploring the Intertwined Neurohumoral Pathways and Therapeutic Prospects. *Neurochemical research*, *49*(4), 847–871.
49. Miranda-Ribera, A., Serena, G., Liu, J., Fasano, A., Kingsbury, M. A., & Fiorentino, M. R. (2022). The Zonulin-transgenic mouse displays behavioral alterations ameliorated via depletion of the gut microbiota. *Tissue barriers*, *10*(3), 2000299.
50. Martorell, P., Alvarez, B., Llopis, S., Navarro, V., Ortiz, P., Gonzalez, N., Balaguer, F., Rojas, A., Chenoll, E., Ramón, D., & Tortajada, M. (2021). Heat-Treated *Bifidobacterium longum* CECT-7347: A Whole-Cell Postbiotic with Antioxidant, Anti-Inflammatory, and Gut-Barrier Protection Properties. *Antioxidants (Basel, Switzerland)*, *10*(4), 536.
51. Orlando, A., Linsalata, M., Notarnicola, M., Tutino, V., & Russo, F. (2014). Lactobacillus GG restoration

of the gliadin induced epithelial barrier disruption: the role of cellular polyamines. *BMC microbiology*, *14*, 19.

52. Daneman, R., & Rescigno, M. (2009). The gut immune barrier and the blood-brain barrier: are they so different?. *Immunity*, *31*(5), 722–735.
53. Hagemeyer, H., Hellwinkel, O. J. C., & Plata-Bello, J. (2024). Zonulin as Gatekeeper in Gut-Brain Axis: Dysregulation in Glioblastoma. *Biomedicines*, *12*(8), 1649.
54. Moser, B., Milligan, M. A., & Dao, M. C. (2022). The Microbiota-Gut-Brain Axis: Clinical Applications in Obesity and Type 2 Diabetes. *Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion*, *74*(6), 302–313.
55. Sircana, A., Framarin, L., Leone, N., Berrutti, M., Castellino, F., Parente, R., De Michieli, F., Paschetta, E., & Musso, G. (2018). Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence?. *Current diabetes reports*, *18*(10), 98.
56. Mostafavi Abdolmaleky, H., & Zhou, J. R. (2024). Gut Microbiota Dysbiosis, Oxidative Stress, Inflammation, and Epigenetic Alterations in Metabolic Diseases. *Antioxidants (Basel, Switzerland)*, *13*(8), 985.
57. Craciun, C. I., Neag, M. A., Catinean, A., Mitre, A. O., Rusu, A., Bala, C., Roman, G., Buzoianu, A. D., Muntean, D. M., & Craciun, A. E. (2022). The Relationships between Gut Microbiota and Diabetes Mellitus, and Treatments for Diabetes Mellitus. *Biomedicines*, *10*(2), 308.
58. Xi, Y., & Xu, P. F. (2021). Diabetes and gut microbiota. *World journal of diabetes*, *12*(10), 1693–1703.
59. Olszanecka-Glinianowicz, M., Chudek, J., Kocełak, P., Szromek, A., & Zahorska-Markiewicz, B. (2011). Body fat changes and activity of tumor necrosis factor α system--a 5-year follow-up study. *Metabolism: clinical and experimental*, *60*(4), 531–536.
60. Mörkl, S., Lackner, S., Meinitzer, A., Mangge, H., Lehofer, M., Halwachs, B., Gorkiewicz, G., Kashofer, K., Painold, A., Holl, A. K., Bengesser, S. A., Müller, W., Holzer, P., & Holasek, S. J. (2018). Gut microbiota, dietary intakes and intestinal permeability reflected by serum zonulin in women. *European journal of nutrition*, *57*(8), 2985–2997.