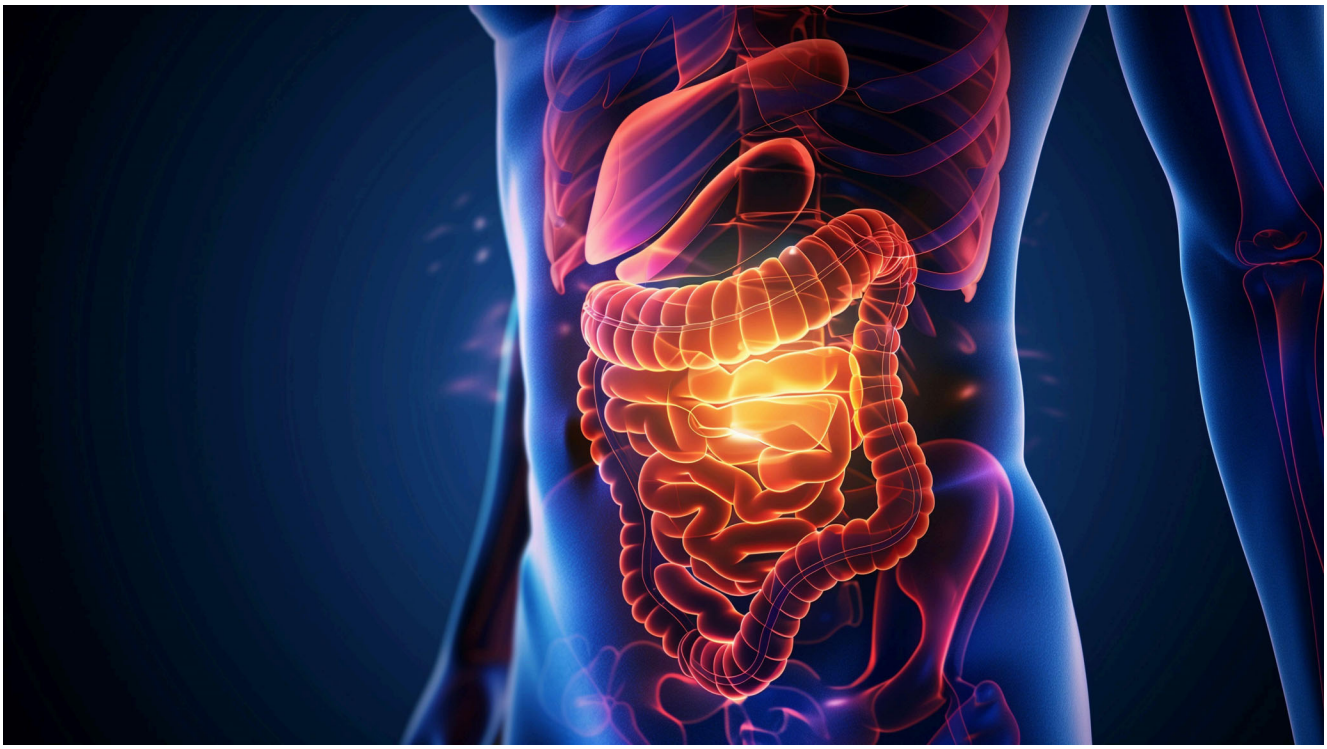
Are Zonulin Tests Useful? Answering the Critics



Zonulin is a protein that modulates the permeability of tight junctions between cells in the wall of the digestive tract that was discovered in 2000 by

Dr. Alessio Fasano and his team at the University of Maryland School of Medicine. The discovery was significant because it provided insight into how the intestinal barrier functions and how its permeability can be regulated and is considered to be a marker of intestinal permeability.

Fasano's team identified zonulin while studying the zonula occludens toxin (Zot) from the cholera pathogen Vibrio cholerae. Zot can bind to a specific receptor in the intestinal epithelium and regulate actin filaments to polymerize by activating protein kinase C (PKC), resulting in increased intestinal permeability. This led to the identification of zonulin as the mammalian analog of Zot. In later research, Fasano would continue to unravel zonulin's place in the scheme of intestinal permeability (sometimes referred to as leaky gut), and its connection to pathways of inflammation not only in the gut, but systemically. Fasano found initially that zonulin was part of the Haptoglobin pathway, meaning it must be associated with inflammation. Because zonulin was detectable in the blood of patients with conditions like celiac disease and IBD, Fasano theorized that it was a traceable and testable marker for inflammation in those conditions.

Later research by Fasano and others identified zonulin as a precursor to haptoglobin-2, that independently acted as a modulator of tight junctions, separately from the involvement in inflammation of haptoglobin-2:

"Through proteomic analysis of human sera, we have now identified human zonulin as the precursor for haptoglobin-2 (pre-HP2). Although mature HP is known to scavenge free hemoglobin (Hb) to inhibit its oxidative activity, no function has ever been ascribed to its uncleaved precursor form. We found that the single-chain zonulin contains an EGF-like motif that leads to transactivation of EGF receptor (EGFR) via proteinase-activated receptor 2 (PAR2) activation. Activation of these 2 receptors was coupled to increased IP. The siRNA-induced silencing of PAR2 or the use of PAR2−/− mice prevented loss of barrier integrity. Proteolytic cleavage of zonulin into its α2- and β-subunits neutralized its ability to both activate EGFR and increase IP. Quantitative gene expression revealed that zonulin is overexpressed in the intestinal mucosa of subjects with celiac disease. To our knowledge, this is the initial example of a molecule that exerts a biological activity in its precursor form that is distinct from the function of its mature form. Our results therefore characterize zonulin as a previously undescribed ligand that engages a key signalosome involved in the pathogenesis of human immune-

mediated diseases that can be targeted for therapeutic interventions." (A Tripathi, et al, Proceedings of the National Academy of Sciences, 2009)

This discovery of independent action by zonulin served to demonstrate how exactly zonulin was involved in inflammation. By being implicated in the loss of barrier integrity, higher zonulin levels in the bloodstream indicate intestinal permeability. The presence of zonulin means a compromised intestinal wall, in that the tight junctions have been loosened and allow other materials that are inflammatory to pass from the gut to the bloodstream, such as tiny particles of food and potential pathogenic bugs from the gut biota. Passage of these materials into the bloodstream can cause an inflammatory response throughout the body, including the brain via the vagus nerve. Indeed, "Zonulin is a master regulator of intestinal permeability that opens the door to several chronic inflammatory conditions." (Craig Sturgeon, Alessio Fasano; Tissue Barriers, Oct 21, 2016).

These inflammatory conditions can be wide-ranging. In fact, Fasano believes that perhaps most or even all autoimmune conditions may begin in the gut. He and Craig Sturgeon illustrate some examples: "Zonulin is found in abundance in rheumatoid arthritis, systemic lupus erythematosus, irritable bowel syndrome, and other autoimmune diseases." (same source as above). The most recent research has demonstrated a connection between rheumatoid arthritis (Heidt, et al, Nutrients, May 19, 2023), and chronic inflammatory diseases including diabetes, autoimmune diseases, and some cancers (Fasano, Physiol Rev. 2011), to name a few. Large amounts of research have confirmed the connection between zonulin and tight junctions, between tight junctions and inflammation in the gut and elsewhere, and the fact that inflammation underpins many chronic conditions.

So what about zonulin testing? Fasano has claimed "By the time we discovered that zonulin was linked, not only to celiac disease, but to a variety of autoimmune diseases, this peptide represented to us a possible tool to eventually treat autoimmune diseases" (Craig Gustafson, Integr Med (Encinitas). 2018 Apr). If autoimmune diseases and other inflammatory diseases begin in the gut, and the origin of that inflammation is intestinal permeability, then if identified as such, preventing autoimmune diseases from forming in the first place is possible by removing the triggers to inflammation and intestinal permeability. Essentially, triggers, such as food allergies, especially gluten, cause inflammation in the gut which triggers zonulin to

loosen tight junctions, which can cause that inflammation to become chronic and systemic. If that inflammation becomes chronic, then various inflammatory conditions can arise. Identifying triggers, the amount of zonulin circulating in the blood, and the degree of inflammation can all greatly increase the effectiveness of intervention by the practitioner.

Precision Point Diagnostics offers the Intestinal Barrier Assessment test that includes measurements of zonulin, as a marker of intestinal permeability; histamine, diamine oxidase, and the histamine diamine oxidase ratio, as markers of histamine balance and tolerance; and LPS, as a confirmatory marker of intestinal permeability and inflammation. We also offer the P88 Dietary Antigen Test, which measures IgE, IgG, IgG4, and C3d reactions for 88 different foods, including gluten, that can act as triggers for gut inflammation and the loosening of tight junctions in the intestinal wall. These two tests are available together as a bundle.

Since the identification of zonulin as prehaptoglobin-2, a precursor to haptoglobin-2, there have been those that have argued that zonulin testing may not actually be useful in discovering intestinal permeability. The arguments have ranged from “the half-life of zonulin in the blood is too short”, to “testing for zonulin isn’t worthwhile because current testing methods test for haptoglobin-2, not prehaptoglobin-2, so intestinal permeability can’t be assessed” to “leaky gut isn’t real” to “only celiac disease and other very extreme gut disturbances can be detected, so testing won’t be able to test early stages of gut dysfunction before autoimmunity sets in”. Let’s take a look at these arguments and see if they are valid. Rather than taking them one by one, it might be more enlightening to take a more holistic approach and make some points that will answer each objection. Keep in mind that as research continues, more and more researchers are convinced of the validity of zonulin testing, just as a large number of practitioners are now convinced of the legitimacy of the condition “leaky gut”, where once that was confined to the integrative medical community.

1. **Testing methodology matters:** The argument that current testing can’t distinguish between prehaptoglobin-2 (zonulin) and haptoglobin-2 isn’t true. The Precision Point Diagnostics and some other labs testing methodologies do indeed distinguish between the two. We are testing

for zonulin specifically and not haptoglobin-2. In this methodology, not only is zonulin specifically tested for (as prehaptoglobin-2) rather than the entire haptoglobin family, but it has also been validated that there is no cross-reactivity between zonulin and haptoglobin-2. What is true is that there are a large number of labs that are still testing for haptoglobin-2 or a combination of zonulin and haptoglobin-2. This is not best practice since haptoglobin-2 is likely a marker of inflammation and not of intestinal permeability. It may be likely that being indiscriminate in testing the haptoglobin family, rather than utilizing a more specific test that isolates zonulin specifically, still will give a zonulin reading, but much diluted. Tests with that methodology are what give rise to the argument that zonulin levels are too low in the blood, as well as the argument that they don't detect intestinal permeability.

2. **The medical community has mostly accepted that intestinal permeability is real:** According to the National Institutes of Health (NIH), "intestinal permeability disturbances are now linked to a variety of chronic diseases, including inflammatory bowel diseases, celiac disease, and type 1 diabetes mellitus" (Di Vincenzo, F., Del Gaudio, A., Petito, V. et al. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. Intern Emerg Med 19, 275–293 (2024)). This growing acceptance is driven by accumulating evidence that highlights the role of the gut barrier in maintaining overall health and preventing disease (Bischoff, S.C., Barbara, G., Buurman, W. et al. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterol 14, 189 (2014)). There will always be some in the medical community who are slow to recognize what the accumulating evidence is clearly establishing. Functional and integrative practitioners are often on the cutting edge of new research and functional testing.
3. **Zonulin testing can make a difference before autoimmunity sets in:** Each new research study adds to our confidence that this statement is true. The trend is clearly toward the fact that zonulin testing (of the right kind) is able to detect zonulin levels in patients who don't necessarily have celiac disease, IBS, or other autoimmune conditions. Although most agree that zonulin is involved in inflammation and tight junction dysfunction in the gut, there are some holdouts that believe that only severe intestinal compromise can be detected by zonulin tests. Again, the right methodology for testing is necessary as a starting

point. Assuming that then the question is is something like non-celiac gluten sensitivity that damages the tight junctions detectable with the correct zonulin test? If so, it means that zonulin testing would likely be able to detect tight junction damage before it advances to a nearly irreversible level. Aside from the accumulating evidence from recent studies on specific conditions, a study in the journal Gut determined that non-celiac gluten sensitivity could be detected with proper zonulin testing: “Zonulin can be considered a diagnostic biomarker in NCGS (non-celiac gluten sensitivity) and combined with demographic and clinical data differentiates NCGS from IBS-D with high accuracy.” (Barbaro, et al, Gut, Nov 2020). Beyond gluten sensitivities, general disturbance of the microbiome may result in gut dysbiosis, intestinal permeability, and inflammation. A very recent review of the literature confirmed this point:

“There is significant interaction of the microbiome and barrier function, including the inflammatory of luminal/bacterial antigens, and anti-inflammatory effects of commensals or probiotics and their products, including short-chain fatty acids…intestinal permeability is a critical factor in protection against gastrointestinal diseases and is impacted by nutrients that preserve or heal and repair the barrier and nurture anti-inflammatory effects.” (Matar, A.; Damianos, J.A.; Jencks, K.J.; Camilleri, M. Intestinal Barrier Impairment, Preservation, and Repair: An Update. Nutrients 2024, 16, 3494.) This means that not only is the right intestinal barrier assessment test extremely useful, but it can set the stage for successful treatment.

Aside from the effectiveness of well-designed zonulin testing that takes into account the objections from the nay-sayers, remember that the Precision Point Diagnostics Intestinal Barrier assessment also includes other markers aside from Zonulin. This comprehensive panel leads in its field in assessing intestinal permeability and assessing this interaction with a multifaceted approach to histamine. We are the only diagnostic laboratory in the country to offer diamine oxidase on a panel, which is important in its own right. We also do zonulin testing as it should be done.