Hypothesis

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Immunopathophysiology of Gluten-Associated Insulin Resistance: A 33-mer Gliadin–CXCR3–Zonulin Axis Driving IRAK4-Centered Innate Immune Amplification, Sulfur Depletion, and Upstream Modulation by *Aspergillus niger* Prolyl Endopeptidase

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Immunopathophysiology of Gluten-Associated Insulin Resistance: A 33-mer Gliadin–CXCR3–Zonulin Axis Driving IRAK4-Centered Innate Immune Amplification, Sulfur Depletion, and Upstream Modulation by *Aspergillus niger* Prolyl Endopeptidase

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Abstract

Insulin resistance is increasingly recognized as a disorder of immunometabolic integration rather than a purely metabolic defect. Although chronic low-grade inflammation is known to impair insulin signaling, the upstream dietary and mucosal drivers sustaining innate immune activation in susceptible individuals remain incompletely defined. Here, we propose an immunopathophysiological hypothesis linking digestion-resistant gliadin peptides particularly the canonical 33-mer fragment to systemic insulin resistance through a gut-initiated innate immune cascade coupled to sulfur and redox dysregulation. The 33-mer gliadin peptide resists gastrointestinal proteolysis, permitting prolonged luminal persistence and sustained epithelial interaction. Experimental evidence demonstrates that gliadin binds the epithelial chemokine receptor CXCR3, inducing zonulin release and transient modulation of tight junctions. This regulated increase in intestinal permeability facilitates enhanced mucosal access of dietary peptides and microbial ligands to innate immune cells. We propose that this co-exposure potentiates MyD88-dependent Toll-like receptor signaling, with IRAK4 functioning as a central signaling hub. IRAK4-driven activation of NF-κB and stress kinase pathways interferes with insulin signaling while simultaneously imposing a chronic oxidative and nitrosative burden. Sustained innate immune activation accelerates glutathione consumption and suppresses transsulfuration pathway capacity, resulting in functional sulfur depletion. This redox imbalance compromises protein disulfide isomerase activity and insulin disulfide bond formation, linking mucosal immune activation to impaired insulin structural integrity, reduced bioactivity, hyperinsulinemia, and systemic insulin resistance. As an upstream experimental intervention, *Aspergillus niger*–derived prolyl endopeptidase is proposed to degrade resistant gliadin peptides prior to epithelial engagement and innate immune amplification. This falsifiable framework supports biomarker-guided stratification and staged validation across luminal peptide degradation, epithelial barrier modulation, innate immune signaling, sulfur metabolism, and tissue-level insulin responsiveness.

Keywords: IRAK4; gliadin 33-mer; CXCR3; zonulin; innate immunity; sulfur metabolism; glutathione; transsulfuration pathway; insulin structural integrity; prolyl endopeptidase (AN-PEP)

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1. Introduction

Insulin resistance constitutes a core molecular abnormality underlying a broad spectrum of metabolic disorders, including type 2 diabetes mellitus, metabolic syndrome, and cardiometabolic disease [1]. Classical paradigms initially framed insulin resistance as a defect intrinsic to insulin-responsive tissues; however, contemporary mechanistic models increasingly identify chronic low-grade inflammation as a dominant upstream driver that precedes and perpetuates impaired insulin signaling [2]. At the cellular level, insulin resistance is characterized by functional disruption of insulin receptor substrate (IRS) proteins, attenuation of phosphatidylinositol 3-kinase (PI3K)–AKT signaling, and defective GLUT4 vesicular trafficking, culminating in reduced glucose uptake and systemic metabolic dysregulation [1, 3]. A growing body of evidence indicates that innate immune signaling pathways are deeply interwoven with metabolic regulation [4].

Pattern-recognition receptors (PRRs), particularly Toll-like receptors (TLRs), operate not only as sensors of microbial products but also as detectors of endogenous danger-associated molecular patterns and diet-derived ligands capable of sustaining inflammatory tone [5, 6]. Persistent engagement of TLR-linked signaling cascades induces stress kinase activation, transcriptional reprogramming, and inflammatory mediator production processes known to directly antagonize insulin signaling fidelity [7, 8]. In this context, dietary proteins are increasingly recognized as biologically active molecular inputs that can modulate mucosal immunity and systemic inflammatory status rather than serving solely as inert nutritional substrates [9, 10]. Gluten, a composite protein network composed of gliadins and glutenins, is of particular relevance due to the distinctive biochemical properties of gliadin-derived peptides. These peptides are enriched in proline and glutamine residues, conferring relative resistance to complete gastrointestinal proteolysis [11]. As a consequence, digestion-resistant gliadin fragments persist within the intestinal lumen, enabling sustained interaction with the epithelial barrier and immune interfaces [12].

Experimental evidence has demonstrated that gliadin can directly engage the chemokine receptor CXCR3 expressed on intestinal epithelial cells, triggering zonulin release and inducing regulated loosening of tight junctions [13, 14]. This mechanism establishes a direct molecular connection between luminal gluten exposure and dynamic modulation of intestinal permeability, thereby increasing antigen trafficking across the epithelial barrier and amplifying exposure of subepithelial immune compartments to luminal immunogenic stimuli [15]. Within innate immune signaling networks, MyD88-dependent pathways downstream of multiple TLRs play a central role in converting extracellular danger signals into intracellular inflammatory programs [16]. A pivotal proximal kinase within this axis is interleukin-1 receptor–associated kinase 4 (IRAK4), which functions as a signal amplification node linking receptor engagement to activation of NF-κB and related transcriptional responses [17]. NF-κB occupies a master regulatory position within this cascade, coordinating cytokine expression, metabolic stress signaling, and immune–metabolic crosstalk [18].

Inflammatory mediators generated through NF-κB–dependent pathways exert well-established inhibitory effects on insulin signaling by promoting serine phosphorylation of IRS proteins, inducing suppressor of cytokine signaling (SOCS) expression, and activating stress kinases that compromise PI3K–AKT pathway integrity [19, 20]. Collectively, these mechanisms form a mechanistic bridge linking innate immune activation to insulin resistance across metabolically active tissues.

Against this background, the present work advances an integrated immunopathophysiological hypothesis in which digestion-resistant gliadin peptides initiate a gut-centered innate immune amplification loop mediated by TLR–MyD88–IRAK4 signaling.

This axis is proposed to propagate inflammatory signals capable of intersecting insulin pathways and promoting systemic insulin resistance. Furthermore, this framework introduces upstream enzymatic modulation via Aspergillus niger–derived prolyl endopeptidase (AN-PEP) as a luminal interception strategy designed to reduce the immunogenic peptide burden prior to epithelial engagement, barrier modulation, and downstream innate immune activation.

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2. Digestion-Resistant Gliadin Peptides and Barrier-Mediated Immune Amplification

Gluten is a heterogeneous protein complex in which gliadin fractions exhibit biochemical features that critically influence their digestive fate. Gliadin-derived peptides contain proline-rich motifs that are inefficiently cleaved by human gastrointestinal proteases, resulting in incomplete digestion and the persistence of relatively large peptide fragments within the intestinal lumen [21]. This resistance to proteolysis constitutes a foundational prerequisite for sustained biological activity at the mucosal interface. Unlike rapidly degraded dietary proteins, digestion-resistant gliadin fragments maintain prolonged luminal residence and repeated contact with the intestinal epithelium, thereby increasing the probability of receptor-mediated signaling events and barrier modulation. A well-characterized example is the 33-mer gliadin peptide, which has been extensively employed as a canonical model of proteolysis-resistant gluten fragments [22, 23]. While frequently studied within the context of gluten-related disorders, the broader significance of the 33-mer lies in its illustration of a generalizable principle: structurally resilient dietary peptides can function as persistent biological stimuli capable of shaping immune and metabolic signaling networks [24].

A critical consequence of prolonged epithelial exposure is the regulated modulation of intestinal barrier function. The intestinal epithelium operates as a dynamic regulatory interface rather than a static physical barrier, with tight junction integrity continuously adjusted in response to physiological and environmental cues [25]. Engagement of epithelial CXCR3 by gliadin peptides induces zonulin release, which orchestrates reversible rearrangement of tight junction complexes and increases paracellular permeability [26, 27]. This process establishes gluten exposure as an upstream modulator of immune accessibility rather than a passive luminal event [28].

Transient loosening of tight junctions facilitates enhanced translocation of luminal antigens and microbial-derived products into the lamina propria [29]. This increased antigenic flux is of particular pathophysiological relevance because heightened exposure of resident immune cells to microbial ligands is a potent driver of Toll-like receptor activation and downstream inflammatory signaling [30]. Among these pathways, TLR4-mediated signaling has been repeatedly implicated in inflammatory programs associated with metabolic dysfunction and insulin resistance across multiple tissue contexts [31].

Within the proposed model, barrier modulation is not conceptualized as an irreversible pathological breach but rather as a regulated, potentially reversible permeability shift that increases the likelihood of simultaneous exposure to gliadin peptides and microbial ligands. This co-exposure provides a mechanistically coherent basis for amplification and prolongation of innate immune signaling, particularly through MyD88-dependent pathways, thereby reinforcing inflammatory circuits capable of intersecting insulin signaling networks. By functioning as a molecular gatekeeper, intestinal barrier dynamics shape both the intensity and duration of downstream immune–metabolic responses.

TLR–MyD88–IRAK4 Axis as a Signal Amplification Hub

Toll-like receptors (TLRs) constitute a central class of pattern-recognition receptors that detect conserved microbial structures and initiate innate immune signaling cascades culminating in transcriptional activation of inflammatory programs [32, 33]. Within the proposed immunopathophysiological framework, increased intestinal permeability establishes conditions under which microbial-derived ligands and digestion-resistant gliadin peptides gain concurrent access to mucosal immune compartments. This spatial and temporal convergence does not necessitate the introduction of a novel ligand-specific signaling pathway but instead increases the probability, amplitude, and persistence of TLR engagement [34].

Downstream of several TLRs, MyD88-dependent signaling organizes a receptor-proximal supramolecular complex that recruits and activates interleukin-1 receptor–associated kinase 4 (IRAK4) [35]. IRAK4 occupies a strategically critical position within this cascade, functioning as a

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convergence and amplification node that integrates upstream receptor engagement with downstream activation of inflammatory transcription factors and stress kinase pathways [36]. Through this role, IRAK4 enables localized mucosal immune activation to be translated into broader inflammatory signaling outputs with systemic reach [37]. Importantly, this framework deliberately avoids asserting direct agonistic activity of gliadin peptides toward TLRs. Instead, the emphasis is placed on potentiation of TLR signaling within a permissive context defined by enhanced ligand accessibility and barrier modulation. This conservative mechanistic positioning strengthens experimental tractability by allowing innate immune activation to be interrogated under conditions of microbial ligand exposure alone versus combined exposure scenarios in which gliadin peptide burden and epithelial permeability are independently manipulated.

3. NF-κB–Centered Inflammatory Signaling as a Metabolic Intermediary

Activation of nuclear factor κB (NF-κB) represents a central integrative event within innate immune signaling and functions as the principal transcriptional engine driving expression of pro-inflammatory cytokines, chemokines, and stress mediators [38].

Within the proposed model, NF-κB serves as the critical node translating receptor-proximal TLR–MyD88–IRAK4 activation into sustained inflammatory outputs that extend beyond the initial gut-centered immune event. Inflammatory signaling programs induced downstream of NF-κB activation are directly relevant to the pathogenesis of insulin resistance. Chronic inflammatory states are well established to interfere with insulin signaling fidelity through multiple convergent mechanisms [39]. These include altered phosphorylation dynamics of insulin receptor substrate (IRS) proteins, induction of inhibitory signaling regulators, and activation of stress kinase pathways that antagonize PI3K–AKT signaling and impair GLUT4 vesicular trafficking [40, 41]. As a result, NF-κB–associated inflammatory outputs are positioned as mechanistic intermediates linking upstream innate immune activation to downstream metabolic dysfunction [42]. By situating NF-κB at this junction, the framework provides a coherent molecular bridge connecting mucosal innate immune amplification to systemic impairment of insulin action.

4. Molecular Disruption of Insulin Signaling Pathways

Insulin sensitivity at both cellular and tissue levels is critically dependent on the integrity of insulin signaling cascades and the efficiency of GLUT4 trafficking to the plasma membrane in insulin-responsive cells. Canonical insulin signaling is initiated by insulin receptor activation and propagated through insulin receptor substrate (IRS) proteins toward the PI3K–AKT axis, which orchestrates downstream metabolic processes governing glucose uptake, storage, and utilization [43]. Disruption at any node within this cascade is sufficient to compromise glucose handling and promote insulin resistance.

Inflammatory signaling states intersect with insulin pathways through multiple molecular interfaces, including cytokine receptor engagement and activation of stress-responsive kinases [44]. These pathways interfere with insulin signal transduction by promoting inhibitory serine phosphorylation of IRS proteins, inducing suppressor molecules, and attenuating AKT-mediated regulation of GLUT4 trafficking. Within the present framework, inflammatory outputs generated downstream of TLR–MyD88–IRAK4 signaling are positioned as proximal drivers of quantifiable defects in insulin signaling efficiency and glucose transport [43, 45]. This directional framing from innate immune activation to metabolic impairment enables direct experimental interrogation using established molecular readouts, including phospho-signaling profiles of IRS and AKT, as well as quantitative assessment of GLUT4 translocation and glucose uptake [46, 47].

5. Systemic Propagation of Immunometabolic Dysfunction

A defining feature of immunometabolic pathophysiology is the capacity for localized innate immune activation to propagate systemically through circulating inflammatory mediators, altered

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immune cell programming, and sustained stress signaling [48]. This principle is consistent with extensive evidence demonstrating elevated innate immune activation and TLR-associated inflammation in metabolic states characterized by insulin resistance [48].

Within the proposed axis, the intestinal compartment functions as the initiating site that shapes systemic inflammatory exposure by regulating epithelial barrier permeability and ligand access to mucosal immune compartments. Once innate immune signaling is engaged and amplified, inflammatory mediators exert distal effects on classical insulin-responsive tissues, including skeletal muscle, liver, and adipose tissue. In these tissues, inflammatory stress signaling disrupts insulin pathway integrity, alters glucose handling, and reinforces insulin-resistant phenotypes. These tissue-specific manifestations align closely with established insulin signaling biology and are summarized in (Table 1), which contextualizes systemic metabolic consequences within the framework of immune-driven signaling disruption.

Table 1. Tissue-Level Manifestations of Immune-Driven Insulin Resistance.

Tissue	Pathophysiological vulnerability	Representative functional phenotype
Skeletal muscle	Primary site of insulin-stimulated glucose uptake via GLUT4 trafficking	Reduced insulin-stimulated glucose disposal [49].
Liver	Central regulator of hepatic glucose production under insulin control	Inadequate suppression of hepatic glucose output [50].
Adipose tissue	Endocrine and lipid storage organ influencing systemic metabolic tone	Altered lipid flux and pro-inflammatory adipokine signaling [51].

6. Innate Immune–Driven Sulfur Depletion as a Determinant of Insulin Structural Integrity

Beyond its classical role in antioxidant defense, sulfur metabolism constitutes a critical integrative node linking innate immune activation, redox homeostasis, and insulin structural biology [52]. The transsulfuration pathway supplies cysteine for glutathione biosynthesis and maintains the redox environment required for optimal protein disulfide isomerase (PDI) activity within the endoplasmic reticulum (ER) [53]. Through these functions, sulfur flux directly influences the fidelity of disulfide bond formation in secretory proteins.

Chronic activation of gut-initiated innate immune signaling, as proposed within the gluten–TLR–MyD88–IRAK4 axis, imposes a sustained inflammatory redox burden characterized by increased production of reactive oxygen and nitrogen species. This state accelerates glutathione consumption and oxidation, progressively depleting intracellular pools of reduced glutathione [54]. Importantly, this depletion reflects not only increased oxidative demand but also impaired capacity for redox recovery.

Inflammatory signaling mediated by IRAK4 and NF-κB further exacerbates sulfur imbalance by functionally constraining sulfur supply.

NF-κB–driven inflammatory programs have been associated with suppression of key transsulfuration enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), thereby limiting cysteine availability for glutathione resynthesis [55]. This coordinated increase in sulfur demand coupled with restricted sulfur flux establishes a state of functional sulfur depletion rather than isolated oxidative stress [56]. Under conditions of glutathione deficiency and altered ER redox tone, PDI-dependent disulfide bond formation becomes inefficient. Insulin biosynthesis is particularly vulnerable to this disruption, as precise pairing of intra- and inter-chain disulfide bonds is essential for insulin structural stability and receptor signaling competence. Consequently, insulin may be synthesized and secreted in preserved or increased quantities yet exhibit impaired bioactivity due to subtle misfolding. This mechanism provides a coherent molecular basis for the coexistence of hyperinsulinemia and insulin resistance observed in inflammatory metabolic states [57].

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The hierarchical relationship between innate immune activation, sulfur metabolism, and insulin structural integrity is summarized in (Table 2).

Table 2. Innate Immune Activation, Sulfur Metabolism, and Insulin Structural Consequences.

Pathophysiological level	Key components	Mechanistic effect	Downstream metabolic outcome
Gut innate immune signaling	TLRs, MyD88, IRAK4	Sustained inflammatory activation and redox stress	Chronic low-grade systemic inflammation
Redox homeostasis	ROS, RNS, glutathione	Accelerated glutathione consumption and oxidation	Loss of cellular redox buffering
Transsulfuration pathway	CBS, CSE, cysteine	Suppressed sulfur flux and reduced cysteine availability	Impaired glutathione resynthesis
ER protein folding	PDI, ER redox state	Inefficient disulfide bond formation	Misfolded or structurally compromised insulin
Insulin biology	Insulin disulfide bonds, receptor activation	Reduced insulin bioactivity despite preserved secretion	Hyperinsulinemia with insulin resistance

7. Prolyl Endopeptidase as an Upstream Immunometabolic Modulator

Aspergillus niger–derived prolyl endopeptidase (AN-PEP) is an orally active enzyme evaluated for its capacity to degrade gluten peptides during gastrointestinal transit. Controlled clinical and experimental studies have demonstrated that AN-PEP can substantially reduce the luminal burden of digestion-resistant gluten peptides under defined exposure conditions, establishing the feasibility of enzymatic peptide interception in humans [58].

The conceptual novelty of the present framework lies in positioning AN-PEP not merely as a digestive adjunct, but as an upstream immunomodulatory lever within a gut-initiated immunometabolic cascade. By degrading gliadin peptides prior to engagement of epithelial CXCR3–zonulin signaling and before potentiation of innate immune activation in a microbial ligand–rich environment, AN-PEP is hypothesized to attenuate initiation and amplification of downstream inflammatory signaling without necessitating systemic suppression of innate immune pathways. This upstream positioning confers mechanistic specificity by targeting the initiating luminal stimulus rather than downstream inflammatory mediators, thereby preserving physiological immune competence while modulating pathological signal amplification.

8. Molecular Consequences of Upstream Luminal Modulation

Reduction of the luminal pool of digestion-resistant gliadin peptides through AN-PEP–mediated degradation is predicted to initiate a series of ordered molecular consequences within the proposed framework. The immediate effect is a decreased probability of CXCR3-mediated zonulin release, resulting in attenuation of transient epithelial permeability shifts. This barrier stabilization is expected to reduce co-exposure of mucosal innate immune cells to gliadin peptides and microbial ligands capable of engaging TLR signaling. At the intracellular signaling level, diminished innate immune activation is predicted to reduce the intensity and duration of NF-κB–dependent inflammatory transcriptional programs. Lower inflammatory mediator output would consequently lessen inhibitory cross-talk with insulin signaling pathways in peripheral tissues. At the metabolic signaling level, reduced inflammatory stress is expected to restore insulin pathway dynamics, improve PI3K–AKT signaling fidelity, and enhance GLUT4 trafficking efficiency, consistent with established models of insulin resistance pathophysiology.

Importantly, these outcomes are intentionally framed as experimentally measurable molecular consequences rather than clinical efficacy claims. The framework thus motivates a hierarchical testing

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strategy encompassing luminal peptide quantification, epithelial barrier function assays, innate immune signaling readouts, sulfur and redox status, and tissue-level insulin responsiveness. This structure supports iterative experimental validation, mechanistic refinement, and rational translational progression.

9. Discussion

The present work integrates four independently substantiated biological domains into a unified immunopathophysiological framework: the persistence of digestion-resistant gliadin peptides, gliadin-triggered CXCR3–zonulin–mediated modulation of intestinal permeability, IRAK4-centered amplification of innate immune signaling, and inflammation-driven disruption of sulfur and redox metabolism.

Collectively, these processes form a coherent mechanistic sequence linking a defined dietary exposure to systemic insulin resistance, extending beyond tissue-intrinsic metabolic defects to include gut-initiated immune–metabolic crosstalk [1, 2, 31]. A central gateway within this framework is the gliadin–CXCR3–zonulin axis, which provides an experimentally validated mechanism by which a specific luminal dietary peptide can transiently and reversibly alter epithelial barrier function [13, 14, 27].

Rather than representing pathological barrier failure, this regulated permeability shift plausibly increases mucosal access of both dietary antigens and microbial-derived ligands, thereby amplifying exposure of resident immune cells to innate immune stimuli [25, 29]. Such co-exposure is particularly relevant in metabolic contexts characterized by elevated baseline microbial ligand burden or subclinical barrier dysregulation [28, 30]. Within this permissive environment, Toll-like receptor signaling emerges not as a novel pathway activated by gliadin per se, but as an amplified response driven by increased ligand accessibility [32, 33]. The positioning of IRAK4 as a proximal signaling hub represents a conceptual strength of the model. As a central kinase within MyD88-dependent TLR signaling, IRAK4 integrates upstream receptor engagement with downstream activation of NF-κB and stress kinase pathways [35, 36, 37]. This places IRAK4 in an optimal position to translate localized mucosal immune activation into sustained inflammatory signaling with systemic metabolic consequences.

Downstream of IRAK4, NF-κB functions as a master regulator coordinating inflammatory transcriptional programs known to interfere with insulin signaling fidelity [18, 38]. NF-κB–driven cytokine production and stress signaling have been repeatedly implicated in insulin resistance through promotion of inhibitory serine phosphorylation of insulin receptor substrate proteins, induction of suppressor of cytokine signaling molecules, and attenuation of PI3K–AKT pathway activity [19, 39]. These mechanisms converge to impair GLUT4 trafficking and glucose uptake in insulin-responsive tissues, establishing a mechanistic bridge between innate immune activation and metabolic dysfunction [40, 41, 42]. Importantly, the present framework extends beyond canonical inflammatory interference with insulin signaling by identifying sulfur and redox metabolism as critical downstream effectors of chronic innate immune activation. Sulfur metabolism, via the transsulfuration pathway, sustains glutathione availability and the redox environment required for efficient protein disulfide isomerase activity within the endoplasmic reticulum [52, 53]. Chronic IRAK4–NF-κB signaling imposes sustained oxidative and nitrosative stress, accelerating glutathione consumption while functionally suppressing transsulfuration enzyme activity [54, 55]. This coordinated increase in sulfur demand coupled with restricted sulfur flux establishes a state of functional sulfur depletion rather than isolated oxidative stress [56].

Under these conditions, endoplasmic reticulum protein folding becomes selectively vulnerable. Insulin biosynthesis is particularly sensitive to redox imbalance due to its strict dependence on precise intra- and inter-chain disulfide bond formation for structural stability and receptor signaling competence. Disruption of this process provides a mechanistic explanation for the paradoxical coexistence of hyperinsulinemia and reduced insulin bioactivity frequently observed in insulin-

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resistant states [57]. This insight reframes insulin resistance not solely as a signaling defect, but also as a disorder of hormone structural quality under inflammatory redox stress.

The translational appeal of this framework lies in its modular testability and upstream therapeutic logic. Aspergillus niger–derived prolyl endopeptidase has demonstrated the capacity to degrade gluten peptides in controlled human studies, establishing feasibility of luminal peptide interception.[58] Importantly, this strategy targets the initiating dietary stimulus rather than downstream inflammatory mediators or metabolic pathways, thereby preserving physiological immune function while attenuating pathological signal amplification. Nevertheless, the model is explicitly hypothesis-generating. The magnitude of gliadin-dependent innate immune amplification is likely to vary substantially across individuals as a function of baseline intestinal permeability, microbial ligand exposure, sulfur metabolic reserve, and innate immune tone [31, 48]. Accordingly, future investigation should prioritize biomarker-guided stratification combined with controlled gluten challenge paradigms capable of disentangling epithelial barrier modulation, innate immune activation, sulfur depletion, and insulin structural outcomes into experimentally separable components. By integrating dietary biochemistry, mucosal immunology, innate immune signaling, redox biology, and insulin structural integrity into a single causal framework, this work provides a testable and mechanistically grounded model for immune-driven insulin resistance that extends beyond traditional metabolic paradigms.

10. Conclusion

This manuscript proposes a pathway-level immunopathophysiological framework in which digestion-resistant gliadin peptides initiate a gut-centered cascade capable of propagating systemic insulin resistance. Central to this model is gliadin engagement of epithelial CXCR3, triggering zonulin release and transient modulation of tight junction integrity, thereby increasing mucosal access of dietary antigens and microbial ligands to innate immune compartments. Within this permissive environment, amplification of MyD88-dependent innate immune signaling, with IRAK4 positioned as a proximal convergence node, drives sustained inflammatory and redox stress programs. These programs extend beyond canonical cytokine-mediated interference with insulin signaling to impose functional disruption of sulfur metabolism.

Inflammation-driven glutathione depletion and suppression of transsulfuration capacity compromise endoplasmic reticulum redox balance and protein disulfide isomerase activity, rendering insulin structural integrity selectively vulnerable.

By linking chronic mucosal innate immune activation to impaired insulin bioactivity, this framework offers a mechanistic explanation for the coexistence of hyperinsulinemia and insulin resistance. Importantly, it remains explicitly hypothesis-generating. Aspergillus niger–derived prolyl endopeptidase is advanced as an upstream luminal intervention supported by human evidence of gluten degradation, enabling direct experimental testing of whether attenuation of luminal gliadin burden modulates barrier dynamics, innate immune activation, sulfur homeostasis, and insulin functional outcomes. The model is intentionally structured to support falsifiable validation, biomarker-guided stratification, and iterative refinement across immunological, metabolic, and structural dimensions of insulin resistance.

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