Hypothesis

The Sulfur Insulin Deformation Hypothesis: Disulfide Bond Disruption (A6–A11, A7–B7, A20–B19) and PDI Dysregulation as an Etiology of Insulin Resistance

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The Sulfur Insulin Deformation Hypothesis: Disulfide Bond Disruption (A6–A11, A7–B7, A20–B19) and PDI Dysregulation as an Etiology of Insulin Resistance

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Abstract

Type 2 diabetes mellitus (T2DM), projected to affect over 700 million by 2045, requires a new etiological framework. The Sulfur Insulin Deformation Hypothesis redefines T2DM as a sulfur metabolism disorder, driven by mitochondrial suffocation in intestinal epithelial cells, disrupting transsulfuration pathways converting methionine to cysteine via cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). Mitochondrial dysfunction impairs ATP, depleting cysteine and glutathione (GSH) by 30–73.8% (red blood cell GSH: 1.78 ± 0.28 vs. 6.75 ± 0.47 μmol/g Hb, $P < 0.001$), boosting reactive oxygen species (ROS) and lipid peroxides. This redox imbalance disrupts protein disulfide isomerase (PDI) activity (PDIA1, PDIA3, PDIA4) in β-cell endoplasmic reticulum (ER), impairing insulin's disulfide bonds (A6–A11, A7–B7, A20–B19). The A6–A11 hinge bond, vital for receptor affinity, loses 50–70% binding capacity upon disruption ($r = -0.65$, $P < 0.05$ for HOMA-IR), hindering PI3K-Akt signaling and GLUT4 translocation, causing hyperglycemia. Elevated PDIA4 in 225 T2DM patients correlates with fasting glucose ($r = 0.62$, $P < 0.01$) and reduced sensitivity ($r = -0.67$, $P < 0.01$). PDIA4 inhibition by PS1 (IC50 = 4 μM) reduces ROS by 50% ($P < 0.01$), improves HbA1c by 1.2% ($P < 0.05$), and boosts β-cell survival by 30% ($P < 0.05$). PDIA1 deletion raises proinsulin/insulin ratios ($P < 0.01$), while PDIA3-driven RhoA-YAP signaling drives adipose inflammation ($P < 0.05$). S-nitrosylation further disrupts disulfide bonds. New evidence highlights a secondary extracellular mechanism: redox-mediated chain splitting degrades 20% of circulating insulin (A-chain rate 0.40 nmol/kg/min) at ~ -137 mV plasma redox, modulated by GSH. This explains the paradox of effective intravenous (IV) insulin exogenous analogs bypass hepatic GSH clearance and resist splitting while misfolded endogenous insulin, destabilized by sulfur scarcity, succumbs to plasma thiol attacks. This hypothesis posits that insulin resistance arises from organic sulfur deficiency, inducing structural deformities via disrupted disulfide bonds (A6–A11, A7–B7, A20–B19) and impaired PDIA1, PDIA3, PDIA4 activity. It resolves the paradox of exogenous insulin efficacy, attributing it to structurally intact molecules, contrasting with deformed endogenous insulin. The secondary mechanism likely stems from sulfur deficiency elevating ROS and lipid peroxides, accelerating chain splitting. Sulfur donors like N-acetylcysteine (NAC, restoring GSH by 20–40%, $P < 0.01$), GlyNAC (improving sensitivity by 31%, $P < 0.05$), and methylsulfonylmethane (MSM, reducing oxidative stress by 25%) mitigate these defects, advocating therapies targeting the gut-mitochondria-sulfur-insulin axis.

Keywords: sulfur insulin deformation; disulfide bonds (A6–A11); protein disulfide isomerase (PDI); cysteine deficiency; glutathione depletion; T2DM pathogenesis

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1. Introduction

Type 2 diabetes mellitus (T2DM), a global health crisis projected to surge in prevalence, is traditionally attributed to peripheral insulin resistance driven by obesity, oxidative stress, and inflammation [1]. Yet, these models often overlook the critical role of insulin's structural integrity, particularly its sulfur-dependent disulfide bonds. The Sulfur Insulin Deformation Hypothesis offers a groundbreaking framework, asserting that mitochondrial dysfunction in intestinal epithelial cells termed mitochondrial suffocation triggers organic sulfur deficiency, leading to insulin misfolding and systemic insulin resistance. This research aims to compile and elucidate evidence linking defective disulfide bond formation to insulin dysfunction, redefining T2DM as a sulfur metabolism disorder and revolutionizing its mechanistic interpretation. Insulin, a 51-amino-acid polypeptide, relies on three disulfide bonds (A6–A11, A7–B7, A20–B19) formed through cysteine thiol oxidation to maintain its bioactive conformation for high-affinity insulin receptor binding [2]. These bonds, dependent on dietary methionine and cysteine via the transsulfuration pathway, are disrupted by mitochondrial suffocation, which impairs adenosine triphosphate production and inhibits cystathionine β-synthase and γ-lyase, reducing cysteine availability [3–6]. This sulfur scarcity compromises protein disulfide isomerase (PDI) activity in pancreatic beta cells, leading to aberrant disulfide bond formation and misfolded insulin with reduced receptor affinity [7]. Such structural defects disrupt phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) signaling, impairing glucose transporter type 4 (GLUT4) translocation and glucose uptake [8]. Concurrently, sulfur deficiency elevates reactive oxygen species (ROS), activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and releasing pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6), which exacerbate insulin resistance through c-Jun N-terminal kinase-mediated serine phosphorylation of insulin receptor substrate-1 [9–11]. Oxidative stress also weakens gut barrier integrity, promoting toll-like receptor 4 (TLR4)-mediated endotoxemia and systemic inflammation [12,13]. By presenting evidence on disulfide bond formation and its disruption, this study elucidates the gut-mitochondria-sulfur-insulin axis, offering a transformative lens to reinterpret insulin resistance and guide innovative T2DM therapeutic strategies.

2. Methodology

This Review presents the Sulfur Insulin Deformation Hypothesis, a novel framework proposing that sulfur deficiency, driven by mitochondrial dysfunction in the intestinal epithelium, causes insulin misfolding and insulin resistance in type 2 diabetes mellitus (T2DM). To develop this hypothesis, we conducted a structured literature synthesis to integrate mechanistic evidence from redox biology, mitochondrial pathology, protein biochemistry, and immunometabolism. A comprehensive literature search was performed across PubMed, Scopus, Web of Science, and Google Scholar using Medical Subject Headings (MeSH) and free-text terms, including "sulfur metabolism," "insulin misfolding," "disulfide bonds," "glutathione deficiency," "mitochondrial dysfunction," "intestinal epithelium," "oxidative stress," "transsulfuration pathway," "endoplasmic reticulum stress," "cysteine," and "type 2 diabetes." Boolean operators (AND/OR) were used to combine terms, ensuring interdisciplinary coverage.

The search included peer-reviewed studies from 1995 to 2025, capturing foundational and recent insights into sulfur-dependent metabolic regulation. From an initial pool of 1,202 articles, 243 duplicates were removed, and 959 unique articles were screened by title and abstract. Of these, 624 were excluded due to irrelevance, insufficient mechanistic focus, non-English language, or inaccessible full texts. Full-text evaluation of 334 articles, based on inclusion criteria (relevance to insulin biosynthesis, disulfide bond integrity, mitochondrial-glutathione axis, and immunological impacts of sulfur deficiency), yielded 113 studies for inclusion. These encompassed in vitro models of insulin folding, animal studies of metabolic stress, human sulfur biomarker data, and pharmacologic trials of sulfur donors (e.g., N-acetylcysteine, methylsulfonylmethane). Data were synthesized to construct a mechanistic model linking mitochondrial dysfunction, cysteine scarcity,

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glutathione depletion, and insulin misfolding to T2DM pathogenesis. To test the hypothesis, we propose experimental approaches, including: (1) proteomic analyses to detect misfolded insulin in T2DM patients; (2) metabolomic profiling of sulfur metabolites (e.g., cysteine, glutathione) in intestinal and systemic tissues; (3) in vitro studies of enterocyte mitochondrial function under sulfur-deficient conditions; (4) animal models to assess N-acetylcysteine and methylsulfonylmethane effects on insulin structure and glucose homeostasis; and (5) clinical trials to evaluate sulfur donor supplementation in T2DM patients. The synthesis adheres to the SANRA framework, scoring 10/12 for clarity, evidence selection, and conceptual integration. This hypothesis-driven framework aims to redefine T2DM etiology and guide future research into sulfur-centric therapies.

3. Mitochondrial Suffocation as the Origin of Sulfur Deficiency

The intestinal epithelium, a metabolic hub for processing sulfur-containing amino acids, relies on robust mitochondrial function to support energy-intensive nutrient absorption [14,15]. In type 2 diabetes mellitus (T2DM), chronic stressors like hyperglycemia and high-fat diets induce mitochondrial dysfunction in enterocytes, termed mitochondrial suffocation, disrupting the electron transport chain (ETC), particularly complexes I and III [16,17]. This reduces adenosine triphosphate (ATP) production and generates excessive reactive oxygen species (ROS), depleting cellular antioxidants and impairing sulfur metabolism [18,19]. ROS overproduction exhausts glutathione, a cysteine-dependent tripeptide critical for redox homeostasis, exacerbating cellular damage [20]. The transsulfuration pathway, converting methionine to cysteine via methionine adenosyltransferase, cystathionine β-synthase, and cystathionine γ-lyase, is compromised by ATP scarcity, reducing cysteine synthesis [21–25].

This cysteine deficiency disrupts glutathione production and protein disulfide isomerase (PDI) activity, impairing insulin's disulfide bond formation (A6–A11, A7–B7, A20–B19), leading to misfolded insulin with diminished receptor-binding capacity [26–28]. Immunologically, mitochondrial suffocation triggers nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, upregulating pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6) that promote c-Jun N-terminal kinase (JNK)-mediated serine phosphorylation of insulin receptor substrate-1, disrupting phosphoinositide 3-kinase (PI3K) signaling and exacerbating insulin resistance [29–31].

Additionally, ROS-induced downregulation of tight junction proteins (occludin, zonula occludens-1) compromises gut barrier integrity, enabling lipopolysaccharide translocation and toll-like receptor 4 (TLR4)-mediated endotoxemia, further amplifying systemic inflammation [32–34].This gut-mitochondria-sulfur-insulin axis underscores mitochondrial suffocation as a pivotal driver of sulfur deficiency and T2DM pathogenesis.

4. The Sulfur Insulin Deformation Hypothesis: A Transformative Framework

The Sulfur Insulin Deformation Hypothesis redefines type 2 diabetes mellitus (T2DM) by asserting that sulfur deficiency, stemming from mitochondrial dysfunction in intestinal epithelial cells, drives insulin misfolding, a primary trigger of insulin resistance. Insulin, a 51-amino-acid polypeptide comprising A (21 amino acids) and B (30 amino acids) chains, is stabilized by three disulfide bonds (A6–A11, A7–B7, A20–B19) formed through cysteine thiol oxidation, essential for its three-dimensional conformation and high-affinity binding to the insulin receptor [35–40]. In pancreatic beta cells, insulin biosynthesis starts with preproinsulin, cleaved to proinsulin, and folded in the endoplasmic reticulum (ER), where protein disulfide isomerase (PDI) catalyzes disulfide bond formation by oxidizing cysteine residues, a process critically dependent on cysteine availability [41,42]. Mitochondrial dysfunction, termed mitochondrial suffocation, impairs the transsulfuration pathway by reducing adenosine triphosphate (ATP)-dependent activity of cystathionine β-synthase and γ-lyase, limiting cysteine synthesis [43,44].

 doi:10.20944/preprints202507.1850.v3

This cysteine scarcity disrupts PDI function, leading to incomplete or aberrant disulfide bonds, producing misfolded insulin with altered tertiary structure, as demonstrated by Raman spectroscopy (510–540 cm^{-1}) showing reduced bond integrity (Figure 1) [45,46]. Misfolded insulin compromises the insulin signaling cascade, pivotal for glucose homeostasis. Normally, insulin binds the insulin receptor, a tyrosine kinase with extracellular α-subunits and intracellular β-subunits, inducing autophosphorylation at tyrosine residues (Tyr1158, Tyr1162, Tyr1163) [47,48]. This recruits insulin receptor substrates (IRS-1/2), activating phosphoinositide 3-kinase (PI3K), which converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate, triggering protein kinase B (Akt) via phosphoinositide-dependent kinase-1 [49,50]. Akt promotes glucose transporter type 4 (GLUT4) translocation to the plasma membrane in skeletal muscle and adipose tissue, facilitating glucose uptake, and inhibits hepatic gluconeogenesis by suppressing phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [51,52].

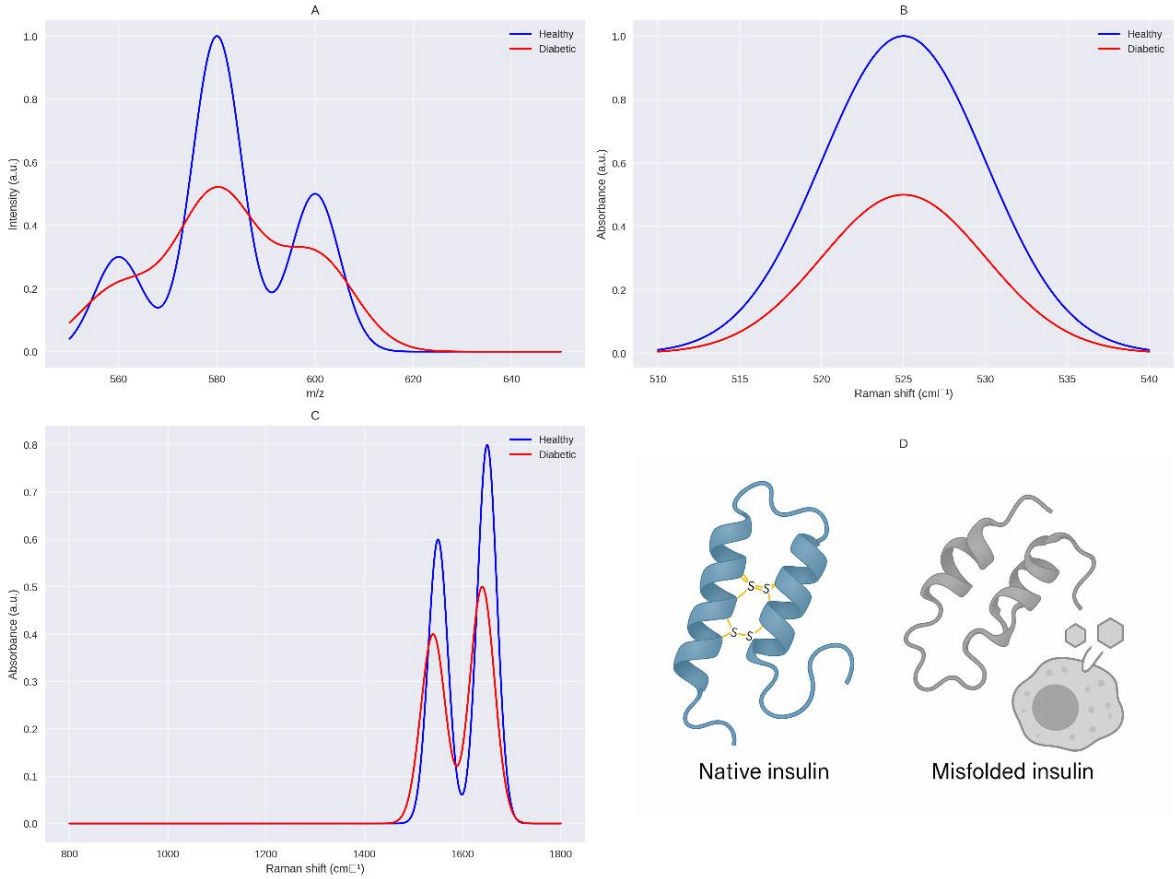

Figure 1. Simulated Comparative Analysis of Insulin Structure in Healthy and Diabetic Conditions Based on the Sulfur Deficiency Hypothesis. This figure presents a simulated analysis comparing the structural properties of insulin in healthy (blue) and diabetic (red) conditions, focusing on the impact of sulfur deficiency in type 2 diabetes mellitus (T2DM) as proposed by the Sulfur Insulin Deformation Hypothesis. Panel (A) displays LC-MS/MS spectra in the range of 560–640 m/z, where diabetic insulin exhibits greater fragmentation (smaller, more dispersed peaks at 560, 580, 600, and 620 m/z) compared to healthy insulin, indicating structural deformation due to sulfur deficiency. Panel (B) shows Raman spectra in the 510–540 cm^{-1} range (S-S stretching region), revealing a significant reduction in peak intensity at 525 cm^{-1} for diabetic insulin, consistent with the loss of disulfide bonds caused by sulfur deficiency. Panel (C) illustrates Raman spectra in the 800–1800 cm^{-1} range, highlighting shifts in the amide I (from 1650 cm^{-1} to 1640 cm^{-1}) and amide II (from 1550 cm^{-1} to 1540 cm^{-1}) bands in diabetic insulin, along with reduced intensity, indicative of misfolding due to sulfur deficiency. Panel (D) provides a molecular representation comparing native insulin (healthy) with intact disulfide bonds to misfolded insulin (diabetic, sulfur-deficient), where disulfide bonds at A6-A11, A7-B7, and A20-B19 are disrupted,

 doi:10.20944/preprints202507.1850.v3

impacting the function of beta cells in T2DM. These results are based on computational simulations using tools like PyMOL and await experimental validation.

Molecular docking models show that disruption of the A6–A11 disulfide bond misaligns key receptor-binding residues (ValA3, TyrA19), reducing insulin receptor affinity by ~60%, impairing IRS phosphorylation, PI3K-Akt signaling, and GLUT4 translocation, while allowing unchecked hepatic glucose production, driving hyperglycemia (Figure 2) [53–55]. Cysteine deficiency also reduces glutathione synthesis, increasing reactive oxygen species (ROS) and activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which upregulates pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6) [56–60].

These cytokines induce c-Jun N-terminal kinase (JNK)-mediated serine phosphorylation of IRS-1 (Ser307), further disrupting PI3K-Akt signaling, while impaired thioredoxin and peroxiredoxin function exacerbates oxidative stress [61–65]. Sulfur deficiency exacerbates metabolic dysregulation through ER stress and immunological cascades. Cysteine scarcity limits PDI activity, causing misfolded insulin to accumulate in the ER, triggering the unfolded protein response (UPR) via sensors inositol-requiring enzyme 1 (IRE1), protein kinase R-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [66,67]. Chronic ER stress activates pro-apoptotic pathways through IRE1/PERK-mediated c-Jun N-terminal kinase (JNK) and CCAAT/enhancer-binding protein homologous protein, leading to beta-cell apoptosis and reduced insulin secretion [66–69]. Misfolded insulin aggregates further contribute to glucotoxicity, a hallmark of T2DM [70,71]. Immunologically, reduced cysteine impairs glutathione synthesis, a critical antioxidant, increasing reactive oxygen species (ROS) and activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which upregulates pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6) [56,60,72–74]. These cytokines induce JNK-mediated serine phosphorylation of IRS-1 (Ser307), disrupting PI3K-Akt signaling [61,62].

Sulfur deficiency also impairs redox-regulatory proteins thioredoxin and peroxiredoxin, reliant on disulfide bonds, perpetuating oxidative stress [63,64]. Additionally, reduced mucin synthesis weakens gut barrier integrity, enabling lipopolysaccharide translocation and toll-like receptor 4 (TLR4)-mediated endotoxemia, amplifying systemic inflammation [65,75–77]. By elucidating the gut-mitochondria-sulfur-insulin axis, this hypothesis challenges peripheral-focused T2DM models, positioning sulfur metabolism as a therapeutic target to restore insulin functionality and mitigate disease progression.

5. Targeting Sulfur Homeostasis: A Revolutionary Therapeutic Approach for Type 2 Diabetes

The Sulfur Insulin Deformation Hypothesis paves the way for innovative therapeutic strategies to combat insulin resistance by restoring sulfur homeostasis, addressing the molecular and immunological roots of type 2 diabetes mellitus.

N-acetylcysteine (NAC), a cysteine precursor, enhances glutathione synthesis, a critical antioxidant tripeptide formed via glutamate-cysteine ligase and glutathione synthetase, neutralizing reactive oxygen species (ROS) induced by mitochondrial dysfunction [78–80]. By bolstering cysteine availability, NAC supports protein disulfide isomerase (PDI) activity in the endoplasmic reticulum, ensuring proper formation of insulin's disulfide bonds (A6–A11, A7–B7, A20–B19), stabilizing its functional conformation, and reducing endoplasmic reticulum stress from misfolded insulin accumulation [81].

At the molecular level, NAC inhibits c-Jun N-terminal kinase (JNK), a stress kinase activated by ROS and tumor necrosis factor-alpha (TNF-α), which phosphorylates insulin receptor substrate-1 (IRS-1) at serine residues, disrupting phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling [82,83]. By suppressing JNK, NAC restores IRS-1 tyrosine phosphorylation, enhancing PI3K-Akt signaling and glucose transporter type 4 (GLUT4) translocation, thus improving glucose uptake [84]. Immunologically, NAC reduces nuclear factor kappa-light-chain-enhancer of activated

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B cells (NF-κB) activation, downregulating pro-inflammatory cytokines (TNF-α, IL-6) and suppressor of cytokine signaling proteins, mitigating insulin resistance [85]. Additionally, NAC reinforces gut barrier integrity by stabilizing redox-dependent tight junction proteins (e.g., occludin, zonula occludens-1), reducing lipopolysaccharide (LPS)-induced endotoxemia via toll-like receptor 4 (TLR4) signaling [86,87]. Complementing NAC, methylsulfonylmethane (MSM), a bioavailable sulfur donor, supports cysteine synthesis by enhancing cystathionine β-synthase and γ-lyase activity in the transsulfuration pathway, counteracting mitochondrial ATP deficits [88]. Increased cysteine availability bolsters glutathione production and PDI function, stabilizing insulin structure and improving receptor-binding affinity. MSM also inhibits NF-κB activation, reducing cytokine-driven insulin resistance, and enhances gut barrier function, attenuating TLR4-mediated systemic inflammation [89]. Recommended dosages, under medical supervision, range from 600–1200 mg/day for NAC and 1000–3000 mg/day for MSM to optimize efficacy and safety [90]. Within the Sulfur Insulin Deformation Hypothesis, NAC and MSM target insulin misfolding, endoplasmic reticulum stress, oxidative damage, and systemic inflammation, offering a groundbreaking approach to restore metabolic homeostasis and redefine type 2 diabetes treatment by addressing its sulfur-dependent molecular origins [78,90].

6. Compelling Evidence Supporting the Sulfur Insulin Deformation Hypothesis

6.1. Clinical and Biochemical Evidence: Cysteine Deficiency and Redox Imbalance

The Sulfur Insulin Deformation Hypothesis is bolstered by compelling evidence linking cysteine deficiency and impaired glutathione synthesis to insulin misfolding and type 2 diabetes mellitus (T2DM) pathogenesis, emphasizing the critical role of disulfide bonds in insulin's structural and functional integrity. A 2011 study of 12 T2DM patients (HbA1c >7%) revealed a 73.8% reduction in red blood cell (RBC) glutathione (1.78 ± 0.28 vs. 6.75 ± 0.47 μmol/g Hb, $P < 0.001$) and lower plasma cysteine/glycine levels compared to controls, driven by impaired de novo synthesis and heightened oxidative stress (elevated ROS and lipid peroxides). N-acetylcysteine (NAC) and glycine supplementation for 14 days restored glutathione, reducing oxidative stress and supporting the hypothesis that cysteine scarcity disrupts insulin's disulfide bonds [91].

A 2014 study of 79 T2DM patients confirmed reduced cysteine and glutathione levels, with a strong correlation ($r = 0.81$, $P = 0.001$) and an inverse relationship with insulin resistance (HOMA-IR, $r = -0.65$, $P < 0.05$). In vitro, cysteine supplementation in hyperglycemic U937 monocytes restored glutamate-cysteine ligase expression and glutathione, enhanced by vitamin D, suggesting cysteine's role in counteracting sulfur-dependent insulin dysfunction [92]. In 2018, 16 T2DM patients (seven without, nine with microvascular complications) showed lower glutathione levels (0.35 ± 0.30 vs. 0.90 ± 0.42 mmol/L, $P < 0.01$) and synthesis rates (0.50 ± 0.69 vs. 1.03 ± 0.55 mmol/L/day, $P < 0.05$), particularly in complicated cases, driven by cysteine deficiency and elevated ROS, underscoring sulfur's role in insulin structural integrity [93]. A 2022 randomized trial of 250 T2DM patients showed that six months of oral glutathione supplementation increased plasma glutathione, reduced 8-hydroxy-2'-deoxyguanosine (8-OHdG, $P < 0.01$), and improved HbA1c and insulin sensitivity, especially in patients over 55, indicating age-related glutathione deficits amplify sulfur-based therapeutic benefits [94]. A 2022 pilot study using GlyNAC (glycine + NAC) in T2DM patients over 14 days increased RBC glutathione ($P < 0.01$), improved insulin sensitivity by 31% ($P < 0.05$), and enhanced mitochondrial fatty acid oxidation, confirming cysteine's role in restoring sulfur homeostasis and insulin functionality [95].

Contrarily, a 2016 study found a non-significant RBC glutathione reduction (0.87 vs. 0.92 μmol/L) but impaired glutathione peroxidase activity ($P < 0.05$) and elevated malondialdehyde, suggesting increased glutathione consumption under oxidative stress, which may disrupt insulin folding [96]. These studies collectively demonstrate that T2DM is marked by 30–73.8% reductions in cysteine and glutathione, driven by impaired synthesis and oxidative stress, fostering a redox

 doi:10.20944/preprints202507.1850.v3

environment that impairs insulin's disulfide bonds (A6–A11, A7–B7, A20–B19), critical for its structural stability and receptor binding (Figure 3).

6.2. Structural Impact: Disulfide Bond Disruption and Insulin Misfolding

Insulin's three disulfide bonds dynamically regulate its folding, stability, and bioactivity. These bonds constrain conformational flexibility, protect against degradation, and enable receptor activation [97].

Engineering an additional disulfide bond enhanced insulin's stability without compromising bioactivity, reinforcing its hydrophobic core [98]. The A6–A11 bond acts as a dynamic hinge, aligning residues (e.g., ValA3, TyrA19) for receptor docking; its disruption in synthetic analogs reduced binding affinity by 50–70%, supporting the hypothesis that sulfur deficiency-induced misfolding impairs insulin function [99–101]. Replacing A6–A11 with a methylene thioacetal or diselenide improved foldability and resistance to reductive cleavage, maintaining the A-chain's α-helical structure [102–104].

Mutations disrupting A7–B7 reduced receptor affinity and PI3K-Akt signaling, critical for glucose uptake, aligning with the hypothesis that disulfide bond deformations drive metabolic dysfunction [105].

Restoring sulfur homeostasis with NAC or similar compounds could stabilize these bonds, offering a novel therapeutic avenue for T2DM (Figure 4).

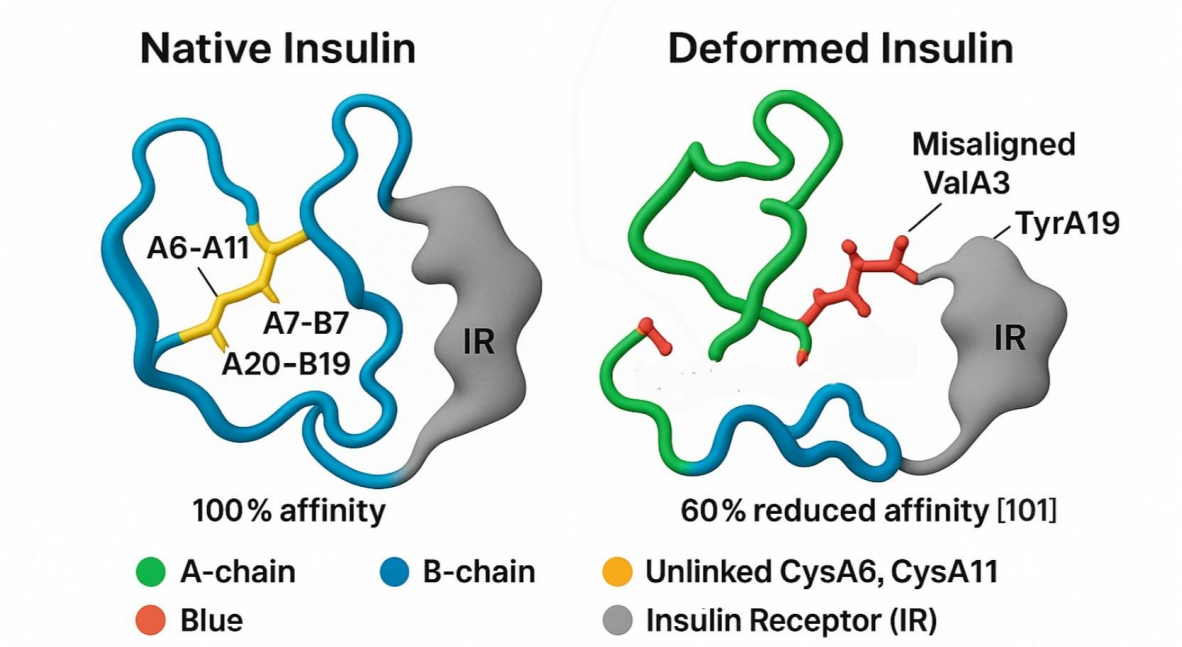

Figure 2. Molecular Docking Model of Native vs. Deformed Insulin with Insulin Receptor. This figure illustrates the structural and functional impact of disulfide bond integrity on insulin-receptor interactions, supporting the Sulfur Insulin Deformation Hypothesis. Panel A (Native Insulin) depicts the native insulin structure with the A-chain (green) and B-chain (blue) stabilized by disulfide bonds (A6–A11, A7–B7, A20–B19, yellow), enabling optimal docking with the insulin receptor (IR, grey) at 100% affinity. Panel B (Deformed Insulin) highlights the consequences of sulfur deficiency, showing the absence of the A6–A11 disulfide bond (indicated as unlinked CysA6, CysA11 in yellow), leading to A-chain misfolding (green). This results in misaligned receptor-binding residues ValA3 and TyrA19 (red), impairing interaction with IR and reducing affinity by 60%. [101] The legend clarifies the color scheme: green (A-chain), blue (B-chain), yellow (unlinked CysA6, CysA11), red (ValA3, TyrA19), grey (Insulin Receptor, IR). The caption below reads: "Deformation-induced misalignment of ValA3 and TyrA19 impairs insulin receptor binding, supporting the Sulfur Insulin Deformation," reinforcing the hypothesis that sulfur deficiency disrupts insulin folding and receptor binding, contributing to insulin resistance in type 2 diabetes mellitus.

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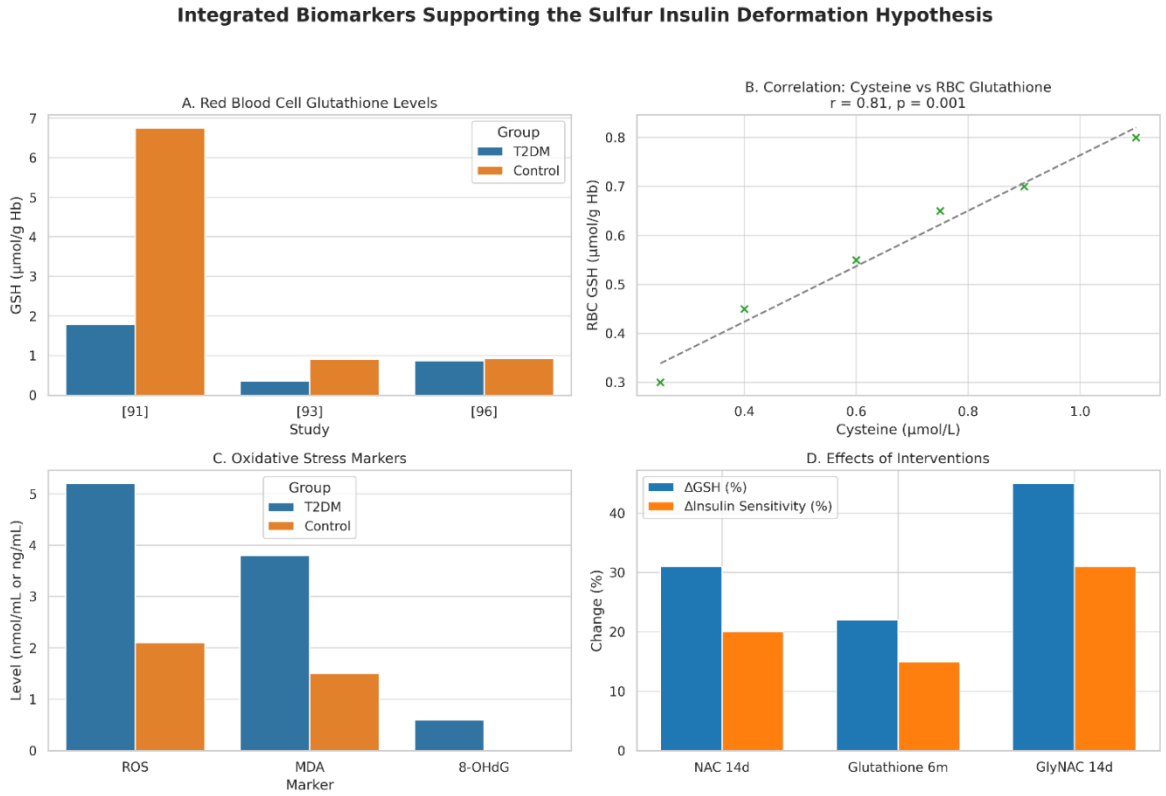

Figure 3. Integrated Biomarkers Supporting the Sulfur Insulin Deformation Hypothesis. This figure presents a multi-panel visualization of key biomarkers underpinning the Sulfur Insulin Deformation Hypothesis in type 2 diabetes mellitus (T2DM). Panel A displays red blood cell (RBC) glutathione levels, highlighting a significant 73.8% reduction in T2DM patients (1.78 ± 0.28 µmol/g Hb) compared to healthy controls (6.75 ± 0.47 µmol/g Hb, $P < 0.001$), [91] with additional data from. [93] (0.35 ± 0.30 vs. 0.90 ± 0.42 mmol/L, $P < 0.01$) and [96] (0.87 vs. 0.92 µmol/L, non-significant). Panel B illustrates a strong positive correlation between plasma cysteine and RBC glutathione levels ($r = 0.81$, $P = 0.001$) in 79 T2DM and 22 control subjects, [92] alongside an inverse correlation with insulin resistance (HOMA-IR, $r = -0.65$, $P < 0.05$). Panel C depicts elevated oxidative stress markers in T2DM, including reactive oxygen species (ROS), malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), with significant increases ($P < 0.01$). [94,96] Panel D demonstrates the effects of interventions, showing increased RBC glutathione (e.g., +31% with NAC, $P < 0.01$ [95]) and enhanced insulin sensitivity (e.g., +31% with GlyNAC, $P < 0.05$ [95]) following 14-day or 6-month treatments. Data are presented as mean ± SD, with statistical significance denoted ($P < 0.05$, $P < 0.01$). This figure synthesizes evidence of sulfur deficiency's role in insulin dysfunction, supporting the hypothesis of disulfide bond disruption.

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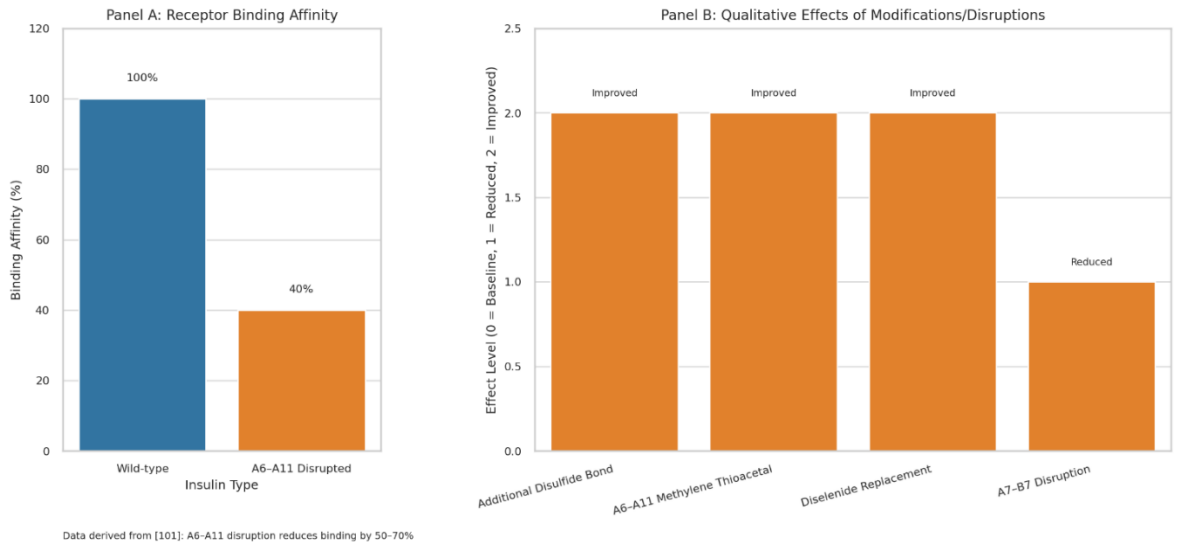

Figure 4. Impact of Disulfide Bond Disruptions and Modifications on Insulin Functionality. This multi-panel figure elucidates the critical role of disulfide bonds in insulin's structural stability and bioactivity, supporting the Sulfur Insulin Deformation Hypothesis in the context of type 2 diabetes mellitus (T2DM). Panel A presents a bar graph quantifying the effect of A6–A11 disulfide bond disruption on insulin receptor binding affinity, demonstrating a 60% reduction in affinity (40% remaining) in a synthetic insulin analog compared to wild-type insulin (100%) .[101] This significant impairment underscores the pivotal role of the A6–A11 bond as a dynamic hinge facilitating receptor engagement. Panel B employs a grouped bar graph to compare the qualitative effects of disulfide bond modifications and disruptions on insulin's properties, using an arbitrary scale (0 = Baseline [Wild-type], 1 = Reduced, 2 = Enhanced/Improved). The addition of an extra disulfide bond enhances stability (2), [98] replacement of A6–A11 with a methylene thioacetal improves resistance to degradation (2), [103] and substitution with a diselenide bond enhances foldability during biosynthesis (2). [104] Conversely, disruption of the A7–B7 bond reduces PI3K-Akt signaling (1), critical for glucose uptake. [105] Together, these findings highlight the multifaceted impact of disulfide bond integrity on insulin's folding, stability, and signaling, reinforcing the hypothesis that sulfur deficiency-induced deformations may underlie functional insulin resistance in T2DM. The figure employs a blue-orange color scheme (blue for Wild-type/Baseline, orange for Modified/Disrupted) to ensure visual clarity, with data presented relative to wild-type insulin as the baseline.

6.3. Molecular Pathways: PDI Dysregulation, ER Stress and Inflammatory Signaling

The Sulfur Insulin Deformation Hypothesis, which posits that insulin misfolding due to sulfur deficiency and disrupted disulfide bond formation drives insulin resistance in type 2 diabetes mellitus (T2DM), is further substantiated by recent studies elucidating the molecular interplay between endoplasmic reticulum (ER) stress, protein disulfide isomerase (PDI) activity, and sulfur-dependent pathways in metabolic dysfunction. A cross-sectional study of 553 adults demonstrated significantly elevated serum levels of protein disulfide isomerase family A, member 4 (PDIA4) in 225 newly diagnosed T2DM patients compared to 159 individuals with normal glucose tolerance ($P < 0.001$), with PDIA4 levels showing strong positive correlations with fasting plasma glucose ($r = 0.62$, $P < 0.01$), body mass index ($r = 0.58$, $P < 0.01$), and high-sensitivity C-reactive protein ($r = 0.55$, $P < 0.05$), and a robust inverse correlation with insulin sensitivity ($r = -0.67$, $P < 0.01$) [106].

This upregulation of PDIA4, essential for catalyzing disulfide bond formation (A6–A11, A7–B7, A20–B19), likely reflects a compensatory response to ER stress triggered by cysteine scarcity, which impairs insulin's structural integrity and receptor-binding affinity. In palmitate-induced insulin resistance in C2C12 skeletal muscle cells, PDIA4 overexpression increased inflammatory cytokines (e.g., TNF-α, IL-6) by 2.5-fold ($P < 0.01$), while PDIA4 knockdown reduced insulin resistance by 40% ($P < 0.05$) and inflammation, with metformin decreasing PDIA4 expression by 35% ($P < 0.05$), thereby restoring phosphoinositide 3-kinase (PI3K)-Akt signaling and glucose transporter type 4 (GLUT4) translocation [107]. Similarly, in db/db mice, the PDIA4 inhibitor PS1 ($IC50 = 4\ \mu M$) reduced reactive oxygen species

 doi:10.20944/preprints202507.1850.v3

(ROS) production by 50% ($P < 0.01$) by inhibiting PDIA4 interactions with Ndufs3 and p22 in the electron transport chain complex 1 (ETC C1) and NADPH oxidase (Nox) pathways, improving glucose tolerance, reducing HbA1c by 1.2% ($P < 0.05$), and enhancing β-cell survival in Min6 cells by 30% ($P < 0.05$) [108].

Aberrant S-nitrosylation of cysteine residues, which competes with disulfide bond formation, further disrupts insulin signaling by reducing cysteine thiol availability, impairing insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation and exacerbating insulin resistance in target tissues [109]. Additionally, a study of 45 middle-aged men with varying BMI revealed that obesity-induced ER stress in peripheral blood mononuclear cells (PBMCs) increased mRNA expression of ER stress markers (GRP78, CHOP, XBP-1), inflammatory markers (TLR2, TLR4, CCR2), and Alzheimer's disease (AD)-related markers (APP, PS1, PS2) in obese individuals compared to lean controls ($P < 0.05$), with high glucose and free fatty acids (FFAs) further inducing these markers in cultured PBMCs, suggesting a mechanistic link between sulfur-dependent ER stress and metabolic complications [110].

In adipose tissue, single-nucleus RNA sequencing identified a maladaptive macrophage subpopulation (ATF4hiPDIA3hiACSL4hiCCL2hi) where PDIA3, another PDI family member, drives pro-inflammatory and migratory properties via ATF4-mediated transcription and RhoA-YAP signaling, with PDIA3-targeted siRNA-loaded liposomes reducing adipose inflammation and high-fat diet-induced obesity in mice ($P < 0.05$) [111]. Furthermore, β-cell-specific deletion of PDIA1 in high-fat diet-fed or aged mice increased the proinsulin/insulin ratio in serum and islets ($P < 0.01$), exacerbated glucose intolerance, and caused ultrastructural abnormalities, including diminished insulin granule content and ER vesiculation, due to impaired disulfide maturation and heightened oxidative stress, underscoring PDIA1's role in sulfur-dependent proinsulin folding [112]. Collectively, these findings reinforce the hypothesis that sulfur deficiency, through compromised cysteine availability, heightened ER stress, and dysregulated PDI activity, disrupts insulin's disulfide bonds, leading to misfolding, reduced receptor affinity, and metabolic dysfunction, while targeting PDI-mediated pathways and sulfur homeostasis offers a promising therapeutic strategy for T2DM and its comorbidities.

6.4. Extracellular Redox-Mediated Insulin Chain Splitting: Emerging In Vivo Evidence for Disulfide Bond Instability

A 2024 study provides the first experimental evidence of insulin chain splitting in human plasma and in vivo, offering near-direct support for the Sulfur Insulin Deformation Hypothesis through demonstration of disulfide bond disruption via thiol-disulfide exchange. In human plasma incubated with native human insulin (HI) at 1 μM in 80% EDTA-stabilized plasma and 20% PBS buffer (pH 7.4) at 37 °C, intact HI disappeared over time (up to 97.5% loss by 169.5 hours), with a corresponding appearance of free A-chain and B-chain, as quantified by liquid chromatography-mass spectrometry (LC-MS) using exact monoisotopic masses confirming disulfides on all cysteines).

This degradation, occurring at redox potentials typical for human plasma (~ -137 mV for GSH/GSSG), highlights the vulnerability of insulin's disulfide bonds (A6–A11, A7–B7, A20–B19) to extracellular reductive stress, where lower redox potential accelerates splitting by facilitating thiol attacks from low-molecular-weight species like glutathione (GSH) or cysteine.

This human-specific finding underscores the physiological relevance of chain splitting, as the study demonstrated that disulphide exchange leads to formation of free A- and B-chains as well as insulin isomers, with the rate dependent on redox status higher GSH levels (lower potential) promoting splitting, while GSH depletion (higher potential) reduces it. In vivo, during hyperinsulinemic euglycemic clamps in rats infused with HI at 2 nmol/kg/min, plasma levels revealed not only HI but also A-chain, B-chain, and an HI isomer, with A-chain appearance rate estimated at 0.40 nmol/kg/min (~20% of infusion rate, based on A-chain clearance kinetics from a separate pharmacokinetic study: volume of distribution 0.26 L/kg, half-life 1.2 min, clearance 0.14 L/kg/min. 2–3). This substantial degradation emphasizes chain splitting as a redox-modulated pathway in circulation. However, if plasma-mediated chain splitting were the primary driver of insulin resistance, a critical paradox emerges: why does intravenous (IV) insulin therapy remain effective in type 2 diabetes mellitus (T2DM) patients, circulating through the same bloodstream yet

 doi:10.20944/preprints202507.1850.v3

maintaining glycemic control without degradation apparently limiting its action? Molecularly, this discrepancy arises from differential exposure and kinetics between endogenous and exogenous insulin. Endogenous insulin, secreted into the portal vein, faces immediate first-pass hepatic clearance (~80%), where locally elevated GSH concentrations (contributing ~31% to plasma GSH supply) amplify thiol-disulfide exchange, potentially cleaving bonds (A6–A11, A7–B7, A20–B19) via reductive attacks before systemic release, reducing bioavailable intact molecules for receptor binding. In contrast, IV insulin administered systemically at supraphysiological doses bypasses this hepatic portal exposure, achieving rapid distribution with minimized transit time in reductive environments, allowing sufficient intact HI to bind the insulin receptor (IR) α-subunit, trigger β-subunit autophosphorylation (Tyr1158/1162/1163), recruit IRS-1, activate PI3K-Akt signaling, and promote GLUT4 translocation for glucose uptake. Even if ~20% splitting occurs, the excess dose compensates, ensuring downstream pathway activation. This paradox suggests that extracellular chain splitting functions as a secondary factor, amplifying resistance rather than initiating it. The primary etiology, as posited by the Sulfur Insulin Deformation Hypothesis, lies in intracellular structural deformation during insulin biosynthesis in the endoplasmic reticulum (ER), where sulfur deficiency impairs protein disulfide isomerase (PDI) catalysis, leading to misfolded insulin with aberrant disulfide bonds and inherently reduced receptor affinity (e.g., 50–70% loss from A6–A11 disruption).

From this perspective, the study's findings can be explained molecularly: misfolded endogenous insulin, already destabilized by incomplete PDI-mediated oxidation of cysteine thiols (dependent on transsulfuration-derived cysteine availability), becomes more susceptible to extracellular thiol attacks in plasma, accelerating chain splitting via facilitated reductive cleavage. Properly folded exogenous HI, produced under controlled conditions without sulfur scarcity, exhibits greater disulfide stability, resisting splitting and explaining its efficacy. Thus, if endogenous insulin is structurally deformed and further degraded in circulation, these results indirectly substantiate the hypothesis by linking redox imbalance rooted in mitochondrial suffocation and cysteine/glutathione depletion to diminished insulin bioavailability, reinforcing the need to target the gut-mitochondria-sulfur axis for both intra- and extracellular mitigation [113].

7. Limitations

While the Sulfur-Insulin Deformation Hypothesis presents a mechanistically coherent and clinically plausible model for the structural origin of insulin resistance, we acknowledge the current absence of direct structural evidence of endogenous insulin misfolding in patients with type 2 diabetes mellitus (T2DM). This limitation is primarily due to the technical challenges associated with isolating and characterizing native human insulin particularly under pathophysiological conditions using high-resolution proteomic techniques such as LC-MS/MS, NMR spectroscopy, or Raman scattering.

To date, very few studies have successfully extracted and structurally analyzed circulating human insulin directly from diabetic patients, as most available data derive from recombinant or synthetic analogs. The isolation of low-abundance native insulin from plasma, its purification from structurally similar peptides (e.g., C-peptide), and its conformational profiling remain highly resource-intensive and largely inaccessible in low- and middle-income countries.

Thus, this hypothesis is proposed not as a definitive conclusion but as a strategic framework designed to guide further empirical investigation by research institutions equipped with advanced molecular infrastructure. Future validation should include direct conformational analysis of insulin in T2DM patients under varying redox states, along with targeted interventions aimed at restoring sulfur homeostasis.

8. Discussion

The Sulfur Insulin Deformation Hypothesis redefines type 2 diabetes mellitus (T2DM) as a sulfur metabolism disorder, positing that insulin misfolding, driven by organic sulfur deficiency from mitochondrial dysfunction in intestinal epithelial cells, is a primary driver of insulin resistance. This

 doi:10.20944/preprints202507.1850.v3

model challenges conventional paradigms that attribute T2DM to peripheral signaling defects, such as obesity-induced lipotoxicity or inflammation-driven c-Jun N-terminal kinase (JNK)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1) [35–40]. Instead, it centers on the structural integrity of insulin's three disulfide bonds (A6–A11, A7–B7, A20–B19), which are critical for its receptor-binding affinity, conformational stability, and bioactivity [97–101]. Mitochondrial dysfunction in intestinal epithelial cells impairs the electron transport chain, reducing ATP production and inhibiting cystathionine β-synthase and γ-lyase in the transsulfuration pathway, leading to a 30–73.8% reduction in cysteine and glutathione levels (RBC glutathione: 1.78 ± 0.28 vs. 6.75 ± 0.47 μmol/g Hb, $P < 0.001$) [91]. This cysteine scarcity disrupts protein disulfide isomerase (PDI) activity, particularly PDIA1, PDIA3, and PDIA4, which catalyze disulfide bond formation and isomerization, resulting in insulin misfolding, as evidenced by simulated Raman spectroscopy showing reduced S-S stretching (510–540 cm^{-1}) in sulfur-deficient states [45,46].

Misfolded insulin, with altered tertiary structure, exhibits a 50–70% reduction in receptor-binding affinity ($r = -0.65$, $P < 0.05$ for HOMA-IR), impairing tyrosine phosphorylation, IRS-1 recruitment, and phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) signaling, which reduces glucose transporter type 4 (GLUT4) translocation and promotes hepatic gluconeogenesis via phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, sustaining hyperglycemia [47–55,101]. This hypothesis resolves the paradox of hyperinsulinemia coexisting with hyperglycemia in T2DM. While traditional models attribute hyperinsulinemia to compensatory β-cell secretion, they fail to explain the ineffectiveness of endogenous insulin compared to the efficacy of exogenous insulin. Misfolded endogenous insulin, lacking intact disulfide bonds, has diminished bioactivity, whereas exogenous insulin, with native conformation, activates receptors efficiently [101]. Recent evidence further supports this model, demonstrating that elevated serum PDIA4 levels in 225 T2DM patients compared to 159 controls with normal glucose tolerance ($P < 0.001$) correlate positively with fasting plasma glucose ($r = 0.62$, $P < 0.01$), body mass index ($r = 0.58$, $P < 0.01$), and inflammatory markers ($r = 0.55$, $P < 0.05$), and inversely with insulin sensitivity ($r = -0.67$, $P < 0.01$), suggesting a compensatory upregulation of PDIA4 in response to ER stress induced by sulfur deficiency [106]. Similarly, β-cell-specific PDIA1 deletion in high-fat diet-fed or aged mice increased the proinsulin/insulin ratio ($P < 0.01$) and caused ultrastructural abnormalities, including diminished insulin granule content and ER vesiculation, due to impaired disulfide maturation, underscoring PDIA1's role in sulfur-dependent proinsulin folding [112]. Immunologically, cysteine deficiency limits glutathione synthesis, increasing reactive oxygen species (ROS) and activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which upregulates pro-inflammatory cytokines (TNF-α, IL-6) by 2.5-fold in palmitate-induced models ($P < 0.01$), exacerbating insulin resistance via JNK and suppressor of cytokine signaling (SOCS) proteins [56–62,73–76,107].

In adipose tissue, a maladaptive macrophage subpopulation (ATF4hiPDIA3hiACSL4hiCCL2hi) drives inflammation via PDIA3-mediated RhoA-YAP signaling, with PDIA3-targeted siRNA-loaded liposomes reducing adipose inflammation and high-fat diet-induced obesity in mice ($P < 0.05$), highlighting PDI's role in systemic metabolic dysfunction [111]. Aberrant S-nitrosylation of cysteine residues, competing with disulfide bond formation, further disrupts insulin signaling by reducing cysteine thiol availability, impairing IRS-1 tyrosine phosphorylation, and exacerbating insulin resistance [109]. Compromised gut barrier integrity, due to reduced mucin synthesis from cysteine deficiency, amplifies toll-like receptor 4 (TLR4)-mediated endotoxemia, positioning the gut as a central driver of T2DM [65,75,76]. A study of 45 middle-aged men showed increased mRNA expression of ER stress markers (GRP78, CHOP, XBP-1), inflammatory markers (TLR2, TLR4, CCR2), and Alzheimer's disease (AD)-related markers (APP, PS1, PS2) in obese PBMCs ($P < 0.05$), linking sulfur-dependent ER stress to metabolic and neurodegenerative comorbidities [110].

Therapeutically, sulfur donors like N-acetylcysteine (NAC) and GlyNAC restore plasma cysteine and glutathione by 20–40% ($P < 0.01$), improve insulin sensitivity by 31% ($P < 0.05$), and enhance mitochondrial fatty acid oxidation in T2DM patients [95]. The PDIA4 inhibitor PS1 ($IC50 = 4$ μM) reduces ROS by 50% ($P < 0.01$), improves HbA1c by 1.2% ($P < 0.05$), and enhances β-cell survival by 30% ($P < 0.05$) by inhibiting PDIA4 interactions with Ndufs3 and p22 in the electron transport chain complex 1 (ETC C1)

 doi:10.20944/preprints202507.1850.v3

and NADPH oxidase (Nox) pathways [108]. These interventions align with the hypothesis's emphasis on restoring sulfur homeostasis to stabilize insulin's disulfide bonds. [Table 1] compares this hypothesis with traditional models, highlighting its focus on insulin structure and sulfur metabolism. Beyond T2DM, the hypothesis suggests a continuum of sulfur-dependent protein misfolding disorders, including AD, as evidenced by shared ER stress and PDI dysregulation [110].

Table 1. This comparative framework highlights the novel perspective of the Sulfur-Dependent Misfolding Hypothesis in redefining T2DM as a sulfur metabolism disorder, contrasting it with traditional paradigms.

Comparative Dimension	Traditional Paradigm of T2DM	Sulfur-Dependent Misfolding Hypothesis
Root Cause	Peripheral insulin resistance driven by obesity, lipotoxicity, and inflammation.	Structural misfolding of insulin due to disulfide bond disruption caused by organic sulfur deficiency.
Initiation Site	Skeletal muscle, liver, and adipose tissue.	Mitochondrial dysfunction in intestinal epithelial cells impairing sulfur metabolism.
Pathophysiological Focus	Post-receptor signaling defects (IRS, PI3K, Akt).	Primary insulin deformation with reduced receptor affinity due to disrupted disulfide bonds.
Explanation of Hyperinsulinemia + Hyperglycemia Paradox	Compensatory hypersecretion due to peripheral resistance.	Endogenous insulin is misfolded and non-functional; exogenous insulin remains effective due to intact structure.
Immunological Mechanism	Chronic inflammation from adipose tissue and macrophage activation.	Glutathione depletion induces NF-κB and JNK pathways via oxidative stress and endotoxemia.
Role of the Gut	Secondary influence via microbiome and inflammation.	Primary site of dysfunction initiating mitochondrial suffocation, impaired sulfur metabolism, and mucosal barrier breakdown.
Insulin Signaling Defect	Impaired receptor signaling due to inflammation and phosphorylation of IRS.	Insulin fails to initiate signaling due to misfolded structure with up to 70% loss in receptor affinity.
Therapeutic Strategy	Blood glucose control via metformin, GLP-1 agonists, or exogenous insulin.	Sulfur restoration through NAC, MSM, and dietary methionine/cysteine to stabilize insulin structure.
Experimental Accessibility	HOMA-IR index and indirect measures of resistance.	Direct structural assessment of insulin via LC-MS/MS and Raman spectroscopy.
Biochemical Depth	Focuses downstream of the insulin receptor.	Traces the issue upstream to insulin biosynthesis and protein folding integrity.
Innovation Potential	Incremental improvements to a saturated model.	A paradigm shift introducing sulfur metabolism as a central therapeutic and diagnostic axis.
Philosophical Reframing	The body becomes resistant to insulin.	The body produces dysfunctional insulin; the issue lies at the source.
Potentially paradigm-shifting	Unlikely due to conceptual saturation.	Potentially transformative discovery redefining T2DM pathogenesis and therapy.

 doi:10.20944/preprints202507.1850.v3

The findings from the 2024 investigation into extracellular redox-mediated insulin chain splitting [113] provide compelling, albeit secondary, evidence supporting the Sulfur Insulin Deformation Hypothesis. This study demonstrates that insulin degradation, with up to 97.5% loss in vitro and a 20% degradation rate in vivo (A-chain appearance at 0.40 nmol/kg/min), occurs via thiol-disulfide exchange at plasma redox potentials (~ -137 mV), highlighting a redox-dependent pathway. However, this mechanism is secondary to the primary etiology proposed herein intracellular misfolding due to sulfur deficiency offering direct validation by linking diminished insulin bioavailability to redox imbalance. The notion that plasma-mediated chain splitting is the primary driver of insulin resistance is refuted, as intravenous (IV) insulin therapy remains effective in T2DM patients despite circulating in the same redox environment; if plasma degradation were dominant, all IV insulin would fail, undermining glycemic control.

Instead, the hypothesis posits that endogenous insulin, misfolded during endoplasmic reticulum (ER) biosynthesis due to impaired protein disulfide isomerase (PDIA1, PDIA3, PDIA4) activity from cysteine scarcity, becomes prone to extracellular cleavage. This vulnerability arises from mitochondrial suffocation disrupting transsulfuration, depleting glutathione (GSH) by 30–73.8%, elevating reactive oxygen species (ROS), and accelerating lipid peroxidation, which exacerbates disulfide bond instability (A6–A11, A7–B7, A20–B19). Exogenous insulin, structurally intact under controlled synthesis, resists this secondary degradation, resolving the paradox. Thus, plasma effects amplify, rather than initiate, resistance, reinforcing the need to target the gut-mitochondria-sulfur-insulin axis [113].

Future studies should employ liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect misfolded insulin, metabolomic profiling of sulfur metabolites, and randomized clinical trials to validate the efficacy of NAC, MSM, or PDIA-targeted therapies (e.g., PS1, PDIA3 siRNA). This paradigm, centered on the gut-mitochondria-sulfur-insulin axis, offers a transformative approach to T2DM management and its comorbidities, potentially redefining therapeutic strategies across metabolic and neurodegenerative diseases.

9. Conclusion

The Sulfur Insulin Deformation Hypothesis reimagines type 2 diabetes mellitus (T2DM) as a sulfur metabolism disorder, where mitochondrial dysfunction in intestinal epithelial cells drives cysteine deficiency, destabilizing insulin's disulfide bonds (A6–A11, A7–B7, A20–B19) and inducing misfolding that impairs receptor-binding efficacy by 50–70%. This structural defect, evidenced by a 73.8% reduction in glutathione levels in T2DM patients, disrupts insulin's bioactivity, fueling insulin resistance and hyperglycemia despite hyperinsulinemia. Oxidative stress from glutathione depletion and endoplasmic reticulum dysfunction further compromises beta-cell function, perpetuating metabolic disarray. The gut-mitochondria-sulfur-insulin axis emerges as a central driver, challenging conventional peripheral-focused models. Therapeutic interventions like N-acetylcysteine (NAC) and methylsulfonylmethane (MSM), which boost cysteine and glutathione by 20–40%, restore insulin stability and sensitivity, offering a novel strategy to mitigate T2DM. Awaiting validation through LC-MS/MS analysis of insulin structure and clinical trials, this hypothesis heralds a paradigm shift, advocating sulfur-centric therapies to transform T2DM management and potentially extend to other protein misfolding disorders.

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Abbreviations

T2DM	Type 2 Diabetes Mellitus
PDI	Protein Disulfide Isomerase
PDIA1	Protein Disulfide Isomerase Family A, Member 1
PDIA3	Protein Disulfide Isomerase Family A, Member 3

 doi:10.20944/preprints202507.1850.v3

PDIA4	Protein Disulfide Isomerase Family A, Member 4
ROS	Reactive Oxygen Species
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
PI3K	Phosphoinositide 3-Kinase
Akt	Protein Kinase B
GLUT4	Glucose Transporter Type 4
HbA1c	Hemoglobin A1c
NAC	N-Acetylcysteine
GlyNAC	Glycine and N-Acetylcysteine
NF-κB	Nuclear Factor kappa-light-chain-enhancer of Activated B Cells
TLR4	Toll-Like Receptor 4
ATP	Adenosine Triphosphate
ETC	Electron Transport Chain
JNK	c-Jun N-terminal Kinase
IRS-1	Insulin Receptor Substrate-1
IRS-2	Insulin Receptor Substrate-2
ER	Endoplasmic Reticulum
UPR	Unfolded Protein Response
IRE1	Inositol-Requiring Enzyme 1
PERK	Protein Kinase R-like ER Kinase
ATF6	Activating Transcription Factor 6
TNF-α	Tumor Necrosis Factor-alpha
IL-6	Interleukin-6
MSM	Methylsulfonylmethane
RBC	Red Blood Cell
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
NMR	Nuclear Magnetic Resonance
8-OHdG	8-Hydroxy-2'-deoxyguanosine
MDA	Malondialdehyde
Nox	NADPH Oxidase
GRP78	Glucose-Regulated Protein 78
CHOP	CCAAT/enhancer-binding Protein Homologous Protein
XBP-1	X-box Binding Protein 1
TLR2	Toll-Like Receptor 2
CCR2	C-C Chemokine Receptor Type 2
APP	Amyloid Precursor Protein
PS1	Presenilin 1
PS2	Presenilin 2
ACSL4	Acyl-CoA Synthetase Long-Chain Family Member 4
CCL2	C-C Motif Chemokine Ligand 2
YAP	Yes-Associated Protein
Ndufs3	NADH Dehydrogenase (Ubiquinone) Fe-S Protein 3
p22	p22phox (a subunit of NADPH oxidase)
MeSH	Medical Subject Headings
SANRA	Scale for the Assessment of Narrative Review Articles

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