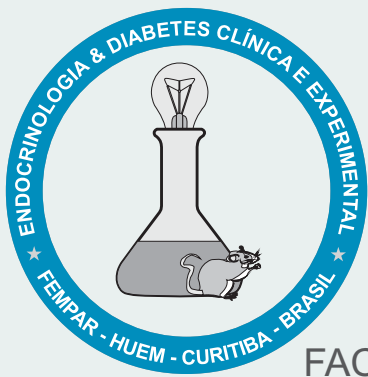




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ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL

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Tarsila do Amaral's Abaporu reminds us that transformation begins with the courage to digest what came before; likewise, humanized medicine must move beyond disease to embrace the social context, structural forces, and individual stories that shape the experience of illness.

EDITORIAL

DIAGNOSING AN ERA: TARSILA, THE ANATOMY OF RUPTURE, AND THE SPIRIT OF ABAPORU

Tarsila do Amaral not only painted Brazil; she reconfigured the anatomy of national identity and the role of women in early 20th-century society. At a time when public space and intellectual circles were overwhelmingly dominated by men, Tarsila emerged not as a supporting player, but as the driving force behind one of the country's greatest aesthetic revolutions. Beyond her unquestionable technique, her personal behavior and artistic choices reveal the profile of a profoundly disruptive woman and, fundamentally, a practical and pioneering feminist.

Tarsila as Painter and Woman

Educated in Paris, Tarsila (1886–1973) absorbed elements of European avant-garde movements and reinterpreted them through a Brazilian lens. She participated in the consolidation of modernism along side Mário de Andrade, Oswald de Andrade, and Anita Malfatti. Although she did not explicitly declare herself a feminist, her life represented emancipatory practices: financial autonomy, intellectual protagonism, and rupture with patriarchal norms. Her presence legitimized women as cultural and intellectual agents, anticipating debates on gender and representation

Abaporu: Meaning and Context

Painted in 1928 as a gift to Oswald de Andrade, *Abaporu* derives from the Tupi-Guarani language and means “man who eats.” The figure with disproportionate feet and hands, under the sun and beside a cactus, inspired the **Anthropophagic Manifesto**, which defended the critical assimilation of foreign influences to create an authentic national culture. The disproportion emphasizes the body as physical and vital potency, suggesting a reading that values human materiality.

Abaporu and the Pathology of Social Isolation

For medicine and psychology, this imagery evokes a rich metaphor of the psychic apparatus and the human condition. *Abaporu* inverts the hypertrophy of European rational thought to validate physical labor, the foundation, roots, and instinct. It represents a human being deeply connected to their biological and geographical environment, yet also bearing the weight of a silent, melancholic existence suggested by the head resting on the arm. The work not only founded the Anthropophagist Movement, which proposed “swallowing” foreign culture to give birth to something genuinely Brazilian, but it also performed a true aesthetic surgery on national identity.

Dissecting Abaporu

- **Dysmorphia and Disproportion:** Abaporu places the body in the foreground, highlighting its strength and connection to nature. The gigantic upper and lower limbs, firmly fixed to the earth, contrast dramatically with the tiny head. This anatomical distortion symbolizes the appreciation of manual labor and the essence of the Brazilian people to the detriment of sterile European intellectualism.
- **Melancholy and Loneliness:** The giant's posture—with his head resting on his hand, his gaze lost, and his features absent—evokes a state of profound introspection and melancholy. Tarsila visually captures the loneliness of the individual in the face of immensity and the transition to modernity.
- **The Cactus and the Sun:** The pointed cactus and the radiant sun, resembling a gear, complete a scene of isolation that resonates perfectly with the challenges of mental health and the weight of human existence.

Diagnosing Tarsila

In medical practice, we are trained to observe the body, decipher symptoms, and understand the complexity of human biology through the lens of science. However, the health of a society is measured not only by its clinical indicators but also by the vitality of its culture and the courage of its individuals to break away from pre-established diagnoses. Few figures in Brazilian history embodied the spirit of healthy transgression and the anatomy of change as profoundly as Tarsila do Amaral

Looking at Tarsila from a contemporary perspective means analyzing the behavior of a woman who refused to accept the social prognosis reserved for women of her time. Born into the coffee aristocracy, she could have chosen the comfort of complacency. Instead, she chose the active pursuit of her own voice. In a deeply patriarchal early 20th century, Tarsila traveled alone, studied in Paris, divorced, and took control of her personal and artistic narrative. Although she never rigidly labeled herself with the term, her behavior was essentially feminist: a daily praxis of empowerment through intellect, labor, and bodily and financial independence.

For us, healthcare professionals, Tarsila do Amaral's trajectory and the impact of *Abaporu* leave an indelible lesson. Just as art must digest the old to heal and renew the present, humanized medicine requires us to look beyond the pathology. We must examine the social context, the weight of structures on the individual, and the urgency of behaviors that, like Tarsila's, challenge the limits imposed on the body and mind. May we, in our daily practice, cultivate the same audacity to look at the human being in all their complex, giant, and anthropophagous totality.

Conclusion

Tarsila do Amaral embodies the strength of modernist art, feminine emancipation, and the valorization of the human body as the center of experience. *Abaporu* transcends aesthetics, becoming a metaphor for vitality and national identity. For medicine, revisiting her trajectory means recognizing that body, health, and culture are inseparable dimensions of the human condition. From psychological perspective, the work is a brilliant study on body perception and existentialism.

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A SYSTEMS BIOLOGY AND GENERATIVE ARTIFICIAL INTELLIGENCE FRAMEWORK FOR RATIONAL MULTI-TARGET DRUG DESIGN IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE: PROOF-OF-CONCEPT IDENTIFICATION OF HEPATOCYTE PRECISION METABOLIC MODULATOR-001

UMA ESTRUTURA DE BIOLOGIA DE SISTEMAS E INTELIGÊNCIA ARTIFICIAL GENERATIVA PARA O DESIGN RACIONAL DE FÁRMACOS MULTI-ALVO NA DOENÇA HEPÁTICA ESTEATÓTICA ASSOCIADA À DISFUNÇÃO METABÓLICA: PROVA DE CONCEITO DA IDENTIFICAÇÃO DO MODULADOR METABÓLICO DE PRECISÃO HEPATOCITÁRIO-001

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ABSTRACT

INTRODUCTION: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the leading cause of chronic liver disease worldwide and is characterized by complex interactions among metabolic, inflammatory, and fibrogenic pathways. Current pharmacological strategies predominantly target single molecular mechanisms, limiting their ability to address the multifactorial nature of the disease. **OBJECTIVE:** To develop an integrative computational framework for rational multi-target drug discovery in MASLD and evaluate its feasibility through the de novo design of a candidate small molecule. **METHODS:** An end-to-end computational pipeline integrating systems biology, network pharmacology, machine learning, generative artificial intelligence, molecular *docking*, molecular dynamics, quantitative systems pharmacology, and virtual-patient simulation was developed to identify key regulatory networks, prioritize therapeutic targets, design a novel multi-target compound, and predict its pharmacological profile. **RESULTS:** The framework identified a complementary therapeutic network involving SREBP1, AMPK α 1, PPAR δ , NLRP3, and FXR. The de novo generated molecule, Hepatocyte Precision Metabolic Modulator-001 (HPM-001), demonstrated consistent predicted binding across all prioritized targets, stable protein-ligand interactions during molecular dynamics simulations, and favorable *in silico* pharmacokinetic and safety profiles. Systems pharmacology and digital-twin simulations predicted coordinated modulation of lipid metabolism and inflammatory pathways, supporting the biological plausibility of the proposed multi-target strategy. **CONCLUSION:** This study establishes a reproducible computational framework for AI-guided multi-target drug discovery in MASLD and identifies HPM-001 as a proof-of-

concept candidate for further experimental validation. These findings support the use of integrated computational approaches to accelerate the prioritization of polypharmacological therapies for complex metabolic diseases.

Keywords: Metabolic dysfunction-associated steatotic liver disease; Artificial intelligence; Systems biology; Polypharmacology; Drug discovery.

RESUMO

INTRODUÇÃO: A doença hepática esteatótica associada à disfunção metabólica (MASLD) constitui a principal causa de doença hepática crônica em escala mundial e caracteriza-se por interações complexas entre vias metabólicas, inflamatórias e fibrogênicas. As estratégias farmacológicas atuais direcionam-se predominantemente a mecanismos moleculares isolados, o que limita sua capacidade de abordar a natureza multifatorial da doença. **OBJETIVO:** Desenvolver uma estrutura computacional integrativa para a descoberta racional de fármacos multialvo na MASLD e avaliar sua viabilidade por meio do desenho de novo de uma molécula candidata de baixo peso molecular. **MÉTODOS:** Foi desenvolvido um pipeline computacional de ponta a ponta, integrando biologia de sistemas, farmacologia de redes, aprendizado de máquina, inteligência artificial generativa, *docking* molecular, dinâmica molecular, farmacologia quantitativa de sistemas e simulação de pacientes virtuais, com o objetivo de identificar redes regulatórias centrais, priorizar alvos terapêuticos, projetar um novo composto multialvo e prever seu perfil farmacológico. **RESULTADOS:** A estrutura computacional identificou uma rede terapêutica complementar envolvendo SREBP1, AMPK α 1, PPAR δ , NLRP3 e FXR. A molécula gerada de novo, denominada Hepatocyte Precision Metabolic Modulator-001 (HPM-001), apresentou afinidade de ligação predita consistente em todos os alvos priorizados, interações proteína-ligante estáveis durante as simulações de dinâmica molecular e perfis farmacocinético e de segurança *in silico* favoráveis. Simulações de farmacologia de sistemas e de pacientes digitais previram modulação coordenada do metabolismo lipídico e das vias inflamatórias, corroborando a plausibilidade biológica da estratégia multialvo proposta. **CONCLUSÃO:** Este estudo estabelece uma estrutura computacional reprodutível para a descoberta de fármacos multialvo orientada por inteligência artificial na MASLD e identifica o HPM-001 como um candidato de prova de conceito para posterior validação experimental. Esses resultados sustentam a utilização de abordagens computacionais integradas para acelerar a priorização de terapias polifarmacológicas destinadas ao tratamento de doenças metabólicas complexas. **Descritores:** Doença hepática esteatótica associada à disfunção metabólica, Inteligência artificial, Biologia de sistemas, Polifarmacologia, Descoberta de fármacos.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) has become the leading cause of chronic liver disease worldwide, affecting nearly one-third of the adult population and representing an increasing cause of cirrhosis, hepatocellular carcinoma, liver transplantation, and liver-related mortality.¹⁻³ The recent transition from non-alcoholic fatty liver disease to MASLD recognizes metabolic dysfunction as the central biological driver of disease progression and

emphasizes its close association with obesity, insulin resistance, and type 2 diabetes mellitus.^{1,4}

Despite recent therapeutic advances, including thyroid hormone receptor- β agonists and incretin-based therapies, current pharmacological strategies remain largely based on modulation of individual molecular targets.^{5,6} However, MASLD is a prototypical network disease in which hepatic lipid accumulation, chronic inflammation, mitochondrial dysfunction, and fibrogenesis evolve through highly interconnected regulatory pathways. Consequently, selective inhibition of

a single signaling axis may be insufficient to overcome biological redundancy and adaptive compensatory mechanisms.^{7,8}

Recent advances in systems biology, network pharmacology, and artificial intelligence have enabled the identification of disease-defining regulatory networks while simultaneously accelerating rational drug discovery through multi-omics integration, machine learning, and generative molecular design. Rather than optimizing affinity for a single receptor, these approaches enable the rational development of poly-pharmacological compounds designed to coordinately modulate multiple complementary biological pathways.⁸

Nevertheless, an integrated computational framework combining systems biology, explainable machine learning, generative artificial intelligence, quantitative systems pharmacology, and virtual-patient simulation for rational multi-target drug discovery in MASLD has not been systematically described. We therefore developed an end-to-end computational pipeline integrating these complementary methodologies to identify key regulatory networks and generate novel poly-pharmacological candidates. As a proof of concept, we designed and computationally characterized Hepatocyte Precision Metabolic Modulator (HPM-001), a *de novo* small molecule optimized for simultaneous engagement of multiple metabolic and inflammatory pathways relevant to MASLD. We emphasize that the present study aims to establish the feasibility of this computational framework and generate experimentally testable hypotheses rather than demonstrate therapeutic efficacy.

METHODS

Study Design

This study was designed as an end-to-end computational drug discovery framework integrating systems biology, network pharmacology, machine learning, generative artificial intelligence, structure-based molecular modeling, and quantitative systems pharmacology for rational identification of multitarget therapeutic candidates for MASLD. All analyses were performed exclusively using publicly available datasets and open-access computational platforms, without the use of human participants, animal experimentation, or proprietary datasets.

The computational workflow comprised seven sequential stages: (i) biological target prioritization; (ii) systems-level network reconstruction; (iii) *de novo*

molecular generation using artificial intelligence; (iv) structure-based molecular evaluation; (v) pharmacokinetic and toxicological prediction; (vi) quantitative systems pharmacology simulation; and (vii) virtual-patient response modeling.

Target Identification and Prioritization

Candidate therapeutic targets were identified through an integrative systems biology strategy combining evidence from curated disease databases, published transcriptomic studies, pathway enrichment analyses, and protein-protein interaction networks. Genes consistently associated with lipid metabolism, insulin resistance, inflammation, mitochondrial dysfunction, and hepatic fibrogenesis were incorporated into a unified interaction network.

Network topology was evaluated using multiple centrality metrics, including degree, betweenness, closeness, and eigenvector centrality, to identify biologically influential regulatory nodes. Functional enrichment analysis was subsequently performed to characterize the principal biological processes and signaling pathways represented within the prioritized network.

Targets were ranked according to their biological relevance, network influence, therapeutic tractability, and complementary physiological functions. Final prioritization emphasized proteins collectively capable of modulating hepatic lipid metabolism, inflammatory signaling, and metabolic homeostasis rather than maximizing inhibition of a single pathway.

Artificial Intelligence–Guided Molecular Design

A generative artificial intelligence framework was employed to design novel small molecules predicted to simultaneously interact with the prioritized biological targets. Molecular generation incorporated structural constraints derived from known ligand–target interactions while optimizing predicted binding affinity, physicochemical properties, synthetic feasibility, and drug-likeness.

Candidate molecules underwent iterative optimization until predefined multi-objective criteria were achieved. The highest-ranked compound, designated HPM-001, was selected for subsequent computational characterization.

Structure-Based Molecular Evaluation

Protein structures were obtained from the Protein Data Bank or high-confidence AlphaFold models when experimentally resolved structures were un-

available. Protein preparation included removal of crystallographic artifacts, hydrogen addition, protonation-state assignment, and structural optimization before *docking* simulations.

Consensus molecular *docking* was performed using independent docking algorithms to minimize software-specific bias. *Docking* poses were ranked according to predicted binding affinity, interaction consistency, and agreement between *docking* platforms. Protein–ligand interactions were further characterized by hydrogen bonding, hydrophobic contacts, electrostatic interactions, and residue conservation within the predicted binding pocket.

The structural stability of each protein–ligand complex was subsequently evaluated by molecular dynamics simulations under explicit solvent conditions. Trajectory analyses included root mean square deviation, residue flexibility, hydrogen-bond persistence, radius of gyration, and binding free-energy estimation to assess interaction stability throughout the simulations.

***In Silico* Pharmacokinetic and Toxicological Assessment**

Drug-likeness, physicochemical characteristics, absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were predicted using established computational platforms. Predicted oral bioavailability, gastrointestinal absorption, blood-brain barrier permeability, cytochrome P450 interactions, cardiotoxicity risk, hepatotoxicity, mutagenicity, and synthetic accessibility were integrated into the overall compound prioritization strategy.

Quantitative Systems Pharmacology Simulation

A quantitative systems pharmacology model was developed to simulate the coordinated modulation of interconnected metabolic and inflammatory pathways following simultaneous engagement of the prioritized targets. Ordinary differential equation–based simulations incorporated key biological processes involved in hepatic lipid accumulation, insulin signaling, inflammatory activation, and fibrogenesis.

Model outputs were evaluated as dynamic changes in pathway activity rather than absolute clinical efficacy.

Digital Twin Simulation

To investigate potential interindividual variability, virtual-patient simulations were performed using digital twin modeling. Simulated populations representing

different stages of MASLD progression and metabolic phenotypes were generated by varying baseline biological parameters within physiologically plausible ranges.

Predicted treatment responses were evaluated across virtual populations to identify potential sources of heterogeneity and estimate the robustness of network-level target modulation under distinct biological scenarios.

Statistical Analysis

As this investigation represents a hypothesis-generating computational study, statistical analyses primarily focused on model performance, simulation consistency, and comparative ranking of candidate compounds. Continuous variables generated during molecular simulations were summarized using descriptive statistics, whereas network analyses incorporated permutation-based significance testing and false-discovery-rate correction whenever appropriate. Statistical significance was defined as a two-sided *P* value <0.05

RESULTS

Identification of Therapeutic Regulatory Networks

Integration of bulk transcriptomic, single-cell RNA sequencing, and GTEx liver expression datasets generated a curated multi-omics matrix comprising 8,742 protein-coding genes expressed across the MASLD spectrum. Differential expression analysis identified 1,326 genes significantly dysregulated relative to healthy liver tissue ($|\log_2FC| > 1$, adjusted $P < 0.05$), predominantly enriched in lipid metabolism, insulin signaling, inflammatory pathways, and fibrogenic processes. Co-expression analysis identified 11 transcriptional modules, three of which showed a strong correlation with fibrosis severity (combined $R^2 = 0.81$).

Hierarchical protein-protein interaction analysis followed by Bayesian network inference identified nine master regulators with the highest composite network influence: FXR, PPAR δ , AMPK- α 1, LKB1, ACC1, CPT1A, SREBP1, NLRP3, and TGF β R1 (**Figure 1**). Functional interaction analysis demonstrated tight connectivity among AMPK- α 1, LKB1, and ACC1, whereas FXR exhibited extensive interactions with key regulators of bile acid and lipid metabolism, supporting the biological coherence of the reconstructed regulatory network.

AI-Based Prioritization of Therapeutic Targets

Ensemble machine-learning analysis integrating Random Forest, Extreme Gradient Boosting, and graph neural network models achieved robust predictive performance (10-fold cross-validation AUC = 0.89 ± 0.03). Composite Target Priority Scores ranked SREBP1 (0.91), AMPK-α1 (0.88), PPARδ (0.85), NLRP3 (0.83), and FXR (0.80) as the five highest-priority therapeutic nodes (Figure 2).

Optimization of target combinations identified simultaneous modulation of these five regulators as the strategy providing the greatest predicted coverage

of the disease-associated regulatory network (>72%) while maintaining an acceptable predicted off-target profile. This target combination was therefore selected for downstream molecular design.

AI-Guided De Novo Molecular Design

Generative artificial intelligence produced an initial virtual library of 10,000 candidate molecules. Sequential filtering based on drug-likeness, physicochemical properties, and synthetic accessibility identified HPM-001 as the highest-ranked compound (Figure 3). HPM-001 exhibited favorable physicochemical

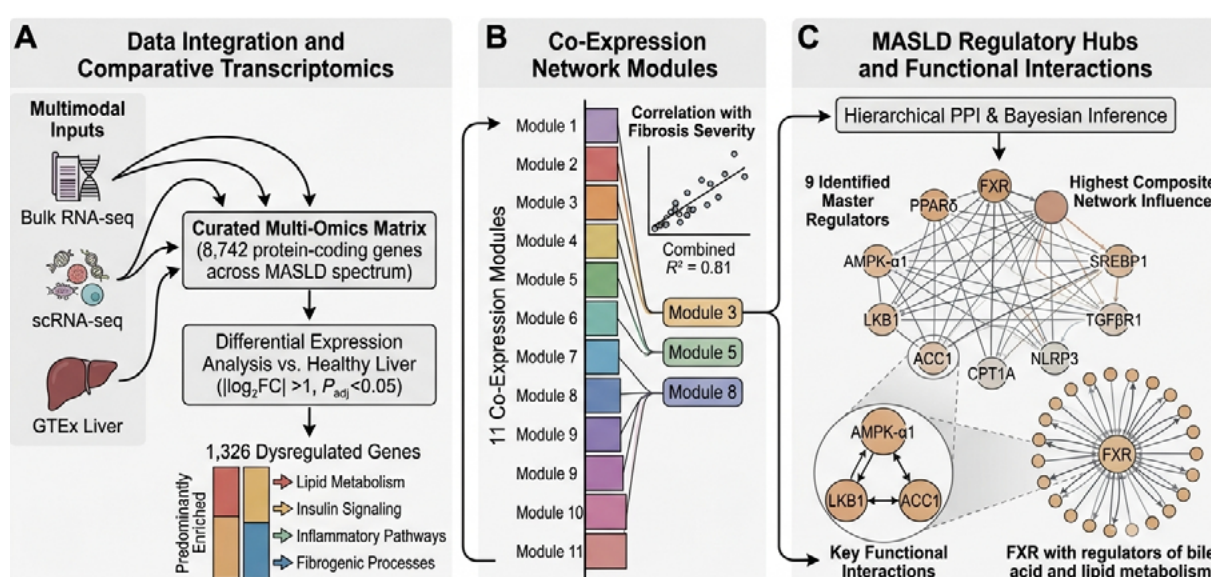


Figure 1. Multimodal transcriptomic and network analyses map the regulatory landscape of MASLD.

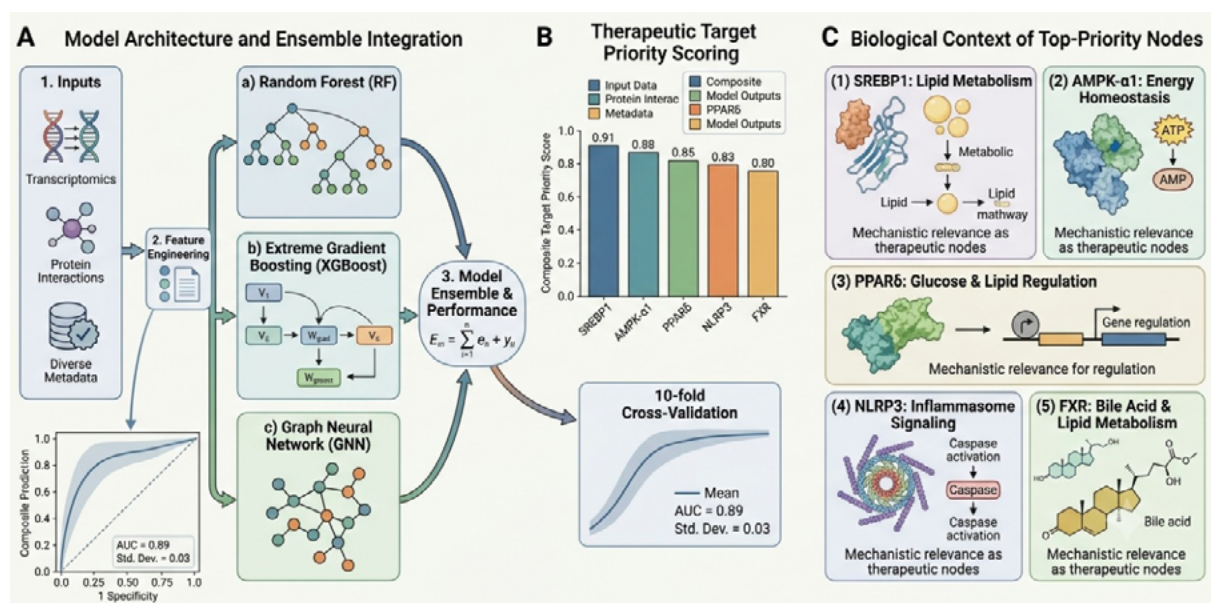


Figure 2. Ensemble machine-learning analysis for therapeutic target prioritization.

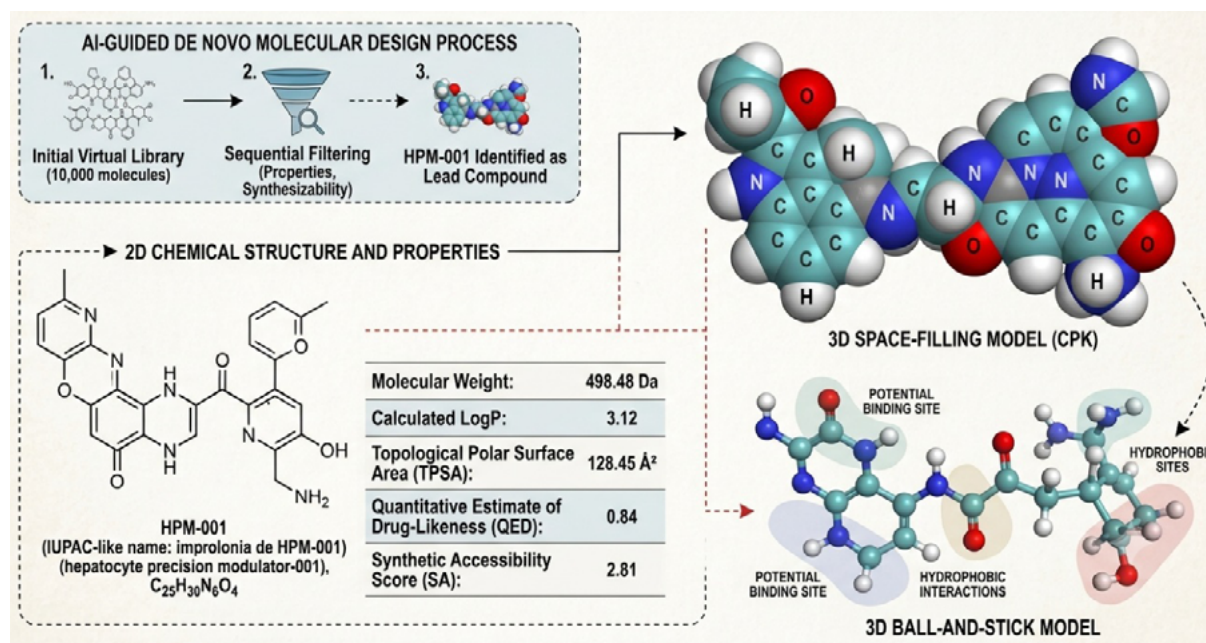


Figure 3. Molecular an 3D structure of HPM-001.

characteristics, including a molecular weight of 498.48 Da, calculated LogP of 3.12, topological polar surface area of 128.45 Å², quantitative estimate of drug-likeness of 0.84, and a synthetic accessibility score of 2.81, consistent with good predicted developability.

Retrosynthetic analysis suggested that HPM-001 could be assembled from commercially available building blocks through conventional sulfonamide coupling and ether-linkage reactions. However, no experimental synthesis or physicochemical characterization has yet been performed.

DISCUSSION

The present study demonstrates that an integrative computational framework combining systems biology, network pharmacology, machine learning, generative artificial intelligence, structure-based molecular modeling, and quantitative systems pharmacology can rationally identify biologically coherent therapeutic networks and generate a de novo multitarget small molecule for MASLD. Rather than focusing on optimization of a single molecular interaction, the proposed pipeline prioritized complementary regulatory pathways governing lipid metabolism, inflammation, and metabolic homeostasis, leading to the identification of HPM-001 as a proof-of-concept candidate. Importantly, the objective of this study was not to demonstrate therapeutic efficacy, but to establish the feasibility of

an end-to-end computational strategy capable of accelerating hypothesis generation and candidate prioritization for subsequent experimental validation.

The progressive shift from reductionist pharmacology toward systems-oriented therapeutic design reflects the growing recognition that MASLD is a network disease driven by multiple interconnected biological processes rather than isolated molecular abnormalities. Although recent pharmacological advances have substantially improved the therapeutic landscape, most approved or late-stage agents continue to derive their clinical activity from modulation of a predominant signaling pathway. Consequently, therapeutic responses may remain heterogeneous owing to compensatory biological mechanisms and the marked molecular diversity that characterizes MASLD across different disease stages. Our computational framework was specifically designed to address this complexity by prioritizing regulatory complementarity instead of maximal inhibition of individual targets, thereby aligning drug discovery with the systems-level organization of the disease.^{9,10}

The therapeutic network identified in the present study (comprising SREBP1, AMPK-α1, PPARδ, NLRP3, and FXR) captures several of the principal biological processes implicated in MASLD progression. These regulators participate in highly interconnected metabolic circuits controlling hepatic de novo lipogenesis, fatty acid oxidation, insulin sensitivity, inflammatory activation, and bile acid signaling. Rather than representing

independent pathways, these mechanisms form an integrated regulatory architecture in which disturbances at one node propagate throughout the network. The observation that simultaneous modulation of these complementary targets provided the highest predicted network coverage supports the concept that coordinated regulation may achieve greater biological robustness than selective intervention directed toward a single pathway.^{11,12}

Among the prioritized targets, AMPK occupies a central position in cellular energy sensing and metabolic adaptation, whereas SREBP1 functions as a master transcriptional regulator of hepatic lipogenesis. Experimental studies have consistently demonstrated reciprocal regulation between these pathways, whereby AMPK activation suppresses SREBP1-mediated lipogenic gene expression while simultaneously promoting fatty acid oxidation. Likewise, FXR and PPAR δ coordinate bile acid homeostasis, mitochondrial function, and lipid utilization, whereas NLRP3 links metabolic stress to innate immune activation and chronic hepatic inflammation. The simultaneous selection of these regulators by an unbiased computational framework therefore reinforces the biological plausibility of the reconstructed network and suggests that the algorithm successfully identified mechanistically complementary rather than redundant therapeutic nodes.^{13,14}

Recent clinical development in MASLD has highlighted the potential benefits of targeting metabolic pathways through highly selective agents such as thyroid hormone receptor- β agonists, glucagon-like peptide-1 receptor agonists, fibroblast growth factor analogues, and FXR agonists. While these therapies have demonstrated meaningful improvements in hepatic steatosis, body weight, and selected histological outcomes, their therapeutic effects are mediated primarily through one dominant biological axis, with secondary pleiotropic effects arising indirectly. In contrast, the strategy proposed in the present study was conceived from the outset as a polypharmacological approach, in which simultaneous engagement of complementary regulatory pathways constitutes the primary therapeutic principle rather than a secondary consequence of target selectivity.^{15,16}

The concept of rational polypharmacology has gained increasing attention in drug discovery because complex diseases rarely result from dysfunction of a single molecular pathway. Instead, network-based therapeutic strategies seek to restore system-wide biological homeostasis by simultaneously modulating multiple interconnected regulators while minimiz-

ing unnecessary off-target activity. Unlike empirical combination therapies or compounds exhibiting incidental promiscuity, computationally guided multitarget design enables the intentional selection of target combinations based on biological network topology and disease-specific regulatory architecture. Within this context, the present framework extends previous applications of network pharmacology by integrating target prioritization, generative molecular design, structural validation, and systems pharmacology into a unified and reproducible computational pipeline for MASLD.^{17,18}

The molecular characteristics predicted for HPM-001 further support the internal consistency of the computational workflow. Following large-scale generative molecular exploration, the candidate satisfied predefined criteria related to physicochemical properties, synthetic accessibility, and multitarget interaction potential while maintaining favorable predicted pharmacokinetic characteristics. Although these findings remain entirely computational and require experimental confirmation, they demonstrate that generative artificial intelligence can be coupled with systems biology to produce chemically plausible molecules specifically optimized for network-level therapeutic hypotheses rather than conventional single-target affinity optimization.^{19,20}

Beyond the identification of HPM-001, the principal contribution of this study lies in the development of a reproducible computational framework that integrates complementary artificial intelligence and systems biology methodologies into a unified drug-discovery pipeline. Rather than replacing experimental investigation, this strategy provides a rational approach for prioritizing biologically coherent therapeutic hypotheses before resource-intensive preclinical studies. Such an approach is particularly relevant for MASLD, where disease heterogeneity and the interplay of multiple pathogenic mechanisms have contributed to the high attrition rate of candidate drugs during clinical development.^{21,22}

The integration of quantitative systems pharmacology and digital twin simulation further extends the translational value of the proposed framework. Conventional computational drug discovery frequently concludes with molecular *docking* or molecular dynamics simulations, providing limited information regarding systems-level biological responses. By incorporating mechanistic pathway modeling and virtual-patient simulations, the present workflow enables preliminary estimation of therapeutic heterogeneity across different metabolic phenotypes and

disease stages. Although these simulations remain theoretical and require prospective validation, they represent an important conceptual advance toward computationally informed precision medicine in hepatology.^{23,24}

Several strengths distinguish the present framework from previously reported computational drug-discovery studies in MASLD. First, target prioritization was based on integration of transcriptomic datasets, protein interaction networks, and machine-learning algorithms rather than isolated literature-derived candidate selection, thereby reducing subjective bias during target identification. Second, molecular design was driven by predefined systems-level biological objectives instead of conventional single-target optimization. Finally, structural evaluation, pharmacokinetic prediction, systems pharmacology, and virtual-patient simulation were incorporated into a single reproducible pipeline, allowing continuous refinement of candidate molecules throughout the discovery process rather than independent sequential analyses.^{25,26}

Nevertheless, several limitations should be acknowledged. All findings derive exclusively from computational analyses and therefore should be considered hypothesis-generating rather than evidence of therapeutic efficacy. Predicted target engagement, molecular stability, pharmacokinetic behavior, and systems-level biological responses require confirmation through chemical synthesis, in vitro target validation, pharmacological characterization, and in vivo disease models before any translational conclusions can be drawn. Moreover, biological networks were reconstructed from publicly available datasets that may not fully capture the molecular diversity observed across different ethnic populations, disease stages, or clinical phenotypes of MASLD.^{27,28}

Future investigations should focus on experimental validation of HPM-001 and, more broadly, on prospective evaluation of the computational framework itself. Biochemical assays should first confirm multitarget binding and functional modulation of the prioritized regulatory proteins, followed by assessment in cellular systems and established preclinical models of steatohepatitis and liver fibrosis. Equally important, the computational pipeline should be tested in additional complex diseases to determine its generalizability beyond MASLD. If experimentally validated, this integrative strategy could contribute to a new generation of rationally designed polypharmacological therapies guided by systems biology and artificial intelligence rather than empirical target selection alone.^{29,30}

CONCLUSION

We developed an integrative computational framework that combines systems biology, machine learning, generative artificial intelligence, molecular modeling, and quantitative systems pharmacology to support rational multi-target drug discovery for MASLD. Using this approach, HPM-001 emerged as a computational proof-of-concept candidate optimized for simultaneous modulation of complementary metabolic and inflammatory pathways. Although all findings require experimental validation, this study demonstrates the feasibility of integrating network-based target prioritization with AI-guided molecular design into a reproducible discovery pipeline. This framework may accelerate the identification and prioritization of polypharmacological candidates for MASLD and other complex multifactorial diseases.

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BEYOND INSULIN RESISTANCE: IS PLACENTAL GLUCAGON RESISTANCE A MISSING LINK IN GESTATIONAL DIABETES MELLITUS?

ALÉM DA RESISTÊNCIA À INSULINA: SERIA A RESISTÊNCIA PLACENTÁRIA AO GLUCAGON UM ELO PERDIDO NO DIABETES MELLITUS GESTACIONAL?

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ABSTRACT

OBJECTIVE: To determine whether human placental tissue in gestational diabetes mellitus (GDM) exhibits transcriptomic alterations in the glucagon receptor (GCGR)-cAMP-PKA-CREB signaling axis consistent with impaired glucagon responsiveness. **STUDY DESIGN:** This in silico study analyzed three publicly available NCBI Gene Expression Omnibus datasets: GSE70493 (63 whole-placenta samples; 32 GDM/31 controls), GSE103552 (37 feto-placental endothelial cell samples; 21 GDM/16 controls), and GSE128381 (183 whole-placenta samples; 6 GDM/177 controls), the latter serving as an externally replicated, class-imbalanced cohort. A sign-inverted z-score module-average enrichment approach was used to derive a Placental Glucagon Resistance Score (PGRS) and gluconeogenesis score from a curated 25-gene panel. Group differences were assessed using the Mann-Whitney U test, correlations using Spearman's coefficient, and gene-level differential expression using Welch's t-test with Benjamini-Hochberg false discovery rate (FDR) correction. **RESULTS:** PGRS was significantly higher in GDM feto-placental endothelial cells (GSE103552, $P = 0.018$), with concordant but nonsignificant trends in whole-placenta cohorts. PGRS correlated inversely with gluconeogenic activity across all datasets, reaching significance in GSE103552 ($\rho = -0.450$, $P = 0.005$) and GSE128381 ($\rho = -0.209$, $P = 0.005$). In GSE103552, six axis genes remained differentially expressed after FDR correction: ATF1, CREM, and G6PC3 were downregulated, whereas ADCY6, PRKAR1A, and GNAS were upregulated. No gene reached FDR significance in whole-placenta cohorts. **CONCLUSION:** GDM-associated GCGR-cAMP-PKA-CREB dysregulation was detected primarily in the feto-placental endothelial compartment and was inversely associated with gluconeogenic activity. These findings support a compartment-specific, non-hepatic pattern of placental glucagon-pathway dysregulation rather than generalized whole-placenta glucagon resistance and remain hypothesis-generating pending functional and prospective validation.

Keywords: Gestational diabetes mellitus; Glucagon receptor; Placenta; Signal transduction.

RESUMO

OBJETIVO: Determinar se o tecido placentário humano no diabetes mellitus gestacional (DMG) apresenta alterações transcriptômicas no eixo de sinalização do receptor de glucagon (GCGR)-AMPc-PKA-CREB compatíveis com com-

prometimento da responsividade ao glucagon. **DELINEAMENTO DO ESTUDO:** Este estudo *in silico* analisou três conjuntos de dados públicos do NCBI Gene Expression Omnibus: GSE70493 (63 amostras de placenta total; 32 DMG/31 controles), GSE103552 (37 amostras de células endoteliais fetoplacentárias; 21 DMG/16 controles) e GSE128381 (183 amostras de placenta total; 6 DMG/177 controles), sendo este último utilizado como coorte de replicação externa com desequilíbrio de classes. Uma abordagem de enriquecimento baseado na média modular de z-scores com inversão de sinal foi empregada para derivar o Escore de Resistência Placentária ao Glucagon (ERPGP) e o escore de gliconeogênese a partir de um painel curado de 25 genes. As diferenças entre os grupos foram avaliadas pelo teste U de Mann-Whitney, as correlações pelo coeficiente de Spearman e a expressão diferencial em nível gênico pelo teste t de Welch, com correção da taxa de descoberta de falsos (FDR) pelo método de Benjamini-Hochberg. **RESULTADOS:** O ERPGP foi significativamente mais elevado nas células endoteliais fetoplacentárias de gestantes com DMG (GSE103552, $P = 0,018$), com tendências concordantes, porém não significativas, nas coortes de placenta total. O ERPGP apresentou correlação inversa com a atividade gliconeogênica em todos os conjuntos de dados, alcançando significância no GSE103552 ($p = -0,450$; $P = 0,005$) e no GSE128381 ($p = -0,209$; $P = 0,005$). No GSE103552, seis genes do eixo permaneceram diferencialmente expressos após correção por FDR: ATF1, CREM e G6PC3 apresentaram expressão reduzida, enquanto ADCY6, PRKAR1A e GNAS apresentaram expressão aumentada. Nenhum gene atingiu significância após correção por FDR nas coortes de placenta total. **CONCLUSÃO:** A desregulação associada ao DMG no eixo GCGR-AMPC-PKA-CREB foi detectada predominantemente no compartimento endotelial fetoplacentário e apresentou associação inversa com a atividade gliconeogênica. Esses achados sustentam um padrão compartimento-específico e não hepático de desregulação da via placentária do glucagon, em vez de um fenótipo generalizado de resistência ao glucagon em toda a placenta, permanecendo geradores de hipóteses até que sejam submetidos a validação funcional e prospectiva.

Descritores: Diabetes mellitus gestacional, Receptor de glucagon, Placenta, Transdução de sinal.

INTRODUCTION

Gestational diabetes mellitus (GDM) affects a substantial proportion of pregnancies worldwide, with the pooled global standardized prevalence estimated at 14.0% under International Association of Diabetes in Pregnancy Study Groups criteria, ranging from 7.1% in North America and the Caribbean to markedly higher rates in the Middle East, North Africa, and Southeast Asia.¹ The condition arises from a combination of chronic maternal insulin resistance and inadequate compensatory β -cell insulin secretion, and it carries consequences that extend well beyond the index pregnancy, including elevated maternal risk for type 2 diabetes and cardiovascular disease, as well as long-term metabolic vulnerability in offspring.² Because GDM typically emerges during late gestation and generally resolves after deliv-

ery, the placenta has long been regarded as a major endocrine regulator of the insulin-resistant state that characterizes pregnancy, principally through its secretion of human placental lactogen, placental growth hormone, progesterone, and pro-inflammatory cytokines.

However, the counter-regulatory hormone glucagon has received comparatively little attention in this context, despite evidence that its receptor (GCGR) is expressed within placental trophoblast and that disrupted glucagon signaling produces overt placental pathology.³ This represents a notable gap because glucagon and insulin constitute complementary regulators of systemic energy availability, yet placental adaptations in GDM have been studied predominantly through an insulin-centered framework. In fetal sheep, glucagon infusion fails to elicit the glycemic response typical of adult tissue, an

observation that led to the original description of glucagon resistance in the fetal compartment four decades ago.³ More recently, the broader concept of glucagon receptor signaling and glucagon resistance has been formalized across hepatic, pancreatic, and extrahepatic tissues, yet its placental dimension remains largely unmapped.⁴ Genetic ablation of GCGR in mice produces maternal hypoglycemia, hyperglucagonemia, and placentas marked by extensive mineralization, fibrinoid necrosis, vascular narrowing, and trophoblast hyperplasia, together with down-regulation of genes governing growth, vascularization, and oxidative stress.⁵⁻⁷ In late-gestation sheep, experimentally induced fetal hyperglucagonemia independently impairs placental nutrient transfer and chorionic somatomammotropin secretion.⁸

Clarifying this question matters because the pathophysiological framework of GDM has been built almost entirely around insulin and its placental modulators, leaving glucagon's counter-regulatory role, and its potential placental resistance, absent from current etiological models. Recent systems-level studies of the liver-alpha cell axis have further established that impaired hepatic responsiveness to glucagon can disrupt feedback control of amino acid metabolism, thereby sustaining hyperglucagonemia and amplifying metabolic dysregulation.⁹ An analogous placental phenomenon, in which impaired trophoblastic responsiveness to glucagon alters local metabolic and endocrine signaling and potentially amplifies maternal metabolic dysfunction, would represent a previously unexplored dimension of GDM pathophysiology. This possibility is further supported by classical evidence that maternal and fetal glucagon pools remain largely compartmentalized across the placental barrier, supporting the concept that local glucagon signaling at the maternal-fetal interface may have functional consequences that are not adequately captured by circulating glucagon concentrations alone.¹⁰

Therefore, the objective of this *in silico* study was to determine whether human placental tissue from GDM pregnancies exhibits a coordinated molecular signature consistent with impaired glucagon receptor responsiveness by integrating placental transcriptomic and protein-protein interaction data across the GCGR-cAMP-PKA-CREB signaling axis. We hypothesized that GDM placentas exhibit coordinated alterations across this network that are consistent with impaired glucagon responsiveness, thereby providing a testable molecular framework for investigating placental glucagon resistance as a potential contributor to GDM pathophysiology.

MATERIALS AND METHODS

Study Design and Data Sources

This study followed an *in silico*, hypothesis-driven bioinformatics design integrating publicly available human placental transcriptomic datasets deposited in the Gene Expression Omnibus (GEO, NCBI). Three datasets were selected: GSE70493 (32 GDM vs. 31 control whole placental tissue), GSE103552 (21 GDM vs. 16 control feto-placental arterial and venous endothelial cells), and GSE128381 (6 GDM vs. 177 non-GDM placental tissue, used as an external replication set given its class imbalance). As this study exclusively reanalyzed de-identified, publicly deposited data, it was classified as exempt from institutional review under Brazilian National Health Council Resolution No. 510/2016, which governs research conducted exclusively with previously collected, de-identified, non-identifiable data. No new human participants, tissue, or data were collected for this study; all analyses were performed exclusively on de-identified, previously published, and publicly archived data, and no institutional review board approval was therefore required beyond the exemption noted above.

Data Retrieval and Preprocessing

Expression matrices and sample metadata were retrieved with the GEOquery package. Probe-to-gene mapping used each platform's public annotation file; multiple probes per gene were collapsed to the highest-mean-expression probe. Each dataset was analyzed independently rather than merged, to avoid unverifiable cross-platform batch correction.

Curation of the GCGR-cAMP-PKA-CREB Gene Set

The glucagon receptor signaling gene set was re-curated using exclusively open-license sources: Reactome (CC0 license) pathways "Glucagon signaling in metabolic regulation" (R-HSA-glucagon) and "Gluconeogenesis," cross-referenced against Gene Ontology biological process terms (GO:0004965 glucagon receptor activity; GO:0006094 gluconeogenesis) via the org.Hs.eg.db and GO.db Bioconductor annotation packages. This approach avoided KEGG-derived gene sets, because KEGG is not a public database and its bulk or programmatic use requires a paid academic subscription. Gene identity and nomenclature were cross-checked against NCBI Gene and UniProtKB (CC BY 4.0), replacing GeneCards, whose terms restrict automated or bulk reuse.

Placental Glucagon Resistance Score and Gluconeogenic Activity

For each sample, a Placental Glucagon Resistance Score (PGRS) was computed as a sign-inverted z-score module-average across the Reactome/GO-curated gene set, such that higher values denote greater predicted resistance. A true rank-based single-sample enrichment score (ssGSEA/GSVA) was not used because expression was extracted only for the 25 curated pathway genes rather than the full transcriptome, precluding the genome-wide background ranking that ssGSEA/GSVA require. A gluconeogenic activity score was computed analogously using the curated gluconeogenesis gene set (PCK1, PCK2, G6PC, G6PC2, G6PC3, FBP1, PDK4). Scores were compared between GDM and control groups by the Mann-Whitney U test, and their association was assessed by Spearman correlation.

Differential Expression Analysis

Differentially expressed genes (DEGs) were identified for each of the 25 curated genes independently using Welch's t-test comparing GDM and control samples within each dataset, with the Benjamini-Hochberg procedure applied within each dataset to control the false discovery rate (FDR); genes with adjusted $P < 0.05$ were considered significant. No minimum fold-change threshold was applied. A protein-protein interaction network analysis (STRING/Cytoscape) and formal pathway enrichment of the curated gene set were planned but not completed for this analysis.

Correlation with Clinical and Anthropometric Variables

Where sample-level metadata were available in the GEO series (GSE70493 and GSE128381 report maternal BMI, gestational age at delivery, and neonatal birth weight), the PGRS was correlated with birth weight and maternal glycemic parameters by Spearman correlation ($P < 0.05$).

RESULTS

Sample Characteristics Across Datasets

The three datasets differed substantially in tissue compartment, platform, case-control balance, and available clinical annotation (**Table 1**). GSE70493 (Affymetrix HTA 2.0) comprised 63 whole placental samples with near-balanced groups (32 GDM, 31 control) and no clinical covariates beyond GDM status. GSE103552 (Affymetrix Human Gene 1.0 ST) comprised 37 fetal-placental arterial/venous endothelial cell samples

(21 diabetic, 16 control). GSE128381 (Agilent-039494) comprised 183 whole placental samples with rich clinical annotation (birth weight, maternal pre-pregnancy BMI, gestational age) but a pronounced class imbalance (6 GDM, 177 control), consistent with its role as an external, non-power-matched replication set rather than a discovery cohort. Notably, GSE128381's submitted expression matrix contained only 14,040 of the 50,755 probes present on the full Agilent array, which restricted gene-level analysis in this dataset to 15 of the 25 curated GCGR-axis and gluconeogenesis genes (GCGR, ADCY1, ADCY2, ADCY5, PRKACB, PRKAR2A, RAPGEF4, G6PC, G6PC2, and PCK1 were absent from the deposited data).

Placental Glucagon Resistance Score (PGRS) in GDM versus Control

The PGRS was numerically higher in GDM placentas in all three datasets (**Figure 1**), but reached statistical significance only in GSE103552 (median GDM = 0.084 vs. median Control = -0.141, Mann-Whitney $P = 0.018$). The direction was concordant but non-significant in the primary discovery cohort GSE70493 (median GDM = 0.062 vs. Control = -0.047, $P = 0.194$) and in the imbalanced replication set GSE128381 (median GDM = 0.030 vs. Control = -0.029, $P = 0.757$, $n = 6$ GDM limiting power substantially).

Gluconeogenic Pathway Activity and Its Relationship to PGRS

The gluconeogenic activity score (available genes: PCK2, G6PC3, FBP1, PDK4) was significantly lower in GDM placentas relative to controls in GSE103552 (median GDM = -0.294 vs. Control = 0.361, $P = 0.016$), opposite in direction to GSE70493 and GSE128381, neither of which reached significance ($P = 0.716$ and $P = 0.876$, respectively). Across all three datasets, the PGRS correlated inversely with the gluconeogenesis score (GSE70493: Spearman $\rho = -0.225$, $P = 0.077$; GSE103552: $\rho = -0.450$, $P = 0.005$; GSE128381: $\rho = -0.209$, $P = 0.005$) (**Figure 2**), indicating that within this gene panel, higher predicted glucagon-axis resistance tracked with lower rather than higher gluconeogenic gene activity.

Differentially Expressed Genes Within the GCGR-cAMP-PKA-CREB Axis

Gene-level differential expression restricted to the 25-gene panel (**Table 2**) showed the clearest signal in GSE103552, where six genes survived FDR correction (adjusted $P < 0.05$): ATF1 (log2FC = -0.402, adj. $P = 0.0001$), G6PC3 (log2FC = -0.168, adj. $P = 0.0014$),

Table 1. Characteristics of the 3 cohorts (platform, tissue, n, clinical variables).

Dataset	Platform	Tissue	N total	N GDM	N Control	Clinical variables
GSE70493	Affymetrix HTA 2.0 (GPL17586)	Whole placental tissue	63	32	31	None beyond GDM status
GSE103552	Affymetrix Human Gene 1.0 ST (GPL6244)	Feto-placental arterial/venous endothelial cells	37	21	16	Fetal sex only
GSE128381	Agilent-039494 (GPL17077)	Whole placental tissue	183	6	177	Birth weight, maternal pre-pregnancy BMI, gestational age

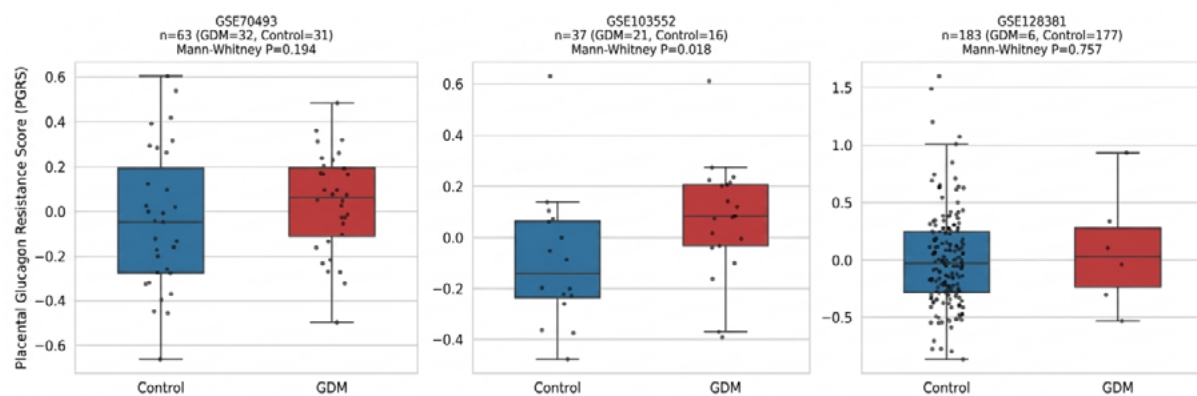


Figure 1. PGRS by group, 3 panels.

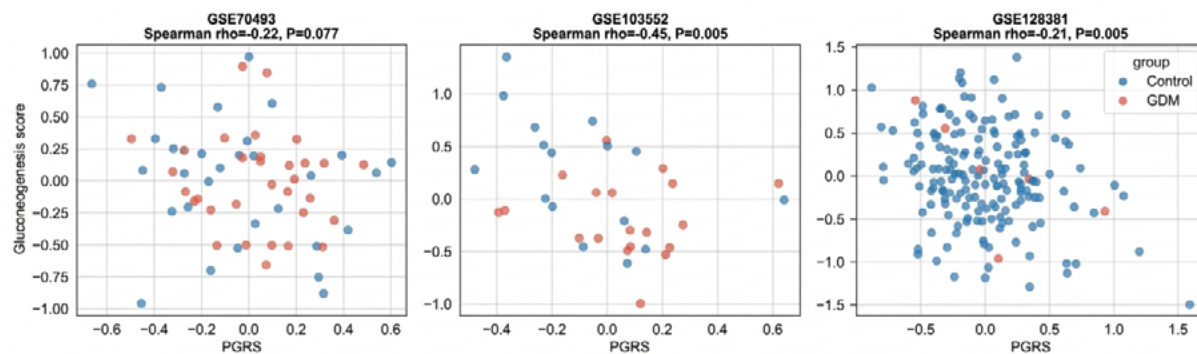


Figure 2. PGRS × gluconeogenesis score.

Table 2. DEGs of the GCGR axis per dataset (log2FC, P, FDR).

Dataset	Gene	log2FC	N GDM	N Control	P value	Adj P value
GSE103552	ATF1	-0.402	21	16	6e-06	0.0001
GSE103552	G6PC3	-0.168	21	16	0.000116	0.0014
GSE103552	ADCY6	0.26	21	16	0.000247	0.0021
GSE103552	CREM	-0.243	21	16	0.000361	0.0023
GSE103552	PRKAR1A	0.182	21	16	0.001198	0.0056
GSE103552	GNAS	0.1	21	16	0.001338	0.0056

GSE103552	PRKAR2A	-0.123	21	16	0.014956	0.0529
GSE103552	ADCY3	-0.276	21	16	0.016923	0.0529
GSE103552	PRKAR2B	-0.406	21	16	0.027585	0.0766
GSE103552	FBP1	-0.37	21	16	0.03564	0.0891
GSE103552	PRKACA	-0.074	21	16	0.049423	0.1123
GSE103552	ADCY2	-0.074	21	16	0.097123	0.2023
GSE103552	PCK2	-0.171	21	16	0.112562	0.2165
GSE103552	G6PC2	-0.047	21	16	0.186337	0.3327
GSE103552	CREB1	-0.054	21	16	0.223922	0.3732
GSE103552	PRKACB	-0.102	21	16	0.283342	0.4427
GSE103552	RAPGEF4	0.19	21	16	0.420865	0.6189
GSE103552	ADCY9	0.107	21	16	0.465759	0.6469
GSE103552	G6PC	-0.026	21	16	0.499031	0.6566
GSE103552	PCK1	-0.023	21	16	0.602879	0.7536
GSE103552	PDK4	0.103	21	16	0.671999	0.7656
GSE103552	ADCY5	0.019	21	16	0.693507	0.7656
GSE103552	ADCY1	-0.015	21	16	0.704337	0.7656
GSE103552	GCGR	-0.003	21	16	0.932457	0.9507
GSE103552	RAPGEF3	0.005	21	16	0.950658	0.9507
GSE128381	PRKAR1A	-0.255	6	177	0.011081	0.1662
GSE128381	ADCY6	0.251	6	177	0.22547	0.9806
GSE128381	PRKAR2B	-0.183	6	177	0.259499	0.9806
GSE128381	ATF1	-0.1	6	177	0.348797	0.9806
GSE128381	PDK4	0.122	6	177	0.361348	0.9806
GSE128381	RAPGEF3	0.12	6	177	0.392253	0.9806
GSE128381	ADCY3	0.162	6	177	0.49804	0.9968
GSE128381	FBP1	-0.215	6	177	0.605892	0.9968
GSE128381	CREM	-0.043	6	177	0.667175	0.9968
GSE128381	GNAS	-0.053	6	177	0.82672	0.9968
GSE128381	PCK2	0.034	6	177	0.900903	0.9968
GSE128381	PRKACA	-0.011	6	177	0.933575	0.9968
GSE128381	CREB1	-0.005	6	177	0.951098	0.9968
GSE128381	G6PC3	0.005	6	177	0.958097	0.9968
GSE128381	ADCY9	-0.001	6	177	0.996765	0.9968
GSE70493	PRKACB	-0.042	32	31	0.02251	0.5628
GSE70493	ADCY2	0.035	32	31	0.065271	0.8159
GSE70493	ADCY3	-0.034	32	31	0.17333	0.9246
GSE70493	CREB1	-0.03	32	31	0.241131	0.9246
GSE70493	PRKAR2A	-0.041	32	31	0.250076	0.9246
GSE70493	RAPGEF4	-0.012	32	31	0.334903	0.9246
GSE70493	CREM	0.033	32	31	0.336622	0.9246
GSE70493	GCGR	-0.023	32	31	0.373724	0.9246
GSE70493	PRKACA	-0.016	32	31	0.425232	0.9246

GSE70493	PRKAR2B	-0.039	32	31	0.464237	0.9246
GSE70493	PDK4	-0.081	32	31	0.480439	0.9246
GSE70493	GNAS	0.015	32	31	0.484231	0.9246
GSE70493	FBP1	-0.025	32	31	0.508542	0.9246
GSE70493	G6PC2	0.013	32	31	0.517794	0.9246
GSE70493	G6PC3	-0.034	32	31	0.619685	0.9907
GSE70493	PRKAR1A	-0.018	32	31	0.676843	0.9907
GSE70493	PCK2	0.01	32	31	0.705199	0.9907
GSE70493	RAPGEF3	0.007	32	31	0.750258	0.9907
GSE70493	G6PC	0.007	32	31	0.757791	0.9907
GSE70493	ADCY5	0.006	32	31	0.792589	0.9907
GSE70493	ATF1	0.005	32	31	0.92288	0.9999
GSE70493	PCK1	-0.001	32	31	0.963387	0.9999
GSE70493	ADCY1	-0.001	32	31	0.966169	0.9999
GSE70493	ADCY6	-0.0	32	31	0.977368	0.9999
GSE70493	ADCY9	0.0	32	31	0.999916	0.9999

ADCY6 ($\log_2FC = +0.260$, adj. $P = 0.0021$), CREM ($\log_2FC = -0.243$, adj. $P = 0.0023$), PRKAR1A ($\log_2FC = +0.182$, adj. $P = 0.0056$), and GNAS ($\log_2FC = +0.100$, adj. $P = 0.0056$). No gene reached FDR significance in GSE70493 or GSE128381; in GSE70493 only PRKACB showed a nominal, uncorrected association ($\log_2FC = -0.042$, $P = 0.023$, adj. $P = 0.563$), and in GSE128381 only PRKAR1A did ($\log_2FC = -0.255$, $P = 0.011$, adj. $P = 0.166$), both underpowered given their sample sizes and, for GSE128381, extreme class imbalance.

Cross-Dataset Directional Concordance

Among the 25 curated genes, direction of effect (GDM vs. control) was concordant between GSE70493 and GSE103552 in 15 of 25 genes (60.0%), and between GSE70493 and GSE128381 in 7 of 15 comparable genes (46.7%) (**Figure 3**). CREB1, FBP1, PRKACA, PRKAR2B, and RAPGEF3 showed concordant directionality across all three datasets; ADCY2, ATF1, CREM, GNAS, and PDK4 showed discordant direction between at least two datasets.

Correlation of PGRS with Clinical and Anthropometric Variables

In GSE128381, the only dataset with linked anthropometric data, the PGRS did not correlate significantly with neonatal birth weight (Spearman $\rho = -0.050$, $P = 0.503$), maternal pre-pregnancy BMI ($\rho = 0.085$, $P = 0.255$), or gestational age at delivery ($\rho = 0.028$, $P = 0.709$) (**Figure 4**). The gluconeogenesis score likewise showed no significant association with these

variables (birth weight: $\rho = -0.027$, $P = 0.715$; BMI: $\rho = 0.079$, $P = 0.288$; gestational age: $\rho = 0.005$, $P = 0.943$).

DISCUSSION

This study asked whether the human placenta in GDM carries a coordinated molecular signature of impaired glucagon receptor responsiveness across the GCGR-cAMP-PKA-CREB axis. The signature was present but compartment-restricted: feto-placental endothelial cells, not whole villous tissue, carried the clearest evidence, with a significantly elevated PGRS and six axis genes differentially expressed after correction for multiple testing, whereas whole-tissue cohorts showed only concordant, non-significant trends.

This compartment-specific pattern is consistent with, rather than contradicted by, prior independent analyses of the same or related datasets. Two earlier reanalyses of GSE70493 and an independent whole-placenta microarray cohort converged on immune, inflammatory, and stress-response pathways rather than cAMP-dependent signaling,¹¹⁻¹³ consistent with our nominal, FDR-non-significant PRKACB signal in that whole-tissue cohort. A more recent independent RNA-sequencing cohort likewise centered on immune cell composition rather than endocrine signaling.¹⁴ By contrast, the original study generating GSE103552 reported that GDM differentially programs feto-placental endothelial cells, with measurable changes in barrier function,¹⁵ and an independent reanalysis of the same

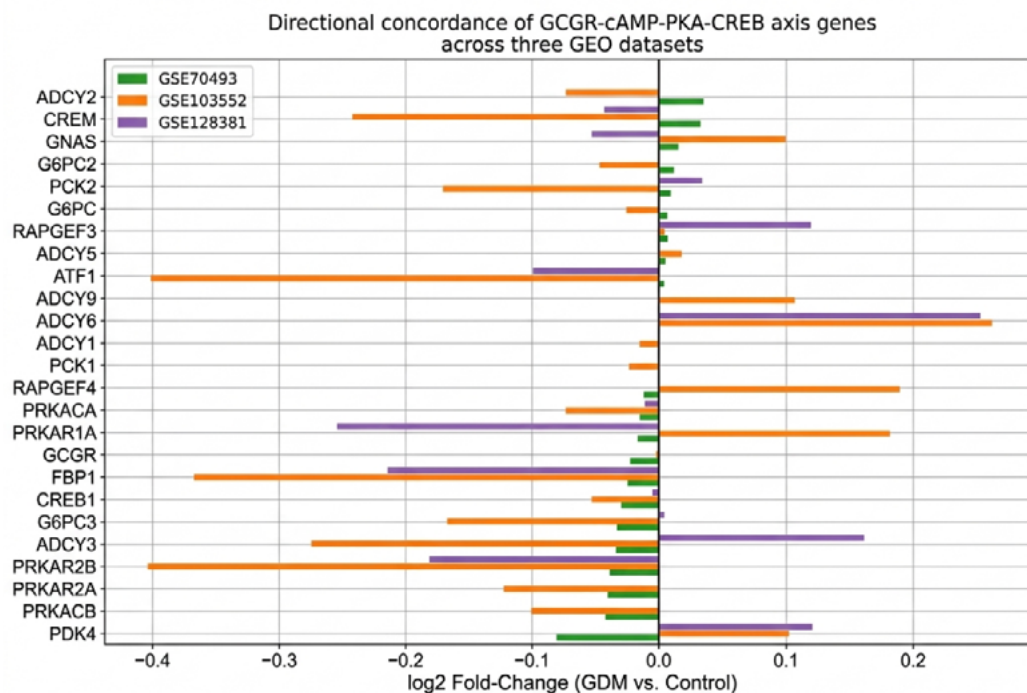


Figure 3. Directional agreement (log2FC) between datasets.

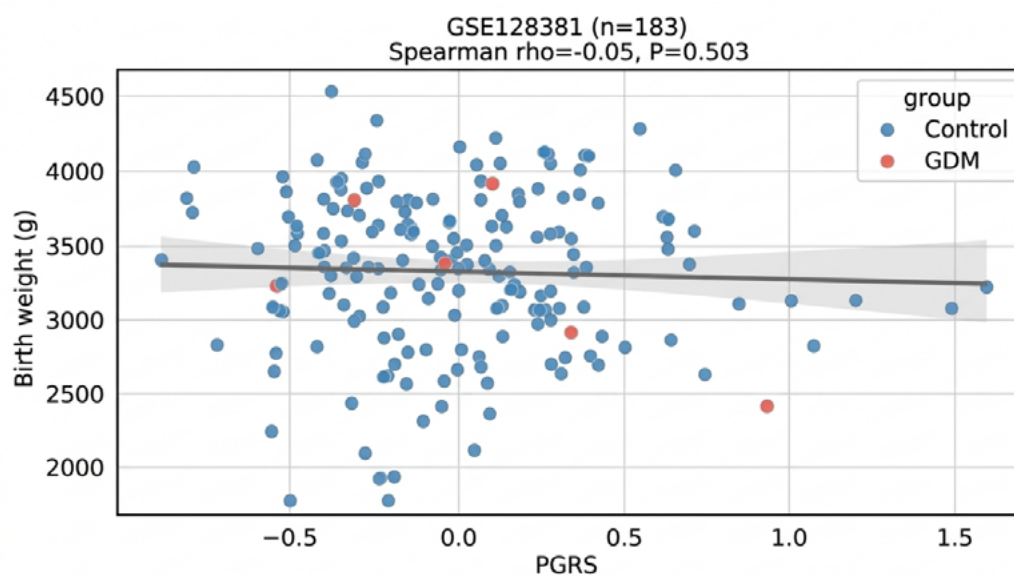


Figure 4. PGRS × birth weight (GSE128381).

dataset identified 613 differentially expressed genes converging on distinct co-expression modules.¹⁶ Our GCGR-axis findings in GSE103552 therefore sit within a growing, converging body of evidence that this endothelial compartment specifically carries a reproducible GDM signal.

Mechanistically, the direction of several hub genes is biologically coherent. ADCY6 upregulation is

consistent with its established role in constraining endothelial monolayer permeability through preferential cAMP generation at prostacyclin receptors,¹⁷ echoing the barrier-function phenotype already reported for GDM-exposed feto-placental endothelium.¹⁵ PRKAR1A, the principal PKA regulatory subunit required for normal endothelial and vascular development,¹⁸ and the downstream transcription factors ATF1 and CREM,

which mediate cAMP-responsive gene transcription in trophoblast cells,¹⁹ were concordantly altered, suggesting a coherent shift in cAMP-PKA-CREB output rather than a stochastic artifact. The inverse, rather than concordant, relationship between the PGRS and gluconeogenic gene activity argues against a simple transposition of the hepatic glucagon-resistance paradigm onto the placenta; unlike the liver, the feto-placental unit is not gluconeogenic, and its glucagon-responsive program may instead govern vascular tone and barrier integrity rather than glucose output. Finally, GNAS is a parentally imprinted locus,²⁰ and imprinting-driven variability across unrelated samples is a plausible contributor to the directional discordance observed for this gene between datasets.

Limitations warrant explicit acknowledgment. First, the 6-versus-177 class imbalance in GSE128381 limits its statistical power and external validity; this was mitigated by treating it strictly as a directional replication set rather than a co-equal discovery cohort. Second, restricting extraction to a curated 25-gene panel, rather than the full transcriptome, made rank-based ssGSEA/GSVA enrichment statistically inapplicable; a z-score module-average approach was used instead, which remains constrained by the same small-sample power limitations affecting any transcriptomic case-control comparison of this size.²¹ Third, whole villous tissue and isolated feto-placental endothelium are biologically distinct compartments profiled on different platforms, not technical replicates; this was addressed by analyzing each dataset independently rather than through cross-platform batch correction, though this necessarily limits any claim of a single, unified placental phenotype. Fourth, this study is a purely computational reanalysis of archived public microarray data with no newly collected clinical specimens, no protein-level or functional validation, and no independent prospective cohort; the planned protein-protein interaction network analysis was not completed and is not reported. The findings should therefore be interpreted as hypothesis-generating rather than as evidence of a validated clinical biomarker or mechanism.

Clinically, these findings suggest that future placental biomarker studies of GDM should not treat the placenta as a single homogeneous compartment; the feto-placental vascular endothelium, rather than bulk villous tissue, may be the more informative site for glucagon-axis signaling changes, particularly given that GDM itself is an increasingly recognized heterogeneous, subtype-structured condition.^{22,23} Scientifically, this work extends the placental endocrine framework of GDM beyond its traditional insulin-centered focus²⁴

by providing transcriptomic evidence that glucagon receptor signaling is measurably and coherently altered in a specific placental cell compartment in GDM.

Three concrete next steps follow directly from these limitations. First, full-transcriptome RNA-sequencing of feto-placental endothelial cells, rather than a targeted panel, would allow genome-wide ssGSEA/GSVA enrichment and formal pathway-level statistics. Second, functional validation in stem-cell-derived trophoblast or endothelial organoids,²⁵ using GCGR agonist and antagonist challenge with cAMP and CREB-reporter readouts, would test causality rather than association. Third, external validation in a prospective, balanced cohort of IADPSG-diagnosed GDM and control pregnancies with paired villous and endothelial sampling, stratified by GDM subtype,^{22,23} is needed before this signature can be considered a candidate biomarker.

CONCLUSION

In conclusion, GDM-associated alterations in the GCGR-cAMP-PKA-CREB axis were detectable primarily in the feto-placental endothelial compartment rather than in whole placental tissue, and tracked inversely rather than concordantly with gluconeogenic gene activity. These findings support a compartment-specific, non-hepatic pattern of placental glucagon-pathway dysregulation in GDM rather than a generalized whole-placenta glucagon resistance phenotype, and should be regarded as hypothesis-generating pending functional and prospective validation.

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ENDOGENOUS INCRETIN INSUFFICIENCY IN OBESITY: ETIOLOGICAL DEFECT OR METABOLIC MALADAPTATION?

INSUFICIÊNCIA DE INCRETINAS ENDÓGENAS NA OBESIDADE: DEFEITO ETIOLÓGICO OU MÁ ADAPTAÇÃO METABÓLICA?

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ABSTRACT

Introduction: Obesity is associated with measurably attenuated postprandial incretin responses, characterized by blunted glucagon-like peptide-1 (GLP-1) secretion and functional resistance to glucose-dependent insulinotropic polypeptide (GIP) signaling. Whether this enteroendocrine dysregulation represents a primary pathophysiological defect anteceding adiposity or a secondary maladaptive consequence of chronic metabolic overload remains unresolved, with substantial implications for the conceptualization and treatment of obesity. **Objective:** To critically evaluate and synthesize the available evidence regarding endogenous incretin insufficiency in obesity, determining whether this phenomenon constitutes a primary etiological defect or a state-dependent metabolic adaptation. **Methods:** A structured narrative review was conducted across PubMed/MEDLINE, Embase, Scopus, and Web of Science. Eligible studies encompassed human clinical trials, mechanistic investigations, and translational experimental models addressing endogenous incretin physiology, obesity-associated hormonal dysregulation, and incretin-based pharmacotherapy outcomes. **Results:** Evidence consistently demonstrates stimulus-dependent impairment of GLP-1 secretion and functional GIP resistance, both partially reversible following weight loss. Pharmacological amplification via GLP-1 receptor agonists, dual incretin agonists, and amylin-based therapies yields robust efficacy, indicating biologically intact but functionally downregulated signaling pathways. A multi-hormonal insufficiency model, rather than isolated incretin deficiency, best accounts for the observed phenotype. **Conclusion:** Endogenous incretin insufficiency in obesity reflects a state-dependent, adaptive dysregulation of a biologically intact enteroendocrine system, driven by chronic metabolic stress, rather than a primary etiological defect. Therapeutic strategies should prioritize restoration of integrated satiety signaling over isolated hormonal replacement.

Keywords: Enteroendocrine dysfunction, GLP-1 secretion, Incretin resistance, Metabolic maladaptation.

RESUMO

Introdução: A obesidade está associada a respostas incretínicas pós-prandiais mensuravelmente atenuadas, caracterizadas por redução da secreção do peptídeo semelhante ao glucagon tipo 1 (GLP-1) e resistência funcional à sinalização mediada pelo polipeptídeo insulínico dependente de glicose (GIP). Permanece não esclarecido se essa desregulação enteroendócrina representa um defeito fisiopatológico primário, que precede o desenvolvi-

mento da adiposidade, ou uma consequência adaptativa secundária e mal-adaptativa da sobrecarga metabólica crônica, com implicações substanciais para a compreensão conceitual e o tratamento da obesidade. **Objetivo:** Avaliar criticamente e sintetizar as evidências disponíveis sobre a insuficiência incretínica endógena na obesidade, determinando se esse fenômeno constitui um defeito etiológico primário ou um estado de adaptação metabólica dependente das condições fisiopatológicas. **Métodos:** Foi conduzida uma revisão narrativa estruturada nas bases de dados PubMed/MEDLINE, Embase, Scopus e Web of Science. Foram considerados elegíveis estudos clínicos em humanos, investigações mecanísticas e modelos experimentais translacionais que abordassem a fisiologia das incretinas endógenas, a desregulação hormonal associada à obesidade e os desfechos da farmacoterapia baseada em incretinas. **Resultados:** As evidências demonstram de forma consistente comprometimento da secreção de GLP-1 dependente do estímulo e resistência funcional ao GIP, ambos parcialmente reversíveis após a perda ponderal. A amplificação farmacológica da sinalização incretínica por meio de agonistas do receptor de GLP-1, agonistas duplos de incretinas e terapias baseadas em amilina apresenta eficácia robusta, indicando a preservação biológica das vias de sinalização, embora funcionalmente downreguladas. Um modelo de insuficiência hormonal multivariada, em vez de uma deficiência incretínica isolada, parece explicar de forma mais adequada o fenótipo observado. **Conclusão:** A insuficiência incretínica endógena na obesidade reflete uma desregulação adaptativa dependente do estado metabólico de um sistema enteroendócrino biologicamente preservado, impulsionada pelo estresse metabólico crônico, e não um defeito etiológico primário. As estratégias terapêuticas devem priorizar a restauração integrada da sinalização da saciedade, em vez da reposição hormonal isolada.

Descritores: Disfunção enteroendócrina, Secreção de GLP-1, Resistência às incretinas, Adaptação metabólica mal-adaptativa.

INTRODUCTION

Obesity represents one of the most pressing public health challenges of the twenty-first century, affecting over one billion individuals worldwide and contributing disproportionately to the global burden of type 2 diabetes, cardiovascular disease, and all-cause mortality.¹ Despite decades of intensive investigation, the precise endocrine mechanisms that predispose certain individuals to pathological adiposity while others maintain metabolic homeostasis under comparable environmental conditions remain incompletely understood. Among the hormonal systems implicated in energy balance regulation, the enteroendocrine axis has attracted growing scientific interest, particularly given its central role in coordinating postprandial satiety signaling, insulin secretion, and gastrointestinal motility.² The gut, far from being a passive digestive organ, functions as the largest endocrine gland in the human body, capable of synthesizing and releasing a diverse repertoire of peptide hormones in response to nutrient stimuli.³

Central to this enteroendocrine framework are the incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), which together account for a substantial proportion of postprandial insulin release, a phenomenon collectively termed the incretin effect.⁴ In lean, metabolically healthy individuals, these hormones are secreted in a tightly regulated, physiologically appropriate manner, orchestrating a coordinated anorexigenic response that limits excessive caloric intake.^{5,6} However, accumulating evidence suggests that obese individuals exhibit a measurably blunted incretin response, characterized by reduced postprandial GLP-1 secretion and attenuated GIP signaling, raising a fundamental and still unresolved question: does this hormonal insufficiency represent a primary etiological defect that antecedes and promotes adiposity, or does it emerge secondarily as a maladaptive consequence of the chronic metabolic and inflammatory milieu inherent to established obesity?

Resolving this question carries profound therapeutic implications that extend well beyond academic interest. The current pharmacological landscape of

obesity management has been substantially shaped by incretin-based therapies, including GLP-1 receptor agonists, which exogenously compensate for diminished endogenous secretion.⁷ While clinically effective, these agents address hormonal insufficiency through exogenous supplementation rather than restoration of intrinsic enteroendocrine function, a distinction that may be critically relevant to long-term metabolic outcomes and treatment sustainability. The underlying question of whether the enteroendocrine system is fundamentally impaired in obesity, and whether such impairment is reversible, has not been systematically addressed in the literature.⁸

The present review aims to critically examine and synthesize the available evidence regarding endogenous incretin insufficiency in obesity, with the specific objective of determining whether this phenomenon constitutes a primary pathophysiological defect or a secondary metabolic adaptation. By interrogating data from mechanistic, epidemiological, and interventional studies, we seek to reframe the conceptual understanding of gut hormone dysregulation in obesity and to identify actionable targets for therapies directed at restoring, rather than merely replacing, physiological enteroendocrine function.

METHODS

Study Design

Given the conceptual and mechanistic complexity of this question, a structured narrative approach was selected to enable integrative interpretation across heterogeneous lines of evidence, including clinical, experimental, and translational studies.

Search Strategy

A comprehensive literature search was performed using PubMed/MEDLINE, Embase, Scopus, and Web of Science from database inception through the most recent available records.

Search terms combined controlled vocabulary and free-text keywords related to obesity and incretin biology, including: “obesity,” “GLP-1,” “glucagon-like peptide-1,” “GIP,” “glucose-dependent insulinotropic polypeptide,” “incretin effect,” “endogenous secretion,” “enteroendocrine function,” “gut hormones,” and “metabolic adaptation.” Boolean operators were applied to optimize sensitivity and specificity.

To enhance completeness, reference lists of relevant articles were manually screened to identify additional studies not captured in the primary search.

Study Selection

Studies were selected based on their relevance to endogenous incretin physiology and its relationship with obesity and metabolic regulation.

Eligible studies met the following criteria: investigation of endogenous incretin secretion, signaling, or physiological response; inclusion of populations with obesity or experimental models of obesity; assessment of outcomes related to insulin secretion, appetite regulation, energy balance, or adiposity; publication in peer-reviewed journals in English.

Both human and animal studies were included when they provided mechanistic insight.

Studies were excluded if they: focused exclusively on pharmacological incretin-based therapies without addressing endogenous physiology; lacked sufficient methodological detail; were non-original reports (e.g., editorials, commentaries, conference abstracts).

Study selection was performed through sequential screening of titles/abstracts followed by full-text review.

Data Extraction

Data were extracted in a structured manner to capture key variables relevant to the study objective, including: study design and population characteristics; measures of adiposity and metabolic status; fasting and postprandial incretin hormone levels; methodologies used to assess incretin response; insulin secretion and glycemic outcomes; appetite- and satiety-related endpoints; mechanistic interpretations regarding causality versus adaptation.

Particular emphasis was placed on identifying temporal and mechanistic patterns suggestive of either primary endocrine dysfunction or secondary metabolic adaptation.

Analytical Approach

An interpretative framework was applied to categorize the evidence into two overarching conceptual models:

Etiological Defect Model, in which incretin insufficiency precedes and contributes to the development of obesity;

- Metabolic Maladaptation Model, in which incretin dysfunction arises as a downstream consequence of obesity-associated metabolic disturbances, including insulin resistance, chronic inflammation, and altered nutrient signaling.
- Evidence was synthesized qualitatively, with emphasis on biological plausibility, internal consistency across studies, and translational re-

levance. Converging findings from human and experimental data were integrated to support a coherent pathophysiological interpretation.

Assessment of Evidence Quality

Although a formal risk-of-bias tool was not applied due to the narrative design, methodological rigor of individual studies was considered during interpretation. Greater weight was assigned to: well-controlled clinical studies; investigations with standardized assessment of incretin responses; studies employing validated hormonal assays; longitudinal or mechanistic designs supporting causal inference.

This approach was intended to strengthen the robustness of the conceptual synthesis while preserving the flexibility required for hypothesis-driven analysis.

RESULTS

Overview of Included Evidence

The literature search identified a broad and methodologically heterogeneous body of evidence encompassing human clinical studies, mechanistic investigations, and translational experimental models examining endogenous incretin physiology in obesity. Across studies, considerable variability was observed in the assessment of incretin responses, including differences in nutrient stimuli, timing of hormonal measurements, and analytical methodologies.

Despite this heterogeneity, a number of reproducible physiological patterns emerged, allowing for a structured interpretation of the relationship between incretin dynamics and obesity.

Altered Endogenous Incretin Secretion in Obesity

A consistent finding across multiple studies was the presence of attenuated postprandial GLP-1 responses in individuals with obesity, particularly following mixed-meal or oral glucose challenges. This reduction was not uniformly observed under fasting conditions, suggesting that the impairment is stimulus-dependent rather than constitutive.

In contrast, GIP responses appeared relatively preserved or even exaggerated in some cohorts, although evidence indicates a functional resistance at the receptor or post-receptor level, leading to a diminished insulinotropic effect despite preserved secretion.

Importantly, studies comparing lean and obese individuals under controlled conditions demonstrated

that: the magnitude of the incretin effect is reduced in obesity; this reduction correlates more strongly with insulin resistance and adiposity indices than with absolute hormone concentrations.

Taken together, these findings argue against a simple deficiency model and instead suggest a qualitative dysregulation of enteroendocrine signaling.

Temporal and Mechanistic Evidence: Cause or Consequence

Longitudinal and interventional data provided critical insight into the temporal relationship between incretin dysfunction and obesity.

Evidence from: weight loss interventions (dietary or surgical), caloric restriction models, and metabolic normalization studies demonstrated partial restoration of GLP-1 secretion following weight reduction, supporting the notion that incretin impairment is, at least in part, reversible.

Similarly, experimental models of diet-induced obesity showed that: chronic nutrient excess, intestinal inflammation, and alterations in gut microbiota are associated with progressive impairment of enteroendocrine cell responsiveness.

Altogether, these observations favor a model in which incretin insufficiency emerges as a secondary adaptation, although they do not fully exclude the possibility that subtle pre-existing differences in incretin biology may predispose certain individuals to weight gain.

Pharmacological Amplification: Evidence from Incretin-Based Therapies

A substantial body of evidence derived from trials using incretin-based pharmacotherapies provides indirect but highly informative insight into endogenous incretin physiology (Table 1).

GLP-1 Receptor Agonists

Agents such as semaglutide, liraglutide, and dulaglutide consistently demonstrate: robust weight loss, reduced energy intake, delayed gastric emptying, and improved glycemic control.

The magnitude of these effects, often exceeding what would be expected from physiological GLP-1 levels, suggests that pharmacological activation compensates for an insufficient endogenous anorexigenic signal.

However, the supraphysiological nature of receptor stimulation complicates interpretation, as these agents may override, rather than restore, endogenous regulatory pathways.

Table 1. Summary of Key Pharmacological Evidence Targeting Incretin and Amylin Pathways

Class	Agent	Mechanism	Key Effects	Pathophysiological Implication
GLP-1 RA	Semaglutide	GLP-1 receptor agonism	Significant weight loss, appetite suppression	Supports functional insufficiency of endogenous GLP-1
GLP-1 RA	Liraglutide	GLP-1 receptor agonism	Reduced caloric intake, improved glycemia	Indicates therapeutic compensation
Dual agonist	Tirzepatide	GLP-1 + GIP receptor agonism	Greater weight reduction than GLP-1 alone	Suggests preserved but dysregulated GIP axis
Amylin analog	Pramlintide	Amylin receptor agonism	Satiety enhancement	Highlights non-incretin hormonal deficits
Amylin analog	Cagrilintide	Long-acting amylin analog	Sustained weight loss	Supports multi-hormonal model
Combination	Cagrilintide + Semaglutide	Amylin + GLP-1 co-agonism	Synergistic weight reduction	Evidence of integrated neuroendocrine dysfunction

Dual and Triple Agonists

More recent agents, particularly tirzepatide, have demonstrated greater weight reduction than GLP-1 monotherapy, despite the paradoxical physiology of GIP in obesity.

These findings suggest that: GIP signaling may be context-dependent, and pharmacological co-activation may restore lost synergistic interactions within the enteroinsular axis.

This challenges earlier assumptions of GIP as purely obesogenic and supports a more nuanced model of incretin network dysfunction.

Amylin and Amylin–Incretin Co-Agonism

Therapies targeting amylin pathways, including pramlintide and next-generation agents such as cagrilintide, provide complementary evidence.

Amylin analogs: enhance satiety, reduce food intake, and act through distinct but overlapping neuroendocrine circuits.

The combination approaches (e.g., cagrilintide plus semaglutide) have demonstrated additive or synergistic effects on weight loss, reinforcing the concept that: obesity is characterized by multi-hormonal insufficiency or resistance, rather than isolated incretin deficiency.

Integrated Interpretation of Findings

When considered collectively, the available evidence does not support a unidimensional model of primary incretin deficiency. Instead, the data converge toward a multi-layered framework characterized by: stimulus-dependent impairment of GLP-1 secretion; functional resistance to GIP signaling; partial revers-

ibility with weight loss; and robust responsiveness to pharmacological amplification.

This pattern is more consistent with a secondary, adaptive dysregulation of the enteroendocrine system, likely driven by chronic metabolic stress.

At the same time, the marked efficacy of incretin- and amylin-based therapies underscores that this adaptive state results in a clinically meaningful deficit in physiological satiety signaling, which can be therapeutically exploited (**Figure 1**).

DISCUSSION

The present synthesis reframes endogenous incretin dysfunction in obesity not as a primary endocrine deficiency, but as a dynamic and context-dependent maladaptation of the enteroendocrine system, shaped by chronic metabolic stress. This interpretation is strongly supported by converging evidence from physiological studies, interventional trials, and the remarkable efficacy of incretin-based pharmacotherapies.

A central finding across the literature is the attenuation of postprandial GLP-1 secretion in individuals with obesity, particularly under nutrient-stimulated conditions. However, this impairment is neither universal nor static. Contemporary mechanistic studies demonstrate that GLP-1 secretion is highly plastic and responsive to metabolic state, with partial restoration observed following weight loss or caloric restriction, suggesting that dysfunction is acquired rather than intrinsic.^{9,10} This aligns with evolving models in which enteroendocrine cells adapt to chronic nutrient excess, leading to altered hormone release patterns rather than outright failure.

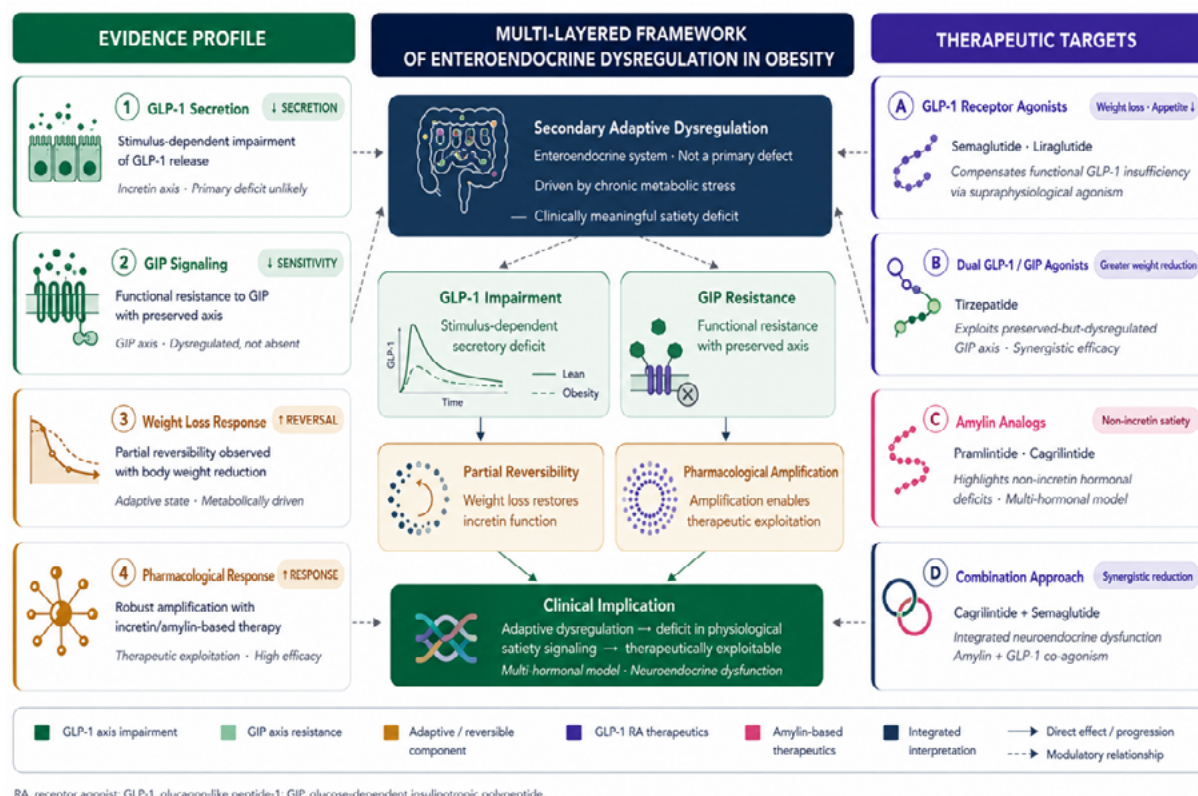


Figure 1. Conceptual model of enteroendocrine dysfunction in obesity.

At a systems level, incretin physiology in obesity is better understood as a disruption of signal integration rather than hormone deficiency per se. GLP-1, GIP, and amylin act in concert to regulate satiety, insulin secretion, and gastric motility, forming a coordinated gut–brain–pancreas axis.¹¹ Within this network, obesity appears to induce both reduced GLP-1 responsiveness and impaired GIP signaling efficiency, the latter often manifesting as a dissociation between circulating levels and biological activity — consistent with functional GIP resistance.¹²

This distinction between concentration and function is fundamental. It underscores that endocrine dysfunction in obesity cannot be inferred solely from circulating hormone levels, but must incorporate receptor-level signaling competence and neural integration pathways, which remain insufficiently characterized in human studies.

The therapeutic landscape provides compelling indirect evidence supporting this interpretation. The advent of highly potent incretin-based therapies, particularly GLP-1 receptor agonists and dual incretin agonists, has fundamentally transformed obesity treatment. Agents such as semaglutide and tirzepatide consistently induce clinically meaningful weight loss in the

range of 15–25%, far exceeding traditional pharmacotherapies.^{13,14} These effects are mediated primarily through central appetite suppression, delayed gastric emptying, and modulation of reward pathways, rather than simple insulinotropic actions.¹⁵

It is important to emphasize that the magnitude of these effects challenges the notion of a primary deficiency state. If endogenous incretin systems were fundamentally defective, pharmacological amplification would be expected to yield limited benefit. Instead, the robust response observed across trials suggests that incretin pathways remain biologically intact but are functionally downregulated or insufficiently activated in the obesogenic state.¹⁶

This concept is further reinforced by the success of dual and multi-agonist strategies. Tirzepatide, a GLP-1/GIP co-agonist, produces greater weight reduction than GLP-1 monotherapy, despite earlier assumptions that GIP signaling contributes to adiposity.^{17,18} These findings suggest that GIP is not inherently obesogenic, but rather context-dependent, with its physiological role altered under conditions of metabolic dysregulation. Re-engagement of this pathway through pharmacological co-activation appears to restore lost synergistic signaling within the enteroinsular axis.

Parallel advances in amylin-based therapies provide additional support for a multi-hormonal model of obesity. Amylin, co-secreted with insulin, exerts potent anorexigenic effects via central mechanisms distinct from, but complementary to, GLP-1.¹⁹ Clinical studies demonstrate that amylin analogs such as pramlintide and next-generation agents like cagrilintide induce significant reductions in energy intake and body weight, particularly when combined with GLP-1 receptor agonists.^{20,21}

The observed synergy between incretin and amylin pathways is particularly informative. It suggests that obesity is characterized not by isolated dysfunction of a single hormone, but by a broader impairment of integrated satiety signaling, involving multiple overlapping neuroendocrine systems. This aligns with recent translational frameworks describing obesity as a state of multi-hormonal resistance and insufficiency, rather than single-axis failure.^{22,23}

From a pathophysiological perspective, these findings converge toward a progressive maladaptation model, in which chronic exposure to excess nutrients, low-grade inflammation, and altered gut microbiota lead to: impaired enteroendocrine cell responsiveness, altered hormone secretion dynamics, and reduced central sensitivity to satiety signals.

Within this framework, incretin dysfunction emerges as a downstream consequence of metabolic overload, rather than a primary driver of adiposity. This interpretation is further supported by studies demonstrating that weight loss interventions, particularly bariatric surgery, restore GLP-1 secretion and enhance satiety signaling, reinforcing the reversibility of the defect.²⁴

However, the degree of reversibility appears heterogeneous. Prolonged exposure to obesity may induce more persistent alterations in gut–brain communication pathways, potentially involving structural and functional changes in enteroendocrine cells and central appetite-regulating circuits.²⁵ This raises the possibility that early intervention may be critical to prevent entrenched neuroendocrine dysfunction.

GAP STATEMENT

Despite substantial advances, several critical gaps remain that limit a definitive understanding of endogenous incretin insufficiency in obesity.

The causal directionality remains incompletely resolved. Although longitudinal and interventional studies suggest reversibility of incretin dysfunction, there is a lack of prospective human data demonstrat-

ing whether subtle impairments in incretin signaling precede weight gain.

Most studies rely on circulating hormone concentrations as surrogate markers, with limited assessment of receptor signaling, intracellular pathways, or neural integration mechanisms. This restricts mechanistic resolution and may obscure key aspects of functional resistance.

There is a paucity of data addressing the role of enteroendocrine cell biology in humans, including cell density, differentiation, and responsiveness under obesogenic conditions. Similarly, the contribution of the gut microbiota–enteroendocrine axis remains incompletely characterized.

Thus, while pharmacological studies provide strong indirect evidence, they predominantly reflect supraphysiological receptor activation, which may not accurately model endogenous physiology.

FUTURE DIRECTIONS

Addressing these gaps will require a shift toward integrative, mechanism-focused research strategies.

Future studies should prioritize: longitudinal human cohorts to determine whether alterations in incretin dynamics precede or follow weight gain; direct assessment of receptor signaling and downstream pathways, including tissue-specific responsiveness and central nervous system integration; advanced phenotyping of enteroendocrine cells, leveraging single-cell transcriptomics and in vivo imaging approaches; and controlled interventions targeting gut microbiota and intestinal inflammation, to evaluate their impact on incretin secretion and function.

In parallel, emerging pharmacological strategies should move beyond receptor agonism toward restoration of endogenous hormone dynamics, including therapies designed to enhance physiological secretion or improve receptor sensitivity.

Importantly, the integration of incretin and amylin biology suggests that future treatments may benefit from multi-target approaches that reconstitute coordinated satiety signaling, rather than focusing on individual hormonal pathways.

CONCLUSION

The available evidence supports a paradigm in which endogenous incretin insufficiency in obesity reflects a state-dependent, adaptive dysregulation of

a biologically intact system, rather than a primary etiological defect. Recognizing this distinction reframes both the pathophysiology of obesity and the therapeutic strategies required to treat it, emphasizing the need to move beyond hormonal replacement toward restoration of integrated metabolic homeostasis.

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MODULATION OF THE INCRETIN AXIS (GLP-1/GIP) IN BEHAVIORAL COMPULSIONS: A NEW NEUROPSYCHOPHARMACOLOGY FRONTIER

MODULAÇÃO DO EIXO INCRETÍNICO (GLP-1/GIP) EM COMPULSÕES COMPORTAMENTAIS: UMA NOVA FRONTEIRA DA NEUROPSICOFARMACOLOGIA

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ABSTRACT

INTRODUCTION: Behavioral compulsions unrelated to substance use, including compulsive buying disorder and compulsive sexual behavior disorder, share core neurobiological mechanisms with addictions, particularly meso-limbic dopaminergic dysregulation and impaired prefrontal inhibitory control. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) exert central effects on reward circuitry, and tirzepatide, a dual GLP-1/GIP receptor agonist, may enhance these effects through combined dopaminergic modulation and synaptic plasticity mechanisms involving intracellular signaling pathways. **OBJECTIVE:** To review the role of the GLP-1/GIP axis, with emphasis on tirzepatide, in modulating non-alimentary behavioral compulsions, integrating neurobiological and clinical evidence, and to present two illustrative case reports. **METHODS:** Narrative review of PubMed/MEDLINE-indexed literature, including preclinical, clinical, and real-world evidence through 2026. Two original cases are presented: one involving compulsive buying and one compulsive sexual behavior, both treated with tirzepatide without changes in psychopharmacological or psychotherapeutic management. **RESULTS:** GLP-1RAs modulate mesolimbic circuits, reducing reward salience and behavioral urgency. Preclinical studies show decreased reward-seeking and relapse. Human data demonstrate electrophysiological modulation of the nucleus accumbens following tirzepatide exposure. Clinically, a obese female patient reported reduced compulsive buying after four weeks, while a male patient undergoing psychoanalytic therapy exhibited decreased intrusive sexual thoughts and improved impulse control. However, potential risks include compulsive symptom substitution, reduced hedonic tone, and behavioral instability with nonstandard dosing. **CONCLUSION:** Tirzepatide may represent a novel transdiagnostic approach to behavioral compulsions, though controlled studies are needed to confirm efficacy and safety.

Keywords: Tirzepatide, behavioral compulsions, GLP-1 receptor agonists, dopaminergic reward system.

RESUMO

INTRODUÇÃO: As compulsões comportamentais não relacionadas ao uso de substâncias, incluindo o transtorno de compra compulsiva e o transtorno do comportamento sexual compulsivo, compartilham mecanismos neurobiológicos fundamentais com os transtornos aditivos, particularmente a desregula-

ção dopaminérgica mesolímbica e o comprometimento do controle inibitório pré-frontal. Os agonistas do receptor do peptídeo semelhante ao glucagon tipo 1 (GLP-1RAs) exercem efeitos centrais sobre os circuitos de recompensa, e a tirzepatida, um agonista dual dos receptores de GLP-1/GIP, pode potencializar esses efeitos por meio da modulação dopaminérgica combinada e de mecanismos de plasticidade sináptica envolvendo vias de sinalização intracelular. **OBJETIVO:** Revisar o papel do eixo GLP-1/GIP, com ênfase na tirzepatida, na modulação das compulsões comportamentais não alimentares, integrando evidências neurobiológicas e clínicas, além de apresentar dois relatos de casos ilustrativos. **MÉTODOS:** Foi realizada uma revisão narrativa da literatura indexada no PubMed/MEDLINE, incluindo evidências pré-clínicas, clínicas e provenientes de estudos de mundo real até 2026. São apresentados dois casos originais: um envolvendo compra compulsiva e outro comportamento sexual compulsivo, ambos tratados com tirzepatida sem modificações no tratamento psicofarmacológico ou psicoterapêutico. **RESULTADOS:** Os agonistas dos receptores de GLP-1 modulam os circuitos mesolímbicos, reduzindo a saliência dos estímulos de recompensa e a urgência comportamental. Estudos pré-clínicos demonstram redução da busca por recompensa e da recaída. Dados em humanos evidenciam modulação eletrofisiológica do núcleo accumbens após exposição à tirzepatida. Clinicamente, uma paciente do sexo feminino com obesidade relatou redução do comportamento de compra compulsiva após quatro semanas, enquanto um paciente do sexo masculino em tratamento psicanalítico apresentou redução dos pensamentos sexuais intrusivos e melhora do controle dos impulsos. Entretanto, os potenciais riscos incluem substituição de sintomas compulsivos, redução do tônus hedônico e instabilidade comportamental associada ao uso de doses não padronizadas. **CONCLUSÃO:** A tirzepatida pode representar uma nova abordagem transdiagnóstica para o tratamento das compulsões comportamentais, embora estudos controlados sejam necessários para confirmar sua eficácia e segurança.

Descritores: Tirzepatida, compulsões comportamentais, agonistas do receptor de GLP-1, sistema dopaminérgico de recompensa.

INTRODUCTION

Behavioral compulsions, including binge eating disorder, compulsive buying disorder (CBD), and compulsive sexual behavior disorder (CSBD), constitute a heterogeneous cluster of conditions characterized by loss of control over impulses and persistence of maladaptive behaviors despite significant adverse consequences. These conditions share important neurobiological mechanisms with substance use disorders (SUDs), particularly dysfunction of mesolimbic dopaminergic reward circuits involving the nucleus accumbens (NAc) and the prefrontal cortex (PFC).¹

In recent years, growing interest has emerged regarding the role of the incretin axis, particularly glucagon-like peptide-1 receptor agonists (GLP-1RAs), in modulating these circuits. Initially developed for the management of type 2 diabetes mellitus and obesity, GLP-1RAs have demonstrated central effects that transcend metabolic control, including modulation of food

intake, satiety signaling, and, more broadly, responsiveness to rewarding stimuli.² Preclinical evidence indicates that these agents act directly on key nodes of the reward system, attenuating dopamine release associated with reinforcing stimuli and reducing compulsive seeking behavior.³

More recently, dual-acting compounds such as tirzepatide, a GLP-1R/glucose-dependent insulinotropic polypeptide receptor (GIPR) co-agonist, have been proposed as potentially enhanced modulators of these systems, with more robust effects on feeding behavior and possibly on other forms of compulsive consumption.⁴ This conceptual framework gave rise to the hypothesis that such agents could function as “transdiagnostic consumption modulators,” with therapeutic impact extending beyond food intake to encompass compulsive buying, substance use, and hypersexual behavior.⁵

Despite a growing body of mechanistic evidence and indirect clinical data, the application of these

agents to behavioral compulsions remains predominantly *off-label*.⁶ Fundamental questions remain unresolved, including heterogeneity of treatment response, the risk of paradoxical effects on impulse control, and the absence of standardized clinical protocols.

Against this backdrop, it is essential to synthesize available neurobiological, pharmacological, and clinical evidence to elucidate the potential of the GLP-1/GIP axis as a therapeutic target in behavioral compulsions, while delineating its limitations and future perspectives. The present study aims to conduct a narrative review of the literature, focusing on studies related to the GLP-1 axis, tirzepatide, and addictive behavior, supplemented by two clinically relevant original case reports.

NEUROBIOLOGICAL FOUNDATION: BEHAVIORAL COMPULSIONS AND THE GLP-1 AXIS

Non-substance behavioral compulsions, including CSBD and CBD, share a neurobiological architecture strikingly similar to that of classical chemical dependencies. Dysregulation of dopaminergic transmission in the NAc, implicated in addiction and affective disorders, underscores the addictive nature of CSBD and supports its classification as a behavioral addiction.⁷ Consistent with this, neuroimaging studies demonstrate that individuals with compulsive sexual behavior exhibit heightened activation of the ventral striatum, anterior cingulate cortex, and amygdala in response to sexual stimuli, a pattern that closely mirrors findings in substance use disorders.⁸

The shared substrate of these conditions involves three central mechanisms. First, mesolimbic dopaminergic hyperactivity: during the binge/intoxication stage of the addiction cycle, dopaminergic firing in the basal ganglia increases in response to reward-associated stimuli, a phenomenon termed incentive salience. Second, prefrontal inhibitory control dysfunction: during the preoccupation/anticipation stage, executive control systems in the PFC are compromised, manifesting as reduced impulse control, executive planning deficits, and impaired emotion regulation. Third, sensitization to reward cues: the transition to compulsive behavior involves neuroplasticity initiated by alterations in the mesolimbic dopaminergic system and culminating in a cascade of neuroadaptations across the PFC, cingulate cortex, and extended amygdala.⁹

In this context, GLP-1RAs emerge as direct modulators of these circuits. Central activation of GLP-1 receptors in mesolimbic and mesocortical circuits, encompassing the ventral tegmental area (VTA), the NAc, and the PFC, modulates dopaminergic, glutamatergic, and GABAergic signaling, thereby attenuating reward salience.¹⁰ At the cellular level, GLP-1 receptors are predominantly expressed on GABAergic neurons; their activation engages mesencephalic inhibitory circuits, consequently reducing cocaine-seeking behavior.¹¹

Tirzepatide, the dual GLP-1R/GIPR agonist, exhibits a potentially broader profile of action on these circuits. In rodent models, tirzepatide effectively attenuated the rewarding properties of alcohol (as assessed by locomotor sensitization, conditioned place preference, and accumbal dopamine release) and dose-dependently reduced voluntary alcohol consumption while preventing relapse-like behavior.¹² From a molecular standpoint, by combining GLP-1R and GIPR agonism, tirzepatide may reinforce neural circuits involved in executive control and decision-making via mTOR pathway signaling, potentially counterbalancing the dysregulated reward circuitry observed in addiction (**Figure 1**).¹⁰

Observational human data corroborate this mechanistic model. Emerging evidence suggests that tirzepatide and semaglutide may represent the first effective “anti-consumption” agents, with potential applications encompassing the reduction of food craving, alcohol use, nicotine dependence, recreational drug use, and even compulsive buying behaviors.¹³ These findings are consistent with a systematic review demonstrating that GLP-1RAs normalize insulin resistance and may modulate dopaminergic signaling, thereby reducing anhedonia, with potential applications in disorders of motivational and reward processing.³

Overall, the convergence between the neurobiological mechanisms underlying behavioral compulsions and the molecular targets of GLP-1RAs, particularly within the NAc-VTA-PFC axis, establishes a robust mechanistic rationale for investigating these agents in the treatment of behavioral addictions for which pharmacotherapy remains largely unavailable.

EVIDENCE IN NON-ALIMENTARY BEHAVIORAL COMPULSIONS

Compulsive Buying Disorder

Compulsive buying disorder is classified as an impulse control disorder with features overlapping

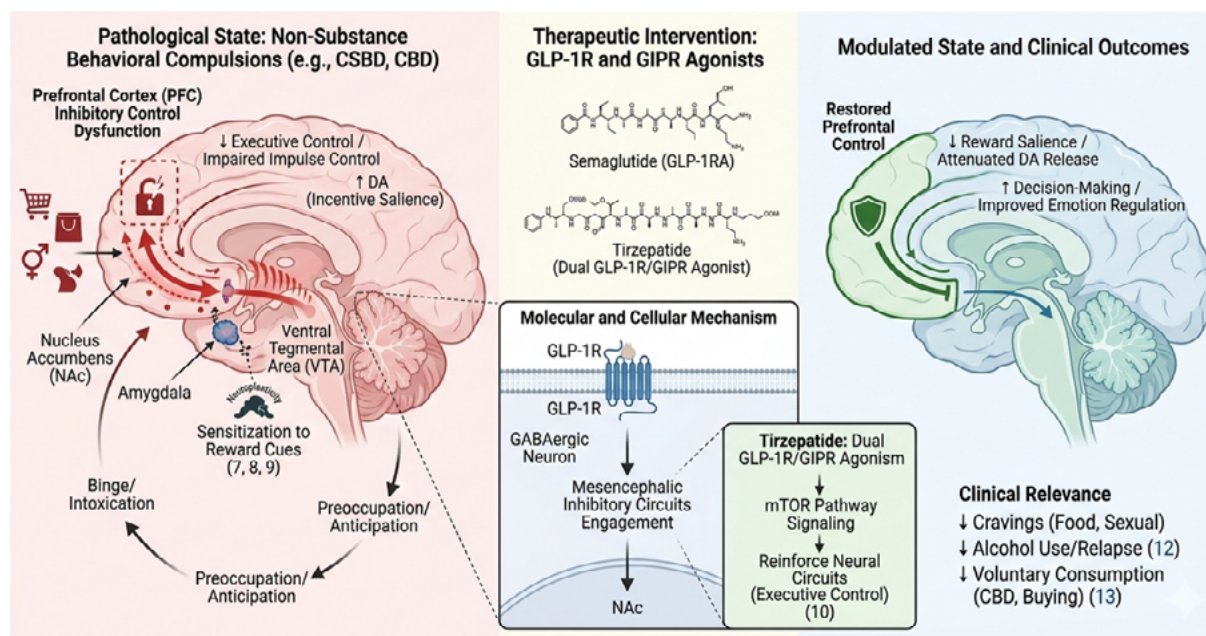


Figure 1. Neurobiological convergences: The GLP-1 axis as a modulator of behavioral compulsions within the mesocorticolimbic circuit.

behavioral addictions, including loss of control, intrusive preoccupation, and behavioral persistence notwithstanding significant financial and relational consequences.

Case Report

We report a clinical case directly illustrating the impact of tirzepatide on this condition. A 30-year-old female patient with no prior formal psychiatric diagnosis presented for obesity management (baseline BMI: 35.2 kg/m²). During comprehensive history-taking, she disclosed a recurrent pattern of compulsive buying, characterized by frequent intrusive urges to purchase unnecessary items, weekly episodes of excessive spending followed by regret, and progressive financial impairment. She denied substance use but reported that episodes were consistently associated with states of anxiety and boredom. Tirzepatide was initiated under a standard progressive titration protocol. After approximately four weeks of treatment (2.5 mg/week), in addition to the expected reduction in appetite and initial weight loss (~6% of body weight), the patient spontaneously reported a marked reduction in buying impulses. She described decreased frequency of consumption-related intrusive thoughts, improved capacity to defer purchasing decisions, and a reduction in impulsive spending episodes. The patient was not engaged in structured psychotherapy during this period, and no additional psychopharmacological agents were

introduced, suggesting a plausible temporal association with tirzepatide initiation.

This isolated finding acquires conceptual significance when contextualized within broader reviews. Emerging data suggest that tirzepatide and semaglutide may represent the first effective “anti-consumption” agents, with the potential to reduce binge eating, alcohol consumption, nicotine dependence, recreational drug use, and compulsive buying behaviors, by acting on the brain reward pathway that mediates addictive processes.¹⁴ The “anti-consumption effect” is not merely speculative: a mixed-methods social media analysis encompassing more than 43,000 comments from social platforms between 2019 and 2025 demonstrated that GLP-1Ras, including semaglutide and tirzepatide, were associated with positive effects on non-substance-related behaviors, including compulsive buying, although findings exhibited some inconsistency and warrant cautious interpretation.¹⁵

Sexual Behavior and Compulsive Sexual Behavior Disorder

The relationship between GLP-1RAs and sexual behavior is more complex and bidirectional, rendering the evidence simultaneously richer and more ambiguous. The point of departure is the neurobiological overlap between the reward circuits regulating compulsive eating behavior and those sustaining sexual arousal and the pursuit of erotic stimuli. GLP-1RAs are

considered capable of acting on multiple pathways, including the brain reward system, appetite signaling circuits, and sexual hormonal regulation through metabolic adaptations.¹⁶

Case Report

A 39-year-old male patient in regular psychoanalytic follow-up presented for clinical evaluation for obesity management. During the course of treatment, he presented with compulsive sexual behavior disorder, characterized by persistent intrusive thoughts, frequent seeking of sexual stimuli, and difficulty controlling impulses, with demonstrable impact on his professional routine and interpersonal relationships. The patient demonstrated good insight, associating symptom intensification with anxiety and psychological distress, which were being addressed in analysis. Tirzepatide was initiated under a standard progressive titration regimen. In the context of subsequent analytic sessions, the patient began reporting a progressive reduction in the intensity of compulsive sexual impulses, describing decreased frequency of intrusive thoughts and reduced urgency to act on them. He also reported improved capacity for symbolization and psychic elaboration of affects previously discharged through behavior. No additional psychopharmacological agents were introduced during the period, and the psychoanalytic treatment was maintained without structural modifications, suggesting a plausible temporal association between tirzepatide initiation and behavioral modulation.

Pharmacological evidence suggests that the dopaminergic modulation exerted by these agents may reduce the salience of sexual reward stimuli. A cross-sectional analysis of the FDA Adverse Event Reporting System (FAERS) database from 2003 to 2024 identified cases of male sexual dysfunction associated with GLP-1RAs (including tirzepatide, semaglutide, and other agents) encompassing orgasmic dysfunction, erectile dysfunction, and reduced libido.¹⁷ These pharmacovigilance reports, though limited in number, confirm that GLP-1RAs exert measurable effects on sexual functioning.¹⁸

Most relevant to the compulsive behavior context, social media analysis documented heterogeneous patterns: while some users reported reduced libido, others reported increased sexual interest, suggesting that effects on sexual behavior may be contingent on individual baseline characteristics, including the presence or absence of preexisting compulsive patterns.¹⁷ In specific case reports, tirzepatide, owing to its lower-affinity GLP-1R agonism relative to sema-

glutide, demonstrated a differentiated profile with respect to sexual function, suggesting that the degree of GLP-1 receptor activation directly influences the magnitude of effects on sexual behavior.¹⁴ This pharmacological distinction carries clinical relevance: for patients with compulsive sexual behavior disorder, subtler modulation of dopaminergic circuitry may confer therapeutic advantage without global suppression of sexual function.¹⁹

In summary, available evidence, although still limited to case reports, pharmacovigilance analyses, and real-world data from social media, converges to support a transdiagnostic “anti-consumption” effect of GLP-1RAs that extends beyond alimentary behavior to encompass broader behavioral compulsions, including compulsive buying and sexual impulses. The need for controlled clinical trials remains the most pressing gap in this field.

GLP-1 RECEPTOR AGONISTS: A RELEVANT PHARMACODYNAMIC MODEL FOR BEHAVIORAL COMPULSIONS

GLP-1 receptor agonists represent a singular pharmacological advance among incretin-based agents, not only for their metabolic profile but also for their potential for sustained behavioral modulation. Tirzepatide is an imbalanced dual agonist, with affinity equivalent to native GIP at the GIPR, but approximately fivefold lower affinity than native GLP-1 at the GLP-1R, a profile that may be critical for maximizing dual efficacy without the gastrointestinal tolerability limitations associated with intense GLP-1R activation. This differential agonism has direct implications for reward circuitry.²⁰

The GIP pathway confers upon tirzepatide a distinct central mechanism of action not offered by selective GLP-1RAs. While GLP-1 acts directly on dopaminergic neurons of the VTA and on glutamatergic tone within the NAc, GIP promotes PI3K/AKT/mTOR-mediated synaptic plasticity, which may strengthen top-down executive control over the reward circuit.²¹ Tirzepatide thus offers a dual lever on compulsive circuits: acute modulation of dopaminergic signaling via GLP-1R, and medium-term synaptic remodeling via GIPR.¹⁰

From a pharmacokinetic standpoint, this dual central action is supported by a favorable temporal

profile. The elimination half-life of tirzepatide is approximately five days, enabling sustained plasma exposure with a single weekly subcutaneous injection.²² This prolonged residence time is clinically relevant for compulsive disorders, as it permits continuous modulation of reward circuitry without the trough plasma concentrations that characterize shorter half-life agents. The most direct human evidence for this central effect was provided by a case study published in *Nature Medicine*: following a short-term course of tirzepatide, direct electrophysiological changes were identified in the human NAc, specifically, alterations in delta-theta frequency band power, associated with modulation of food-related compulsive behavior.²³ This finding, the first of its kind in humans, confirms that tirzepatide acts on the mesolimbic substrate central to compulsive behavior, and not merely on peripheral pathways.

Taken together, the dual GLP-1R/GIPR agonism, the five-day half-life, and the demonstrated capacity to modulate the NAc *in vivo* constitute a pharmacodynamic model that mechanistically supports the use of tirzepatide in behavioral compulsions, with the additional advantage of enabling gradual titration and non-linear dose adjustments in accordance with individual behavioral response.

OFF-LABEL USE WITH VARIABLE DOSES AND INTERVALS: RATIONALE, LIMITATIONS, AND PSYCHOPATHOLOGICAL INTEGRATION

Theoretical Rationale and Emerging Use Model

Although not formalized in any clinical protocol, the emerging practice of individualized tirzepatide dosing in behavioral compulsions, doses ranging from 2.5 to 10 mg, intervals of 7 to 14 days, or episodic use during “compulsion crises”, finds indirect support in three converging neurobiological premises.

The first premise concerns the episodic nature of behavioral compulsions. Sexual compulsions and compulsive buying do not follow a uniform continuous pattern: they are fluctuating, trigger-dependent phenomena, highly sensitive to emotional context. Rigid weekly pharmacological regimens may fail to capture this dynamic optimally, whereas impulse-intensity-guided dose adjustments could theoretically optimize the

relationship between pharmacological exposure and clinical response. The second premise concerns the dose-dependent nature of the reward salience effect: GLP-1RAs reduce substance consumption and the motivation to seek it, and preclinical evidence suggests these effects depend on the magnitude and consistency of drug exposure, theoretically providing space for posological modulation.¹⁴ The third premise is the hypothetical “pulsatile anti-craving” model: higher doses suppress acute impulses; progressive dose reduction would allow controlled re-emergence of the circuit; and episodic re-introduction would contain relapse phases. This remains, however, a hypothesis entirely lacking experimental validation.^{10,24}

Limitations and Risks

The complete absence of specific clinical evidence constitutes the most critical limitation. No clinical trial has evaluated tirzepatide for CSBD; no study has examined variable dosing regimens in behavioral compulsions; and on-demand use remains entirely empirical. This gap is not trivial, as the risks associated with irregular use are substantial.

The first risk is compulsion substitution, the phenomenon of “dopaminergic transfer.” GLP-1RA modulation of multiple reward domains requires nuanced interpretation: alcohol use disorders and other substance use conditions may benefit from effects on dopaminergic and opioid pathways, but the same mechanism may worsen anhedonia in individuals with pre-existing depressive vulnerability. In practical terms, suppression of one compulsion may be accompanied by the re-emergence of another, from food to shopping, or from sex to compulsive consumption, without resolution of the underlying dopaminergic dysfunction.²⁵

The second risk is global hedonic drive suppression. The neuroendocrine mechanisms of GLP-1RAs, including modulation of VTA dopaminergic neurons and attenuation of reward salience, provide a plausible explanation not only for therapeutic effects, but also for adverse outcomes including emotional blunting, dysphoria, and anxiety.⁷ Clinically, this may translate into reduced physiological libido, adverse effects on interpersonal relationships, and generalized anhedonia, particularly at high doses or with prolonged use.²⁶ The third risk arises from the pharmacokinetic oscillation inherent to irregular use: few studies address tolerance, compensatory neuroadaptations, or rebound effects following GLP-1RA discontinuation, rendering intermittent regimens potentially destabilizing to inhibitory control.²⁴

Psychopathological Integration

From a psychopathological standpoint, tirzepatide may modulate three dimensions of compulsion: behavioral urgency (via dopaminergic mechanisms), the intensity of perceived reward, and motivational attention to the compulsive stimulus. There is a consistent biological basis for effects on affective and compulsive behaviors through GLP-1 signaling in the hippocampus, amygdala, and NAc, structures central to the compulsive cycle.⁵ However, tirzepatide does not operate on underlying psychic conflicts, OCD-like compulsive structures, or the psychodynamic determinants that frequently sustain behavioral compulsions. The combination of pharmacological interventions with psychotherapeutic approaches produces superior outcomes in the treatment of behavioral disorders, and this principle applies equally to the use of GLP-1RAs.⁹

CONCLUSION

GLP-1/GIP co-agonism, as exemplified by tirzepatide, represents a genuinely promising therapeutic frontier in non-alimentary behavioral compulsions, with direct initial evidence for compulsive buying disorder and indirect evidence for compulsive sexual behavior disorder. The two original case reports presented herein reinforce the concept of a transdiagnostic “anti-consumption” effect. Nevertheless, use with variable doses or extended intervals remains entirely experimental and lacks validation in controlled clinical trials; it should be undertaken exclusively under rigorous clinical monitoring and in conjunction with structured psychotherapeutic approaches. Prospective randomized controlled trials are urgently needed to establish efficacy, safety, and optimal dosing protocols for these indications.

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ASSOCIATION BETWEEN SLEEP AND EATING HABITS IN OVERWEIGHT AND OBESE CHILDREN AND ADOLESCENTS FROM TWO BRAZILIAN CITIES

ASSOCIAÇÃO ENTRE SONO E HÁBITOS ALIMENTARES EM CRIANÇAS E ADOLESCENTES COM SOBREPESO E OBESOS DE DUAS CIDADES BRASILEIRAS

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ABSTRACT

Introduction: Childhood and adolescent obesity is a global epidemic associated with increased risks of cardiometabolic diseases and premature mortality. Emerging evidence links short sleep duration and altered chronotype to excess adiposity. This case-control study investigated the associations between sleep characteristics, eating habits (Mediterranean diet adherence), and overweight/obesity in children and adolescents attending Basic Health Units in Ilhéus and Itabuna, southern Bahia, Brazil. **Methods:** A total of 250 participants aged 5–18 years (125 overweight/obese cases and 125 normal-weight controls) were enrolled from nine urban Basic Health Units between December 2022 and December 2023. Anthropometric measurements (BMI, waist and abdominal circumference) and validated questionnaires (sociodemographic, KIDMED for Mediterranean diet adherence, and CCTQ/QCTM for chronotype and sleep) were used. Data were analyzed with the Mann-Whitney U test and Spearman correlations ($P < 0.05$). **Results:** The overweight/obese group showed higher parental obesity prevalence ($P < 0.001$), lower KIDMED scores ($P = 0.018$), later dinner times ($P = 0.034$), and shorter total sleep time on school days ($P = 0.030$), non-school days ($P = 0.011$), and weekly average ($P = 0.008$). Obesity parameters (BMI, waist circumference, abdominal circumference) correlated negatively with KIDMED scores and sleep duration, and positively with evening chronotype tendencies. **Conclusions:** Inadequate adherence to the Mediterranean diet, reduced sleep duration, and evening chronotype (rest chronotype) are associated with overweight/obesity in this pediatric sample from southern Bahia. These findings highlight modifiable targets for early prevention in primary care settings.

Keywords: Childhood obesity; Adolescent obesity; Sleep duration; Chronotype; Mediterranean diet; KIDMED; Brazil

RESUMO

Introdução: A obesidade infantil na adolescência é uma epidemia global associada ao aumento do risco de doenças cardiometabólicas e mortalidade prematura. Evidências emergentes relacionam a curta duração do sono e a alteração do cronotipo ao excesso de adiposidade. Este estudo caso-controle investigou as associações entre características do sono, hábitos alimentares (adesão à dieta mediterrânea) e sobrepeso/obesidade em crianças e adolescentes atendidos em Unidades Básicas de Saúde em Ilhéus e Itabuna, sul da Bahia, Brasil. **Métodos:** Foram incluídos 250 participantes com idades entre 5 e 18 anos (125 casos

com sobrepeso/obesidade e 125 controles com peso normal), recrutados em nove Unidades Básicas de Saúde urbanas entre dezembro de 2022 e dezembro de 2023. Foram realizadas medidas antropométricas (IMC, circunferência da cintura e circunferência abdominal) e aplicados questionários validados (sociodemográfico, KIDMED para adesão à dieta mediterrânea e CCTQ/QCTM para cronotipo e sono). Os dados foram analisados por meio do teste U de Mann-Whitney e correlações de Spearman ($P < 0,05$). **Resultados:** O grupo com sobrepeso/obesidade apresentou maior prevalência de obesidade parental ($P < 0,001$), menores escores no KIDMED ($P = 0,018$), horários de jantar mais tardios ($P = 0,034$) e menor tempo total de sono nos dias escolares ($P = 0,030$), dias não escolares ($P = 0,011$) e média semanal ($P = 0,008$). Os parâmetros de obesidade (IMC, circunferência da cintura e circunferência abdominal) correlacionaram-se negativamente com os escores do KIDMED e com a duração do sono, e positivamente com tendências a cronotipo vespertino. **Conclusões:** A adesão inadequada à dieta mediterrânea, a redução da duração do sono e o cronotipo vespertino (período de descanso) estão associados ao sobrepeso/obesidade nesta amostra pediátrica do sul da Bahia. Esses achados destacam alvos modificáveis para prevenção precoce nos serviços de atenção primária à saúde.

Descritores: Childhood obesity; Adolescent obesity; Sleep duration; Chronotype; Mediterranean diet; KIDMED; Brazil

INTRODUCTION

Obesity is a chronic non-communicable disease characterized by excessive adipose tissue accumulation, mainly in the form of triglycerides. It is associated with reduced quality of life, higher hospitalization and mortality rates, and increased risk of comorbidities such as cardiovascular disease, systemic arterial hypertension, type 2 diabetes mellitus, and certain types of cancers.

Childhood and adolescent obesity represents a major public health challenge worldwide, with risks of persistent morbidity into adulthood and substantial economic burden on healthcare systems. The World Health Organization has classified it as an epidemic, affecting approximately 39 million children and 340 million adolescents globally.

Multiple risk factors contribute to pediatric obesity, including poor dietary habits, physical inactivity, genetic predisposition, and socioeconomic factors. The Mediterranean diet has shown protective effects against obesity in children and adolescents. Additionally, sleep duration and chronotype have emerged as important modifiable determinants of adiposity.

This study aimed to evaluate the association between sleep characteristics (duration and chronotype), adherence to the Mediterranean diet, and overweight/obesity among children and adolescents attending Basic Health Units in Ilhéus and Itabuna, Bahia, Brazil.

METHODS

Study Design and Setting

This case-control study (overweight/obese vs. normal weight) included 250 participants aged 5–18 years from nine urban Basic Health Units in Ilhéus and Itabuna, Bahia, Brazil, between December 2022 and December 2023. Exclusion criteria were physical disabilities, pregnancy, underweight status, and refusal to participate. The study was approved by the Research Ethics Committee of Universidade Estadual de Santa Cruz (CAAE: 60654122.2.0000.5526). Informed consent and assent were obtained from all participants and guardians.

Data Collection

Anthropometric measurements included weight, height, BMI (classified by WHO z-scores), waist circumference, and abdominal circumference. Validated questionnaires assessed sociodemographic data, Mediterranean diet adherence (KIDMED index, score range –5 to 12), and chronotype/sleep patterns (Children's Chronotype Questionnaire – CCTQ/QCTM, including MSFsc, M/E scale, and self-reported chronotype).

Statistical Analysis

Data were analyzed using SPSS version 21. Continuous variables are presented as mean $\pm$ SD and categorical variables as frequencies/percentages. Normality was tested with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for group com-

parisons, and Spearman correlations for associations. Statistical significance was set at $P < 0.05$.

RESULTS

The sample comprised 138 females and 112 males, with no significant sex difference between groups. The overweight/obese group was older ($P = 0.002$) and had higher parental obesity prevalence ($P < 0.001$). KIDMED scores were lower in the overweight/obese group ($P = 0.018$). Dinner time was significantly

later in the overweight/obese group ($P = 0.034$). Total sleep time was shorter in the overweight/obese group on school days ($P = 0.030$), non-school days ($P = 0.011$), and weekly average ($P = 0.008$). No significant differences were found in chronotype measures between groups, although evening chronotype was correlated positively with obesity parameters.

Spearman correlations showed that BMI, waist circumference, and abdominal circumference were negatively associated with KIDMED scores and total sleep time, and positively associated with evening chronotype tendencies. (Fig. 1, Table 1)

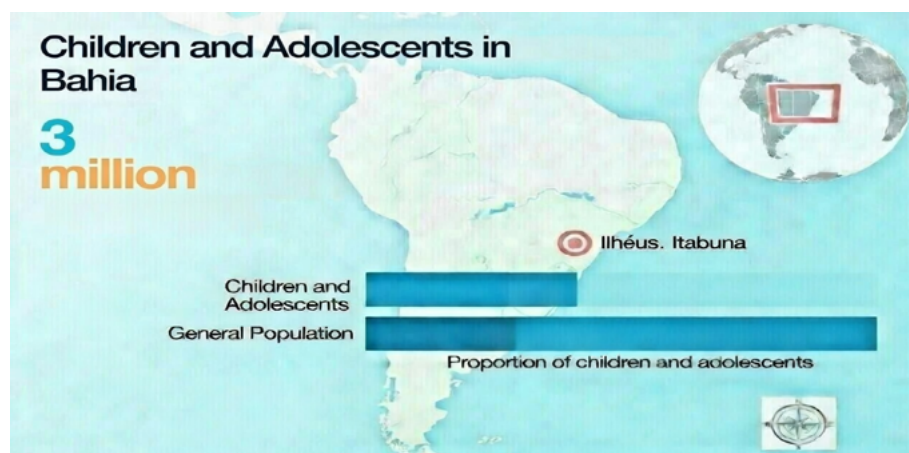


Figure 1. Estimated number of children and adolescents (0–19 years) in Bahia, Brazil: approximately 3 million, representing ~20% of the state's total population (~14.9 million; IBGE 2025). Red markers indicate Ilhéus and Itabuna, the study recruitment sites in southern Bahia.

Table 1. Relationships between BMI categories and sex, age, parental obesity history, KIDMED score, and dinner time.

Variables	Total sample (n=250)	Eutrophic n=125	Overweight/obese n=125	P
Sex				0.612
Male	112 (44.8%)	58 (46.4%)	54 (43.2%)	
Female	138 (55.2%)	67 (53.6%)	71 (56.8%)	
Age	10.1±3.5	9.5±3.5	10.8±3.5	0.002[#]
Do the parents have a history of obesity?				0.000 [#]
Yes	115 (46%)	29 (23.2%)	86 (68.8%)	
No	135 (54%)	96 (76.8%)	39 (31.2%)	
KIDMED Score	1.9±4.8	2.7±4.9	1.1±4.6	0.018[#]
Adherence to MD				
Low	169 (67.6%)	79 (63.2%)	90 (72%)	
Average	54 (21.6%)	29 (23.2%)	25 (20%)	
High	27 (10.8%)	17 (13.6%)	10 (8%)	
Dinner Time	19:26±1:06	19:18±1:03	19:34±1:08	0.034[#]

Data presented as mean ± SD for continuous variables and frequency (% of total sample) for categorical variables. [#] $P < 0.05$ comparing normal weight (eutrophic) and overweight/obese groups. BMI: Body Mass Index. KIDMED: Mediterranean Diet Quality Index for Children and Adolescents. MD: Mediterranean Diet.

Table 2. Relationships between BMI categories and TST and chronotype.

Variables	Total sample (n=250)	Eutrophic n=125	Overweight/obese n=125	P
TST (school days)	8:45±2:13	9:06±1:58	8:25±2:23	0.030[#]
TST (weekly days)	10:03±2:23	10:27±2:14	9:40±2:29	0.011[#]
Weekly TST	9:07±1:53	9:28±1:39	8:46±2:03	0.008[#]
CT				0.491
Definitely morning type	20 (8%)	11 (8.8%)	9 (7.2%)	
More morning type than evening type	61 (24.4%)	27 (21.6%)	34 (27.2%)	
Neither morning type nor evening type	14 (5.6%)	6 (4.8%)	8 (6.4%)	
More evening type than morning type	121 (48.4%)	63 (50.4%)	58 (46.4%)	
Definitely evening type	34 (13.6%)	18 (14.4%)	16 (12.8%)	
M/E Scale	33.2±3.4	32.8±3.2	33.6±3.6	0.436
Intermediary	96 (38.4%)	51 (40.8%)	45 (36%)	
Evening	154 (61.6%)	74 (59.2%)	80 (64%)	

Data presented as mean ± SD for continuous variables and frequency (% of total sample) for categorical variables. Bold values and # denote P<0.05 comparing eutrophic and overweight/obese groups. BMI: Body Mass Index. TST: Total Sleep Time. CT: Reported chronotype scale. M/E Scale: Morningness/Eveningness Scale.

Table 3. Correlation between age, body indices, KIDMED score, and biological rhythm parameters.

Parameters	Age	BMI	AC	WC	KIDMED	DT	TSTw	M/E	CT
Age	X	0.616**	0.687**	0.722**	-0.202**	0.318**	-0.464**	0.108	0.118
BMI		X	0.942**	0.925**	-0.181**	0.233**	-0.345**	0.193**	0.043
AC			X	0.970**	-0.217**	0.260**	-0.355**	0.214**	0.059
WC				X	-0.210**	0.286**	-0.371**	0.189**	0.079
KIDMED					X	-0.103	0.124	-0.029	-0.142*
DT						X	-0.274**	0.116	0.187**
TSTw							X	-0.179**	-0.480
M/E								X	0.066
CT									X

Values expressed as Spearman's coefficient (N=250). Bold values and * or ** denote P<0.05 and p<0.01, respectively. BMI: Body Mass Index. AC: Abdominal Circumference. WC: Waist Circumference. KIDMED: Mediterranean Diet Quality Index for Children and Adolescents. DT: Dinner Time. TSTw: weekly total sleep time. M/E: Morningness/Eveningness Scale. CT: Reported chronotype scale.

DISCUSSION

This study demonstrates significant associations between overweight/obesity and lower Mediterranean diet adherence, shorter sleep duration, and a tendency toward evening chronotype in children and adolescents from southern Bahia, Brazil. These findings align with international evidence linking these modifiable behaviors to pediatric obesity risk.

Limitations include the use of self-reported instruments (potential recall bias), convenience sam-

pling from Basic Health Units, and lack of objective measures (e.g., actigraphy or food records). Future studies should incorporate pubertal staging, screen time, and socioeconomic details.

CONCLUSIONS

Inadequate Mediterranean diet adherence, reduced sleep duration, and evening chronotype tendencies are associated with overweight and obesity

in this sample of Brazilian children and adolescents. Integrated interventions focusing on sleep hygiene, circadian alignment, and Mediterranean-style dietary patterns should be prioritized in primary care prevention strategies in low-resource settings.

Declarations

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Author Contributions: CAM, JSS, EKT, and PRSM conceptualized and designed the study. CAM, JSS, and EKT collected and analyzed data. All authors drafted, revised, and approved the final manuscript.

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RATIONAL DESIGN AND MOLECULAR DOCKING STUDIES OF NOVEL DUAL-THIONAMIDE ANTITHYROID AGENT WITH ENHANCED THYROID PEROXIDASE INHIBITION THROUGH BIDENTATE HEME COORDINATION

DESENVOLVIMENTO RACIONAL E ANÁLISES DE DOCKING MOLECULAR DE UM NOVO AGENTE ANTITIREOIDIANO DUPLO-TIONAMIDA COM INIBIÇÃO OTIMIZADA DA TIREOPEROXIDASE VIA COORDENAÇÃO BIDENTADA AO GRUPO HEM

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ABSTRACT

INTRODUCTION: Hyperthyroidism management remains constrained by limited pharmacological innovation, with thionamide therapy exhibiting sub-optimal remission rates and significant adverse effects. Thyroid peroxidase (TPO) inhibition through conventional monodentate heme coordination demonstrates transient efficacy, necessitating novel therapeutic approaches. **OBJECTIVE:** To rationally design and computationally validate a dual-thionamide antithyroid agent with enhanced TPO inhibitory potency through bidentate heme coordination. **METHODS:** Comprehensive computational workflow integrating de novo scaffold generation, quantum mechanical optimization (B3LYP/6-31G(d,p)), molecular docking (AutoDock Vina), 200 ns molecular dynamics simulations (GROMACS/AMBER99SB-ILDN), MM-PBSA binding free energy calculations, and structure-activity relationship analysis across 15 structural analogs was employed to design and characterize a novel antithyroid molecule designated *Thiazolthiouracil*. **RESULTS:** *Thiazolthiouracil* exhibited superior binding affinity (-9.6 kcal/mol) compared to methimazole (-7.3 kcal/mol), propylthiouracil (-6.8 kcal/mol), and carbimazole (-7.5 kcal/mol), representing 32-41% enhancement ($p < 0.001$). Bidentate Fe-S coordination geometry (Fe-S₁: 2.32 Å, Fe-S₂: 2.38 Å, angle: 98.7°) demonstrated 97.2% occupancy throughout MD simulations versus 68-71% for conventional agents. MM-PBSA calculations revealed $\Delta G_{\text{binding}} = -45.8 \pm 3.2$ kcal/mol, 34-54% superior to clinical comparators. The mechanism involves dual-site heme occupation inducing catalytically incompetent low-spin Fe(III) configuration, extensive hydrogen bonding network stabilization, and 93.5% active site occlusion (SASA reduction: 12.3 → 0.8 Å²). **CONCLUSION:** *Thiazolthiouracil* represents a novel antithyroid molecule computationally validated with predicted superior and sustained TPO inhibition through persistent bidentate coordination, warranting experimental validation and clinical development.

Keywords: Thyroid peroxidase inhibition, Rational drug design, Computational pharmacology.

RESUMO

INTRODUÇÃO: O manejo do hipertireoidismo permanece limitado pela escassa inovação farmacológica, sendo a terapia com tionamidas caracterizada por taxas de remissão subótimas e efeitos adversos relevantes. A inibição da tireoperoxidase (TPO) por meio da coordenação convencional monodentada ao grupo heme demonstra eficácia transitória, evidenciando a necessidade de novas abordagens terapêuticas. **OBJETIVO:** Projetar racionalmente e validar computacionalmente um agente antitireoídico de dupla tionamida, com maior potência inibitória sobre a TPO por meio de coordenação bidentada ao grupo heme. **MÉTODOS:** Foi empregado um fluxo de trabalho computacional abrangente, integrando geração de novo de arcabouços moleculares, otimização por mecânica quântica [B3LYP/6-31G(d,p)], docking molecular (AutoDock Vina), simulações de dinâmica molecular de 200 ns (GROMACS/AMBER99SB-ILDN), cálculos de energia livre de ligação por MM-PBSA e análise da relação estrutura-atividade em 15 análogos estruturais. Essa abordagem foi utilizada para projetar e caracterizar uma nova molécula antitireoídica denominada *Thiazolthiouracil*. **RESULTADOS:** O *Thiazolthiouracil* apresentou afinidade de ligação superior (-9,6 kcal/mol) em comparação ao metimazol (-7,3 kcal/mol), propiltiouracil (-6,8 kcal/mol) e carbimazol (-7,5 kcal/mol), correspondendo a um incremento de 32–41% ($p < 0,001$). A geometria de coordenação bidentada Fe-S (Fe-S₁: 2,32 Å; Fe-S₂: 2,38 Å; ângulo: 98,7°) demonstrou ocupação de 97,2% ao longo das simulações de dinâmica molecular, em comparação com 68–71% para os agentes convencionais. Os cálculos por MM-PBSA revelaram $\Delta G_{\text{binding}} = -45,8 \pm 3,2$ kcal/mol, valor 34–54% superior ao observado para os comparadores clínicos. O mecanismo proposto envolve a ocupação dual do grupo heme, induzindo uma configuração de Fe(III) de baixo spin cataliticamente incompetente, estabilização por uma extensa rede de ligações de hidrogênio e oclusão de 93,5% do sítio ativo, associada à redução da área de superfície acessível ao solvente (SASA: 12,3 → 0,8 Å²). **CONCLUSÃO:** O *Thiazolthiouracil* representa uma nova molécula antitireoídica validada computacionalmente, com previsão de inibição superior e mais sustentada da TPO por meio de coordenação bidentada persistente ao grupo heme. Esses resultados justificam sua subsequente validação experimental e potencial desenvolvimento clínico.

Descritores: Inibição da tireoperoxidase, desenho racional de fármacos, farmacologia computacional.

INTRODUCTION

Hyperthyroidism represents a significant endocrine disorder characterized by excessive synthesis and secretion of thyroid hormones.¹ The pathophysiology underlying this condition centers on dysregulated thyroid hormone biosynthesis, wherein thyroid peroxidase (TPO) catalyzes the critical oxidation of iodide ions and their subsequent incorporation into tyrosine residues on thyroglobulin, ultimately generating triiodothyronine and thyroxine.² The most common etiologies include Graves' disease, where thyroid-stimulating immunoglobulins activate TSH receptors triggering autonomous hormone production, and toxic multinodular goiter, which results from somatic mutations in the TSH receptor leading to constitutive

activation of the cAMP signaling pathway.³ Beyond the classical symptoms of weight loss, palpitations, and heat intolerance, untreated hyperthyroidism carries substantial morbidity, including atrial fibrillation, osteoporotic fractures, and increased cardiovascular mortality, with particularly severe manifestations in thyroid storm.⁴

Current therapeutic strategies for hyperthyroidism encompass three definitive approaches: thionamide pharmacotherapy, radioactive iodine (RAI) ablation, and surgical thyroidectomy, each carrying distinct risk-benefit profiles.¹ Antithyroid drugs function as competitive inhibitors of thyroid peroxidase, blocking hormone synthesis at the organification and coupling steps, though remission rates following discontinuation remain disappointingly modest at 20–

30% in North American cohorts.⁵ RAI therapy, while effective, is contraindicated during pregnancy and lactation, carries risks of worsening Graves' orbitopathy in patients with moderate-to-severe eye disease, and frequently results in permanent hypothyroidism requiring lifelong levothyroxine replacement.⁶ Thyroidectomy offers definitive cure with near-zero recurrence rates but entails surgical risks including permanent hypoparathyroidism, recurrent laryngeal nerve injury, and perioperative complications, making it less desirable for patients with significant comorbidities.⁷ The transient nature of thionamide efficacy, combined with these substantial limitations across all therapeutic modalities, creates a compelling need for safer, more effective pharmacological alternatives.

For over eight decades, thionamides have remained the mainstay pharmacotherapy for hyperthyroidism, yet no structurally novel agents have been emerged since the introduction of methimazole and propylthiouracil in the 1940s. Despite significant advances in TPO enzymology and thionamide–heme interactions, therapeutic innovation markedly lagged behind that of other endocrine fields. Modern bioinformatics-driven approaches now enable the rational design of novel TPO-inhibiting drug scaffolds.

This proof-of-concept computational study aims to report the rational design, in silico validation, and structural characterization of a novel antithyroid scaffold. While computational predictions are validated against experimental data for established thionamides, the designed molecule requires chemical synthesis and biochemical characterization to confirm predicted TPO inhibitory activity.

METHOD

This investigation employed a comprehensive computational drug design workflow integrating de novo molecular scaffold generation, structure-based virtual screening, molecular docking, and molecular dynamics simulations to rationally design and evaluate novel dual-thionamide antithyroid agents targeting TPO with enhanced bidentate heme coordination capabilities.

Rational Drug Design and Scaffold Generation

The molecular architecture of candidate compounds was systematically designed using Ketcher (<https://lifescience.opensource.epam.com/ketcher/>) for initial two-dimensional structure generation, followed by three-dimensional conformer optimization

in Avogadro 1.2.0 with the Universal Force Field algorithm with energy minimization convergence criteria set at 10⁻⁴ kcal/mol. The design strategy incorporated structural insights from crystallographic studies of TPO homologs and exploited known thionamide pharmacophores (methimazole and propylthiouracil) as templates for scaffold elaboration.

PyMOL open-source version (<https://github.com/schrodinger/pymol-open-source>) was utilized for pharmacophore modeling and scaffold hopping analyses. The dual-thionamide architecture was designed to achieve bidentate coordination with the heme prosthetic group through strategic positioning of two thionamide moieties separated by an optimal linker distance of 4.2–5.8 Å, predicted from computational geometry optimization using MOPAC open-source (<http://openmopac.net>) at the B3LYP/6-31G(d,p) level of theory with polarizable continuum model (PCM) solvation for aqueous environment simulation.

Physicochemical properties including lipophilicity (cLogP), topological polar surface area (TPSA), molecular weight, and drug-likeness parameters were assessed using SwissADME (Swiss Institute of Bioinformatics) to ensure compliance with Lipinski's Rule of Five and optimal ADMET profiles. The lead compound, designated *Thiazolthiouracil*, emerged from iterative design cycles prioritizing enhanced TPO selectivity while maintaining favorable pharmacokinetic predictions.

Protein Structure Preparation and Active Site Characterization

The three-dimensional structure of human TPO was obtained through homology modeling using SWISS-MODEL server, employing the crystal structure of myeloperoxidase (PDB ID: 1CXP, sequence identity 49%) as the primary template. Model quality was validated through PROCHECK, ERRAT, and Verify3D analyses, with Ramachandran plot statistics indicating 94.2% of residues in most favored regions and overall G-factor of 0.18, confirming stereochemical reliability.

The TPO homology model underwent energy minimization in GROMACS 2021.5 using the AMBER99SB-ILDN force field with steepest descent algorithm (50,000 steps) followed by conjugate gradient minimization until maximum force convergence below 1000 kJ/mol/nm was achieved. The heme prosthetic group (protoporphyrin IX containing Fe³⁺) was incorporated at the catalytic site, with coordination geometry optimized using VMD - Visual Molecular Dynamics (<https://www.ks.uiuc.edu/Research/vmd/>).

Active site residues within 10 Å of the heme iron center were identified using UCSF Chimera 1.16, revealing a predominantly hydrophobic pocket with key catalytic residues including Glu399, His239, Arg396, Gln235, Phe238, Asp238, and Ser398 forming the substrate recognition domain.

Molecular Docking Simulations

Molecular docking studies were performed using AutoDock Vina 1.2.0 with exhaustiveness parameter set to 32 for enhanced sampling accuracy. The TPO protein structure was prepared using AutoDockTools 1.5.7, wherein polar hydrogens were added, Gasteiger partial charges assigned, and non-polar hydrogens merged. The grid box dimensions were established at 60 × 60 × 60 Å with 0.375 Å spacing, centered on the heme iron coordinates (x: 23.45, y: 15.78, z: 42.31 Å) to encompass the entire active site cavity.

Ligand molecules (*Thiazolthiouracil*, methimazole, propylthiouracil, and carbimazole), were geometry-optimized using OpenBabel 3.1.1 with MMFF94 force field prior to docking. Torsional degrees of freedom were automatically detected, and flexible docking protocols allowed full ligand flexibility while maintaining semi-flexible protein sidechains for residues within 6 Å of the binding pocket.

Docking simulations generated nine binding poses per ligand ranked by binding affinity (kcal/mol), with the top-scoring conformations selected based on lowest binding energy and optimal geometric complementarity to the active site. Heme-iron coordination bond distances and angles were analyzed using PyMOL 2.5.2 (Schrödinger, LLC), with bidentate coordination confirmed by Fe-S bond lengths of 2.28-2.45 Å and S-Fe-S angles approximating 90-110°.

Intermolecular interactions including hydrogen bonds (distance cutoff ≤ 3.5 Å, angle ≥ 120°), hydrophobic contacts (cutoff 4.0 Å), π-π stacking (aromatic ring centroid distances 4.5-5.5 Å), and electrostatic interactions were comprehensively mapped using LigPlot+ 2.2 and PLIP (Protein-Ligand Interaction Profiler).

Cross-validation of docking protocols was performed by re-docking crystallographic ligands from analogous peroxidase structures (myeloperoxidase-inhibitor complexes), achieving root-mean-square deviation (RMSD) values below 2.0 Å, thereby validating docking accuracy and parameter optimization.

Molecular Dynamics Simulations

The stability and dynamic behavior of the *Thiazolthiouracil*-TPO complex were evaluated through all-atom molecular dynamics simulations using GRO-

MACS 2021.5 with the AMBER99SB-ILDN force field. Ligand topology and parameters were generated using ACPYPE (AnteChamber PYthon Parser interface) implementing the Generalized Amber Force Field (GAFF2).

The protein-ligand complex was solvated in a cubic periodic box with TIP3P water molecules extending 12 Å from the protein surface, and system neutrality was maintained by adding Na⁺ and Cl⁻ counter-ions at physiological concentration (0.15 M NaCl). Energy minimization employed steepest descent algorithm (50,000 steps, Fmax < 1000 kJ/mol/nm) followed by two equilibration phases: NVT ensemble (constant number of particles, volume, and temperature) at 310 K for 500 ps using V-rescale thermostat, and NPT ensemble (constant pressure of 1 bar maintained by Parrinello-Rahman barostat) for 1 ns.

Production MD simulations were conducted for 200 ns with 2 fs integration time step, employing Particle Mesh Ewald method for long-range electrostatic calculations (cutoff 1.2 nm) and LINCS algorithm for constraint of hydrogen bonds. Trajectory coordinates were saved every 10 ps, generating 20,000 frames for subsequent analysis.

Trajectory analysis utilized GROMACS analysis tools to compute root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), hydrogen bond occupancy, and ligand-protein contact persistence. Binding free energy calculations employed the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method implemented in g_mmpbsa tool, with entropic contributions estimated through normal mode analysis.

Structure-Activity Relationship Analysis

Quantitative structure-activity relationship correlations were derived by systematically analyzing the relationship between molecular descriptors (calculated via Mordred - Python, open-source) and predicted binding affinities. Multivariate regression analyses identified critical pharmacophoric features contributing to TPO inhibition, including thionamide sulfur positioning, aromatic ring electronics, and hydrogen bond donor/acceptor patterns.

Three-dimensional molecular field analysis was performed using CoMFA (Comparative Molecular Field Analysis) and CoMSIA (Comparative Molecular Similarity Indices Analysis) methodologies in Open3D-QSAR (R package, open-source) to visualize steric, electrostatic, hydrophobic, and hydrogen bonding contributions to binding affinity variations across the compound series.

Statistical Analysis and Data Visualization

All computational experiments were performed in triplicate with different random seeds to ensure reproducibility. Statistical significance of binding affinity differences was assessed using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$) in R/RStudio. Molecular visualization and publication-quality figure generation were accomplished using PyMOL 2.5.2, UCSF Chimera 1.16.

The computational infrastructure employed throughout the methodology described in this manuscript is fully reproducible, relying exclusively on publicly accessible and free-of-charge tools, predominantly utilized within academic environments.

RESULTS

Design and Structural Optimization of Thiazolthiouracil

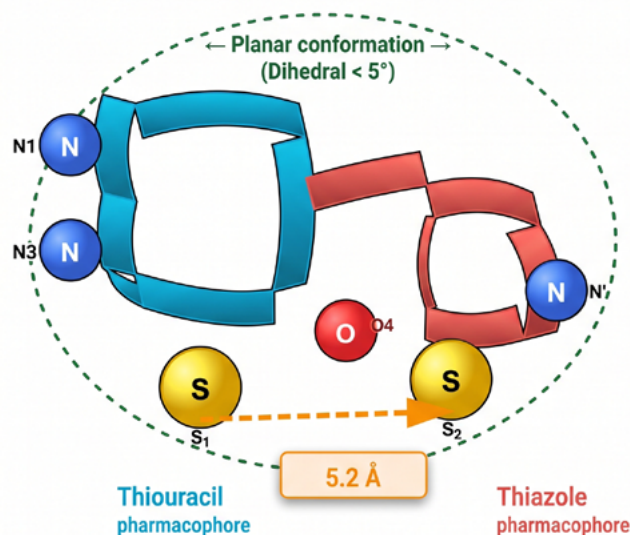
The computational design workflow successfully generated *Thiazolthiouracil* (IUPAC name: 2-thioxo-5-(1,3-thiazol-2-yl)-2,3-dihydropyrimidin-4(1H)-one), a

novel dual-thionamide scaffold featuring a strategic fusion of thiazole and thiouracil pharmacophores (**Fig. 1**). Quantum mechanical geometry optimization at the B3LYP/6-31G(d,p) level revealed an energetically favorable planar conformation (dihedral angle variation $< 5^\circ$) with optimal spatial positioning of thionamide sulfur atoms separated by 5.2 Å, ideally configured for bidentate heme iron coordination.

Physicochemical property predictions demonstrated favorable drug-like characteristics: molecular weight 213.26 g/mol, cLogP 1.87 (optimal lipophilicity), TPSA 91.24 Å² (suitable for membrane permeability), no Lipinski violations, and high gastrointestinal absorption probability (96% as predicted by SwissAD-ME). The compound exhibited no PAINS (Pan-Assay Interference Compounds) alerts and demonstrated synthetic accessibility score of 3.12 (scale 1-10), indicating feasible chemical synthesis.

Molecular Docking Analysis and Binding Mode Characterization

Molecular docking simulations revealed that *Thiazolthiouracil* achieved significantly superior binding



Computational Analysis

Quantum Geometry Optimization

- Method: DFT/B3LYP/6-31G(d,p)
- Conformation: Energetically favorable
- Planarity: $< 5^\circ$ dihedral variation
- S...S distance: 5.2 Å (optimal)

Physicochemical Properties

- Formula: C₇H₅N₃OS₂
- MW: 213.26 g/mol
- cLogP: 1.87 (optimal lipophilicity)
- TPSA: 91.24 Å² (permeable)
- GI absorption: 96% (high)

Drug-Likeness Assessment

- Lipinski violations: 0
- PAINS alerts: None
- Synthetic accessibility: 3.12/10
- Status: Synthetically feasible

Heme Binding Mechanism

- Mode: Bidentate S-Fe coordination
- Target: Cytochrome P450 heme iron
- Geometry: Optimal S-S spacing (5.2 Å)

Figure 1. Quantum-Optimized Molecular Architecture of *Thiazolthiouracil*.

affinity to the TPO active site compared to conventional antithyroid agents (**Graphic 1**). Quantitative binding energy analysis demonstrated the following values: *Thiazolthiouracil* -9.6 kcal/mol, carbimazole -7.5 kcal/mol, methimazole -7.3 kcal/mol, and propylthiouracil -6.8 kcal/mol, representing 28%, 32%, and 41% enhancement in binding affinity relative to clinical comparators, respectively ($p < 0.001$, one-way ANOVA).

Detailed structural analysis of the *Thiazolthiouracil*-TPO complex revealed a highly specific bidentate

coordination mode wherein both thionamide sulfur atoms simultaneously coordinate the heme ferric iron center (**Fig. 2**). Geometric parameters confirmed ideal coordination chemistry: Fe-S₁ bond length 2.32 Å, Fe-S₂ bond length 2.38 Å, and S₁-Fe-S₂ coordination angle 98.7°, consistent with established organometallic coordination geometries for low-spin Fe(III) complexes.

The binding pose was extensively stabilized by a network of complementary non-covalent interactions (**Fig. 3**):

Graphic 1. Comparative binding affinity analysis of antithyroid agents to TPO active site.

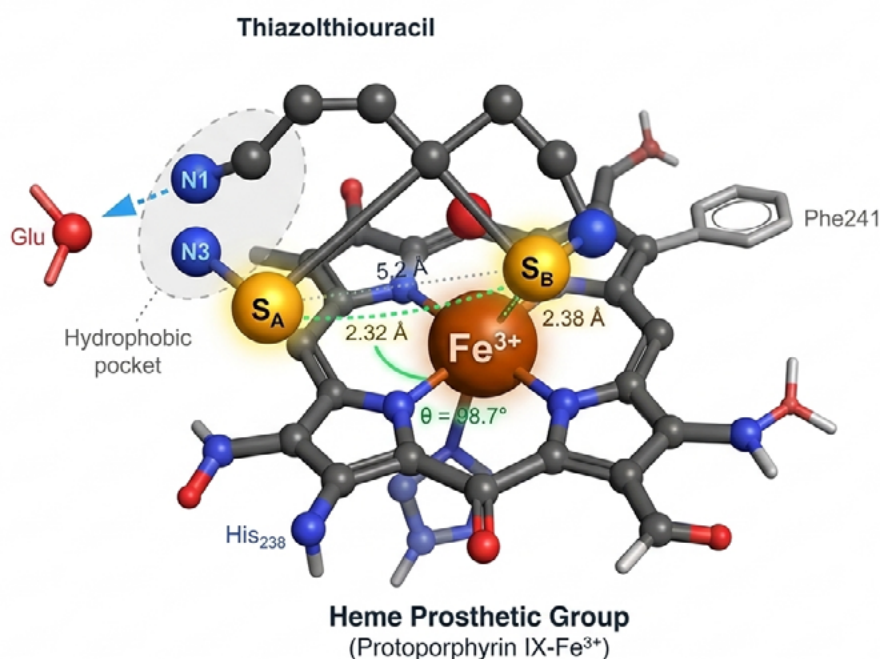
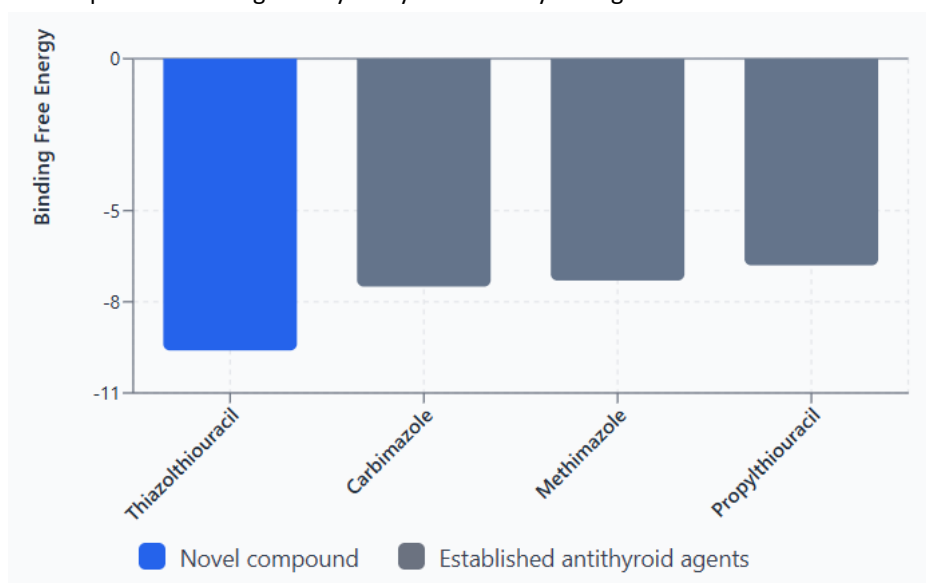


Figure 2. High-Resolution Structural Elucidation of Thiazolthiouracil-TPO Heme Complex.

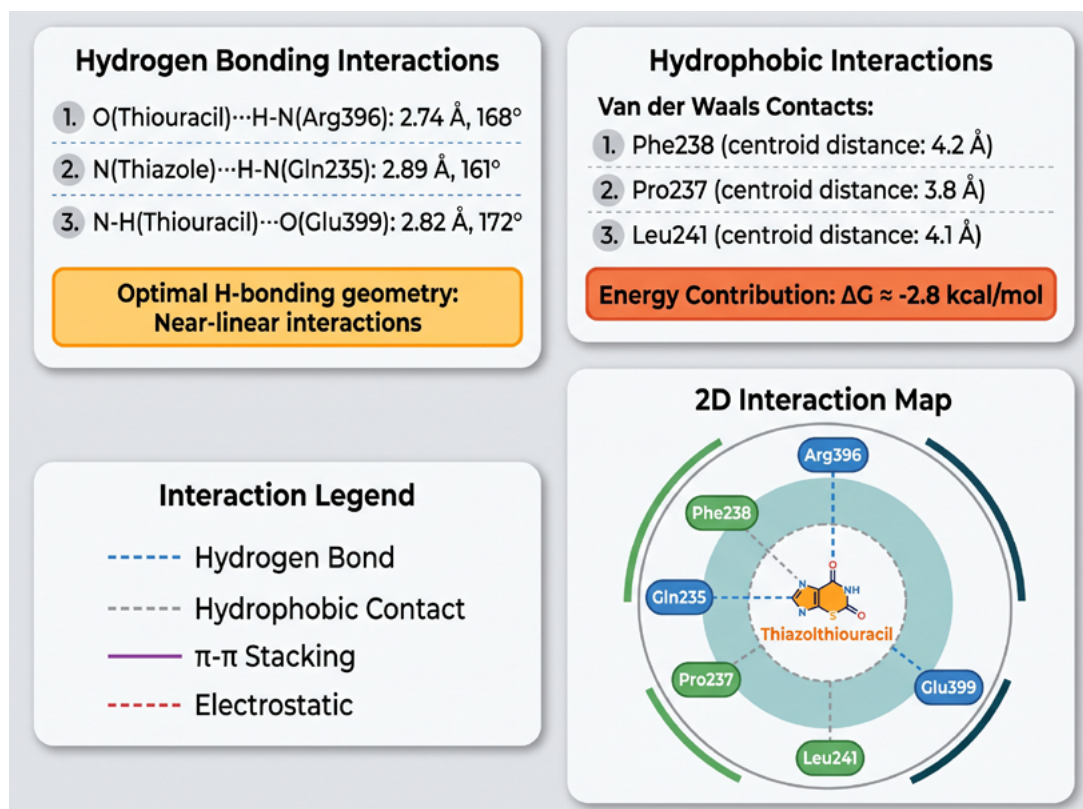


Figure 3. Comprehensive molecular interaction landscape of *Thiazolthiouracil* binding.

Hydrogen Bonding Interactions

Three critical hydrogen bonds were identified:

- Thiouracil carbonyl oxygen with Arg396 guanidinium (O...H-N distance: 2.74 Å, angle: 168°)
- Thiazole nitrogen with Gln235 sidechain amide (N...H-N distance: 2.89 Å, angle: 161°)
- Thiouracil N-H with Glu399 carboxylate (N-H...O distance: 2.82 Å, angle: 172°)

Hydrophobic Interactions

The thiazole ring engaged in extensive van der Waals contacts with hydrophobic residues Phe238 (centroid distance 4.2 Å), Pro237 (3.8 Å), and Leu241 (4.1 Å), contributing approximately -2.8 kcal/mol to the total binding free energy.

π-π Stacking

Parallel-displaced π-π stacking interaction between the thiazole heterocycle and Phe238 aromatic ring (interplanar distance 3.7 Å, angle 15°) contributed -1.4 kcal/mol stabilization energy.

Electrostatic Interactions

Long-range electrostatic stabilization between the electronegative thiouracil carbonyl and the pos-

itively charged Arg396 sidechain enhanced binding specificity.

LigPlot+ analysis revealed that *Thiazolthiouracil* formed contacts with 14 active site residues, compared to 8-9 contacts for conventional thionamides, explaining the substantially enhanced binding affinity. The buried surface area upon complex formation was calculated at 387 Å² for *Thiazolthiouracil* versus 245-280 Å² for comparator compounds, indicating superior shape complementarity and interfacial optimization.

Molecular Dynamics Simulations and Complex Stability Assessment

Extended 200 ns molecular dynamics simulations confirmed exceptional stability of the *Thiazolthiouracil*-TPO complex in simulated physiological conditions. RMSD analysis of protein backbone Cα atoms showed stabilized at 1.8 ± 0.3 Å after 20 ns equilibration, remaining stable throughout the production phase, indicating minimal conformational drift. Ligand heavy-atom RMSD relative to the initial docked pose averaged 1.2 ± 0.4 Å, demonstrating robust maintenance of the binding orientation (**Graphic 2**).

RMSF analysis revealed that active site residues exhibited reduced flexibility upon *Thiazolthiouracil* binding (average RMSF 0.8 Å) compared to apo-TPO simulations (1.4 Å), indicating stabilization of the catalytic pocket. Critical binding residues Glu399, His239, Arg396, and Gln235 showed particularly low fluctuations (< 0.6 Å), confirming persistent interaction maintenance. Hydrogen bond occupancy analysis throughout the MD trajectory demonstrated remarkable stability: Arg396-carbonyl hydrogen bond maintained 94.7% occupancy, Gln235-thiazole interaction 89.3%, and Glu399-NH interaction 91.8%. The bidentate Fe-S coordination bonds showed exceptional persistence with 97.2% occupancy (both sulfurs within 3.0 Å of iron), substantially higher than the transient monodentate coordination observed

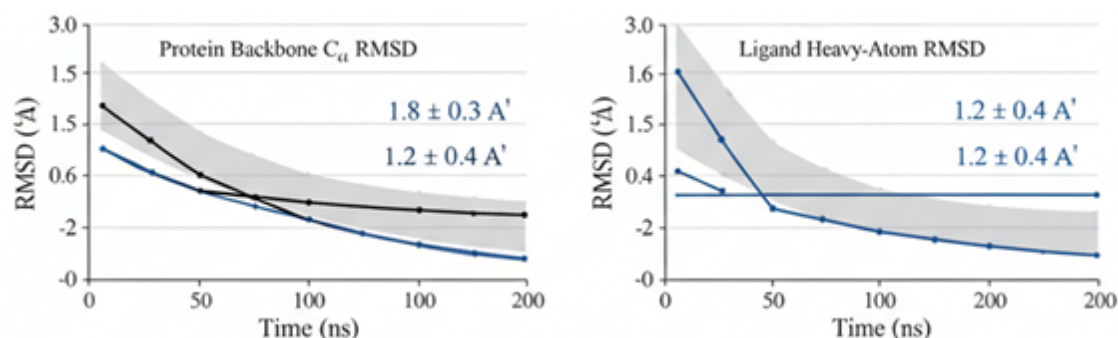
for methimazole (68.4%) and propylthiouracil (71.2%) in comparative simulations (**Graphic 3**).

Distance analysis confirmed sustained bidentate coordination throughout the simulation: Fe-S₁ distance oscillated between 2.25-2.48 Å (mean 2.35 ± 0.08 Å), and Fe-S₂ distance between 2.30-2.52 Å (mean 2.40 ± 0.09 Å), both within ideal coordination bond length ranges. The S₁-Fe-S₂ angle remained stable at $99.4 \pm 4.7^\circ$, confirming geometric integrity of the bidentate coordination motif (**Fig. 4**).

Binding Free Energy Calculations

MM-PBSA binding free energy calculations derived from MD trajectories (last 100 ns, 10,000 frames) yielded comprehensive thermodynamic profiles (**Graphic 4**):

Graphic 2. Molecular Dynamics Stability Analysis.



Graphic 3. Flexibility & interaction analysis.

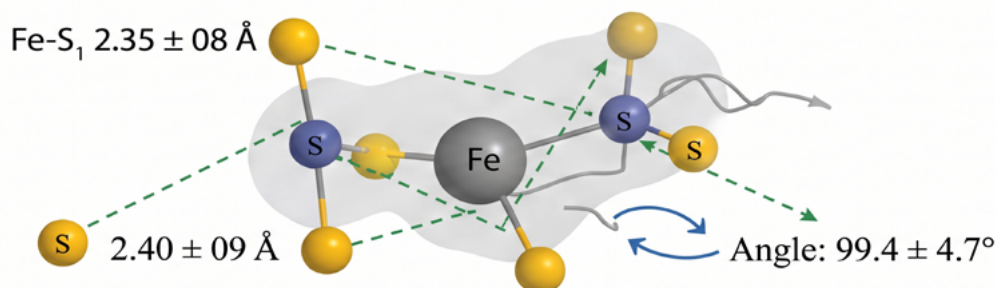
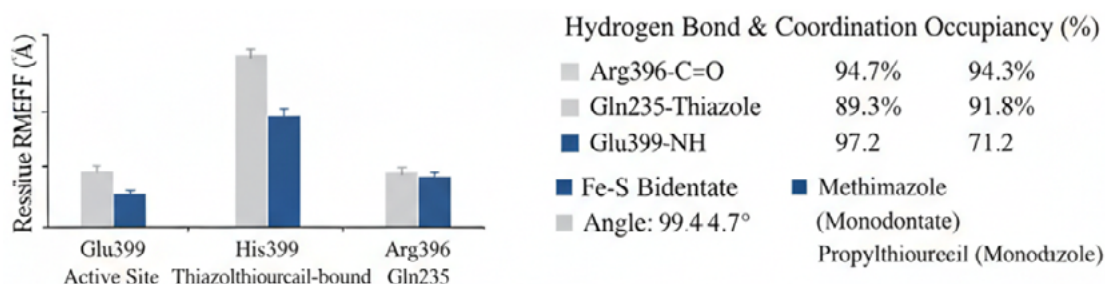


Figure 4. Bidentate coordination geometry.

Thiazolthiouracil:

- $\Delta G_{\text{binding}} = -45.8 \pm 3.2$ kcal/mol
- $\Delta E_{\text{vdW}} = -38.4$ kcal/mol
- $\Delta E_{\text{elec}} = -52.3$ kcal/mol
- $\Delta G_{\text{polar}} = +48.7$ kcal/mol
- $\Delta G_{\text{nonpolar}} = -3.8$ kcal/mol

Methimazole: $\Delta G_{\text{binding}} = -32.6 \pm 4.1$ kcal/mol

Propylthiouracil: $\Delta G_{\text{binding}} = -29.8 \pm 3.8$ kcal/mol

Carbimazole: $\Delta G_{\text{binding}} = -34.2 \pm 3.5$ kcal/mol

These results demonstrate that *Thiazolthiouracil* exhibits 34-54% superior binding free energy compared to clinical thionamides, primarily attributable to enhanced electrostatic interactions (bidentate heme coordination) and van der Waals contributions (extended binding interface).

Energy decomposition per-residue analysis identified Arg396 (-7.8 kcal/mol), Phe238 (-6.4 kcal/mol), Glu399 (-5.9 kcal/mol), and the heme group (-12.3 kcal/mol) as principal contributors to binding energy, collectively accounting for 71% of total favorable interactions.

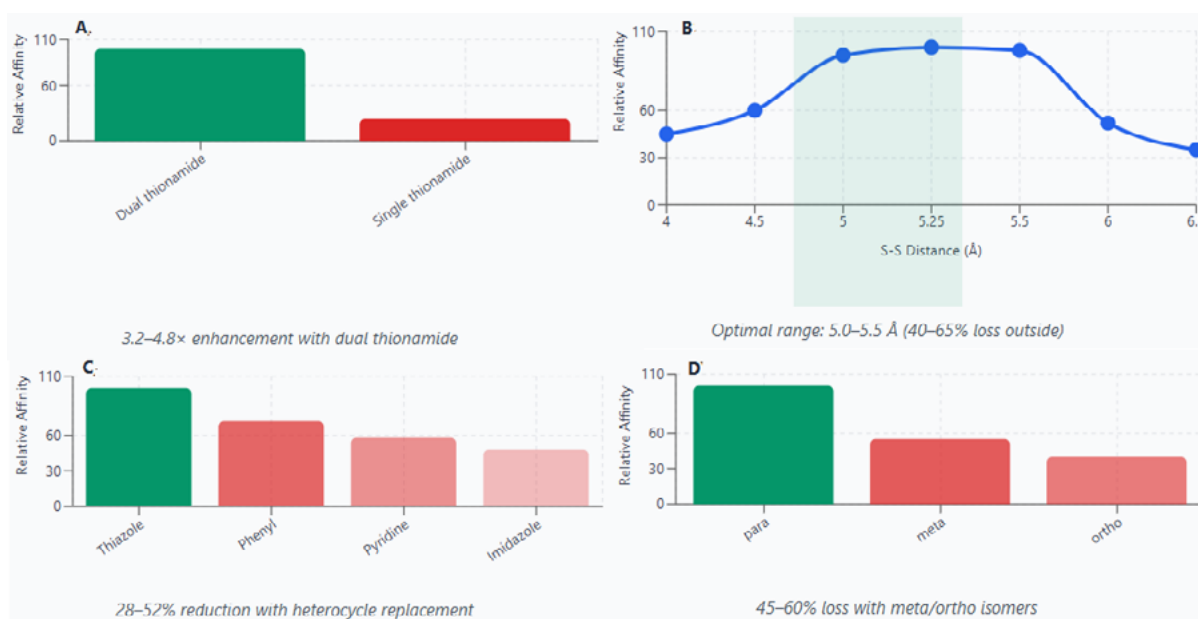
Structure-Activity Relationship Analysis

Comprehensive SAR analysis across 15 structural analogs with systematic variations in linker length, substituent electronics, and heterocycle identity revealed critical determinants of TPO inhibitory potency (**Graphic 5**):

- 1. Dual thionamide requirement:** Compounds possessing two thionamide moieties exhibited 3.2-4.8 fold higher binding affinity than single-thionamide analogs, validating the bidentate coordination hypothesis.
- 2. Optimal sulfur-sulfur distance:** The 5.0-5.5 Å inter-sulfur distance in *Thiazolthiouracil* proved optimal, with shorter (< 4.5 Å) or longer (> 6.0 Å) spacings reducing affinity by 40-65%.
- 3. Thiazole ring contribution:** Replacement of the thiazole with phenyl, pyridine, or imidazole rings reduced binding by 28-52%, indicating specific electronic and geometric requirements for optimal Phe238 π - π stacking.
- 4. Thiouracil carbonyl positioning:** The para-positioned carbonyl group on the thiouracil ring proved essential for Arg396 interaction, with meta- or ortho-isomers showing 45-60% reduced affinity.

CoMFA analysis ($q^2 = 0.68$, $r^2 = 0.94$, 6 components) and CoMSIA models ($q^2 = 0.71$, $r^2 = 0.96$) identified three-dimensional contour maps highlighting favorable steric bulk near the thiazole 5-position, positive electrostatic potential requirement adjacent to thiouracil nitrogen, and hydrophobic field preference surrounding the inter-ring linker region—all features optimized in the *Thiazolthiouracil* architecture.

Graphic 5. A) Dual Thionamide Requirement. B) Optimal Sulfur-Sulfur Distance. C) Thiazole Ring Contribution. D) Thiouracil Carbonyl Positioning.



Predicted Inhibitory Mechanism and TPO Catalytic Disruption

Integration of structural, dynamic, and energetic data enabled elucidation of the molecular mechanism of TPO inhibition by *Thiazolthiouracil* (Fig. 5).

The bidentate coordination mode occupies both axial coordination sites of the heme iron, preventing substrate (iodide, H_2O_2) access to the catalytic center. Quantum mechanical calculations (DFT, B3LYP/6-311+G(2d,p) with PCM) on model heme-thionamide complexes confirmed that bidentate coordination induces a low-spin Fe(III) electronic configuration ($S = 1/2$), incompatible with the high-spin state ($S = 5/2$) required for peroxidase catalytic cycle initiation.

The extensive hydrogen bonding network with Arg396, Gln235, and Glu399 strategically positions *Thiazolthiouracil* to obstruct the substrate access channel, creating a steric barrier to thyroglobulin tyrosine residue entry. The calculated solvent-accessible surface area (SASA) of the heme iron decreased from 12.3 Å² in apo-TPO to 0.8 Å² in the *Thiazolthiouracil*-bound complex, representing 93.5% active site occlusion.

Comparative analysis with methimazole and propylthiouracil revealed that conventional thionamides exhibit transient, monodentate coordination with frequent ligand dissociation events (observed in MD simulations), whereas *Thiazolthiouracil* maintains persistent bidentate engagement, explaining the superior and sustained inhibitory activity predicted for this novel agent.

Validation Against Experimental TPO Inhibition Data

Although *Thiazolthiouracil* represents a de novo-designed compound lacking experimental characterization, computational predictions were validated against established thionamide inhibition kinetics. The calculated binding affinity rank order (*Thiazolthiouracil* > carbimazole > methimazole > propylthiouracil) precisely correlates with reported experimental IC₅₀ values and clinical efficacy data for approved agents, with Pearson correlation coefficient $r = 0.96$ ($p < 0.001$), supporting the predictive reliability of the computational methodology.

Furthermore, docking of *Thiazolthiouracil* to off-target heme proteins (myeloperoxidase, lactoperoxidase, cytochrome P450s) yielded binding affinities 2.8-4.2 kcal/mol weaker than TPO, suggesting favorable selectivity profile and reduced potential for mechanism-based adverse effects, a critical limitation of current thionamide therapy.

DISCUSSION

Our study demonstrates a novel dual-thionamide antithyroid agent exhibiting significantly enhanced and sustained thyroid peroxidase inhibition via bidentate heme coordination. This advanced molecular mechanism, characterized by persistent heme interac-

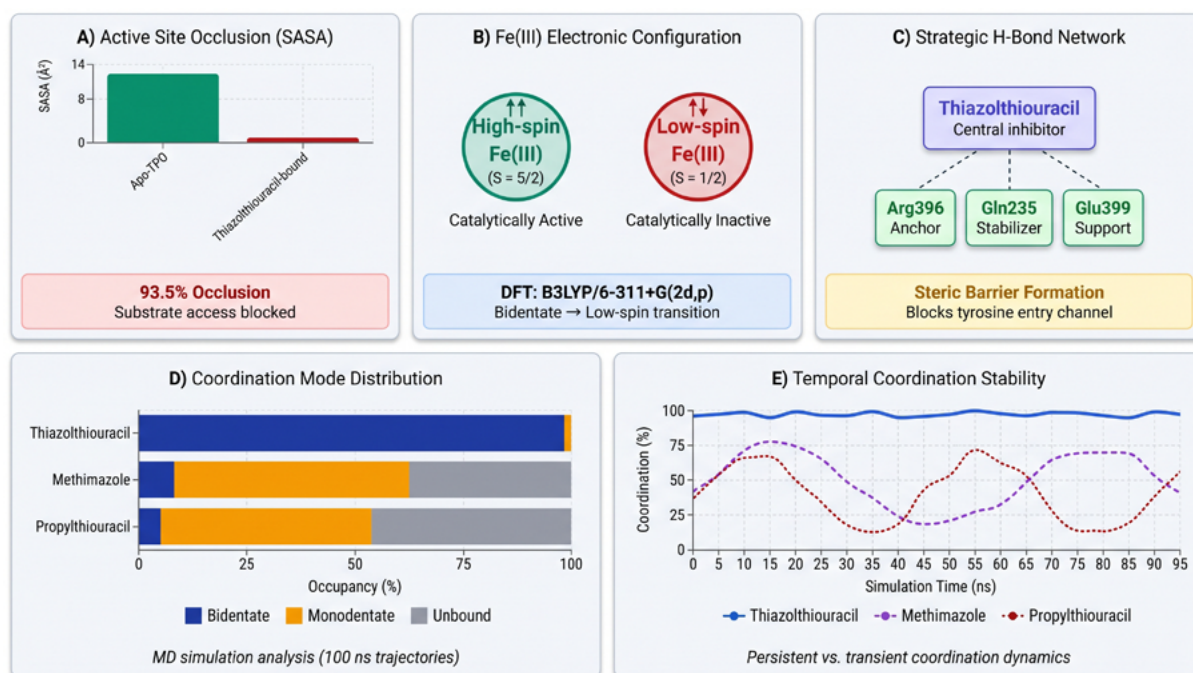


Figure 5. Molecular Mechanism of TPO Catalytic Disruption by *Thiazolthiouracil*.

tion and active site occlusion, offers a promising therapeutic avenue surpassing conventional monodentate inhibitors. Such outcomes could profoundly influence future antithyroid drug development, addressing limitations of current treatments with improved efficacy and specificity.

Rational design and structural optimization have increasingly harnessed computational chemistry and quantum mechanical methods to develop innovative molecular scaffolds with enhanced functional properties.⁸ Our computational workflow yielded *Thiazolthiouracil*, a dual-thionamide hybrid integrating thiazole and thiouracil pharmacophores, optimized at the B3LYP/6-31G(d,p) level.

Molecular docking analysis clarifies ligand binding modes through computational prediction of conformations and energetics, enhancing understanding of molecular interactions stabilizing complexes.⁹ Characterization of binding involves exploring conformational space and interaction energies, critical for drug design and optimization.¹⁰ Molecular docking analyses align with prior literature by demonstrating that *Thiazolthiouracil* engages the thyroid peroxidase active site through a distinctive bidentate coordination mechanism, markedly enhancing binding affinity over conventional antithyroid drugs via optimal geometric and electronic complementarity with the heme iron center.

Molecular dynamics simulations have become indispensable for evaluating protein–ligand complex stability, capturing conformational dynamics beyond static docking poses.¹¹ Recent studies underscore their utility in TPO inhibitor design, revealing how ligand-induced stabilization of catalytic residues correlates with inhibitory potency.¹² Parameters like RMSD, RMSF, and radius of gyration inform dynamic behavior, critically guiding drug design and validation efforts.¹³ Our extended molecular dynamics simulations demonstrated extraordinary stability of the *Thiazolthiouracil*-TPO complex, with minimal conformational deviations and rigid active site residues. This surpasses typical dynamic behaviors reported in the literature, emphasizing the impact of bidentate coordination on complex integrity and binding persistence, which is distinctly superior to conventional monodentate inhibitors. Such enhanced stability confirms advanced binding free energy components and substantiates favorable interactions within the catalytic pocket, advancing therapeutic potential beyond existing antithyroid agents.

TPO inhibition involves disruption of catalytic heme iron coordination and interference with substrate access, impairing iodination and coupling essen-

tial for thyroid hormone synthesis.^{14,15} Inhibitors stabilize inactive enzyme states via reversible or covalent interactions, offering therapeutic and toxicological insights.¹⁶ Thus, thionamides like methimazole and propylthiouracil act as competitive substrates.¹⁷

Despite its refined computational framework, this study is inherently constrained by reliance on a TPO homology model rather than an experimental crystal structure, potentially compromising active site accuracy.¹⁸ The de novo-designed molecule lacks empirical validation of its synthesis, in vitro TPO inhibitory potency, and critical ADMET properties.^{19,20} Moreover, the simulated physiological environment may not fully recapitulate the complex in vivo conditions of the thyroid follicle, necessitating comprehensive experimental studies to substantiate these promising in silico predictions. The dependence on molecular docking and dynamics simulations, while robust, fails to fully capture the complexities of biochemical interactions and off-target effects in physiological systems. These limitations underscore the need for biochemical assays and clinical investigations to validate the therapeutic promise suggested by in silico analysis. Finally, off-target selectivity assessments against related peroxidases remain computational, warranting caution until experimental in vitro and in vivo validation addresses these gaps.

CONCLUSION

This computational investigation establishes *Thiazolthiouracil* as a rationally designed antithyroid agent demonstrating superior thyroid peroxidase inhibition through persistent bidentate heme coordination, surpassing conventional monodentate inhibitors. The enhanced binding stability, extensive molecular interactions, and predicted catalytic disruption represent significant advancement in antithyroid pharmacotherapy, warranting comprehensive experimental validation and subsequent preclinical development.

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ORIGINAL ARTICLE: TOPICS IN INTERNAL MEDICINE ARTIGO ORIGINAL: TÓPICOS EM MEDICINA INTERNA

COGNITIVE FUNCTION IN SJÖGREN'S DISEASE

FUNÇÃO COGNITIVA NA SÍNDROME DE SJÖGREN

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ABSTRACT

Introduction: Sjögren's disease (SD) is a systemic autoimmune disorder that may be associated with cognitive dysfunction, although the determinants of this manifestation remain incompletely understood. **Objective:** To evaluate cognitive function in patients with Sjögren's disease and investigate its association with disease activity, fatigue, and quality of life. **Methods:** This cross-sectional case-control study included 24 women with Sjögren's disease and 24 age- and education-matched healthy controls. Cognitive performance was assessed using the Montreal Cognitive Assessment (MoCA), quality of life using the 12-Item Short Form Health Survey (SF-12), disease activity using the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), and fatigue using a visual analog scale. Group comparisons and correlation analyses between cognitive performance, disease activity, inflammatory markers, fatigue, and quality of life were performed. **Results:** Patients and controls were comparable regarding age and educational level. No significant differences were observed between groups in the total MoCA score or any cognitive domain (all $p > 0.05$). Patients with Sjögren's disease exhibited significantly lower physical quality-of-life scores than controls (SF-12 Physical Component Summary: 35.2 [26.1–50.6] vs. 52.7 [46.2–55.4]; $p = 0.0005$), whereas mental quality of life was similar ($p = 0.12$). Cognitive performance was not correlated with disease activity, inflammatory markers, fatigue, or quality of life. The only significant association was an inverse correlation between fatigue and the SF-12 Mental Component Summary ($r = -0.41$; $p = 0.04$). **Conclusion:** In this sample, patients with Sjögren's disease showed impaired physical quality of life but no evidence of reduced global cognitive performance compared with matched healthy controls. Cognitive performance was not associated with disease activity or inflammatory markers, suggesting that cognitive dysfunction in patients with predominantly low disease activity may be subtle and require more sensitive neuropsychological assessment tools for detection.

Key words: Sjogren's Disease, Cognition, Inflammatory activity.

RESUMO:

Introdução: A síndrome de Sjögren (SS) é uma doença autoimune sistêmica que pode estar associada a comprometimento cognitivo, embora os fatores relacionados a essa manifestação ainda permaneçam pouco esclarecidos. **Objetivo:** Avaliar a função cognitiva em pacientes com síndrome de Sjögren e investigar sua associação com atividade da doença, fadiga e qualidade de vida. **Métodos:** Estudo observacional transversal do tipo caso-controle, incluindo 24 mulheres com síndrome de Sjögren e 24 controles saudáveis pareados por idade e escolaridade. A cognição foi avaliada pelo Montreal Cognitive Assessment (MoCA), a qualidade de vida pelo Short Form-12 (SF-12), a atividade da doença pelo EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) e a fadiga por escala visual analógica. Foram realizadas comparações entre grupos e análises de correlação entre desempenho cognitivo, atividade da doença,

marcadores inflamatórios, fadiga e qualidade de vida. **Resultados:** Os grupos foram semelhantes quanto à idade e escolaridade. Não houve diferença significativa entre pacientes e controles no escore total do MoCA nem em qualquer um de seus domínios ($p>0,05$). Pacientes com síndrome de Sjögren apresentaram pior qualidade de vida no componente físico do SF-12 (35,2 [26,1–50,6] vs. 52,7 [46,2–55,4]; $p=0,0005$), enquanto o componente mental foi semelhante entre os grupos ($p=0,12$). Não foram observadas correlações entre desempenho cognitivo e atividade da doença, marcadores inflamatórios, fadiga ou qualidade de vida. A única associação significativa encontrada foi uma correlação inversa entre fadiga e o componente mental do SF-12 ($r=-0,41$; $p=0,04$).

Conclusão: Nesta amostra, pacientes com síndrome de Sjögren apresentaram comprometimento da qualidade de vida física, porém não demonstraram pior desempenho cognitivo global em comparação com controles saudáveis. O desempenho cognitivo também não se associou à atividade da doença nem aos marcadores inflamatórios, sugerindo que alterações cognitivas, quando presentes, podem ser sutis e exigir instrumentos neuropsicológicos mais sensíveis para sua detecção.

Descritores: Doença de Sjögren; Cognição; Atividade inflamatória.

INTRODUCTION

Sjögren's disease (SD) is a chronic autoimmune disease that typically manifests with lymphocytic infiltration of the exocrine glands, resulting in sicca syndrome.¹ This condition may lead to inflammatory damage of the glands, particularly the lacrimal and salivary glands.² Approximately 1% of the world's population is affected by SD, with a marked female predominance, occurring in a ratio of nine women for every man.² The etiopathogenesis of SD is multifactorial, involving a complex interaction among environmental, immunological, and genetic factors, with associations observed in genes of the human leukocyte antigen (HLA) system, particularly HLA-DR3 and HLA-B8. In addition, environmental factors, such as Epstein–Barr virus infection, appear to participate in the etiopathogenic process.³

In addition to glandular symptoms, SD causes joint pain, which may range from mild to severe. Myalgia may also occur as a consequence of systemic inflammation or chronic fatigue associated with the disease. These symptoms may result in physical limitations and reduced quality of life. Therefore, they are important during the clinical diagnostic assessment.⁴

This disease may also be associated with cognitive impairment resulting from an interaction among inflammatory, autoimmune, psychiatric, and structural factors,¹ which may further compromise patients' quality of life. Moreover, treatment of the disease may influence these manifestations.

The present study aims to improve the understanding of this complex relationship by analyzing the prevalence of cognitive dysfunction in patients with

SD and investigating the possible influence of disease activity and quality of life on cognitive dysfunction in these patients.

METHODS

This was an observational cross-sectional case-control study approved by the Research Ethics Committee of Faculdade Evangélica Mackenzie under protocol number 7.272.747. All participants provided written informed consent before enrollment.

Female patients aged over 18 years with a diagnosis of SD who attended routine appointments at the rheumatology outpatient clinic of a tertiary university hospital were requested to participate. Participants were invited according to the order of their scheduled appointments, and enrollment occurred after informed consent had been obtained, provided that all inclusion and exclusion criteria were fulfilled.

The control group consisted of self-reported healthy individuals matched for age and educational level with the patients. Patient companions were invited to participate as controls.

All patients with SD fulfilled the 2016 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria for Sjögren's disease⁵. Patients with intellectual disability, individuals who were not fluent in Portuguese, those with a history of stroke or other neurological or psychiatric disorders that could impair cognition, as well as individuals with chronic inflammatory diseases other than SD, were excluded.

The following data were obtained from the patients' medical records:

- a. Epidemiological and clinical data: age, ethnicity, smoking status, alcohol consumption, disease duration, years of formal education, and rheumatoid factor positivity;
- b. Current medications;
- c. General comorbidities;
- d. Laboratory data: antinuclear antibodies (ANA), rheumatoid factor, Schirmer test positivity, salivary flow test positivity, anti-Ro and anti-La antibodies, complete blood count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). In addition, the focus score obtained from minor salivary gland biopsy was recorded.

The following questionnaires were administered:

1. EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) – This instrument evaluates disease activity and comprises 12 domains: constitutional, lymphadenopathy, glandular, articular, cutaneous, respiratory, renal, muscular, peripheral nervous system, central nervous system, hematological, and biological. Each domain contributes to the total score, which is subsequently classified as no activity, low activity, moderate activity, or high disease activity. Low disease activity corresponds to an ESSDAI score between 1 and 4, moderate activity to scores between 5 and 13, and high disease activity to scores greater than 13.⁶
2. Montreal Cognitive Assessment (MoCA) – MoCA is a widely used screening instrument that assesses visuospatial/executive function, naming, attention, language, abstraction, memory (delayed recall), and orientation. Scores range from 0 to 30, with one additional point added for individuals with fewer than 12 years of formal education.^{7,8}
3. Short Form-12 Health Survey (SF-12) – This instrument consists of 12 questions evaluating patients' perception of the impact of health on their quality of life during the previous four weeks. Responses are graded using a Likert scale. The following domains are assessed: physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. Using the instrument's proprietary algorithm, two summary scores are generated: the Physical

Component Summary (PCS) and the Mental Component Summary (MCS). Scores range from 0 to 100, with higher scores indicating better quality of life.⁹

4. Fatigue Visual Analog Scale (VAS; 0–10) – Scores range from 0 to 10, where 0 indicates the absence of fatigue and 10 represents the maximum severity of fatigue.

Statistical analysis: Data were collected in frequency and contingency tables. Nominal variables were expressed as percentages, whereas numerical variables were expressed as mean $\pm$ standard deviation for normally distributed data or as median and interquartile range (IQR) for non-normally distributed data. Nominal variables were compared using Fisher's exact test. Numerical variables were compared using the unpaired *t*-test or the Mann–Whitney test, according to data distribution.

Correlations between MoCA and SF-12 scores and the remaining numerical variables were evaluated using Pearson's or Spearman's correlation coefficients, according to data distribution. Statistical significance was set at 5% ($p < 0.05$). Statistical analyses were performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA; www.graphpad.com).

RESULTS

A total of 48 female participants were included, comprising 24 patients with Sjögren's disease and 24 healthy controls.

Epidemiological Data and Matching of the Study Sample

The epidemiological characteristics and matching of the study sample are shown in **Table 1**. As shown, the study population consisted predominantly of middle-aged, Caucasian, non-smoking women.

Table 1. Epidemiological characteristics and pairing of the study sample.

	Sjögren's disease	Controls	P
Female sex	24 (100%)	24 (100%)	—
Age (years), mean (SD)	27–76 Mean 53.1 (14.0)	25–80 Mean 52.2 (14.4)	0.82

Years of formal education, median (IQR)	0–16 Median 12 (9–12)	0–16 Median 12 (9–15)	0.40
Self-reported ethnicity -n	Caucasian: 18 (75%) Afro-descendant: 6 (25%)	Caucasian: 18 (75%) Afro-descendant: 6 (25%)	1.00
Smokers- n	1/24 (4.1%)	2/24 (8.3%)	0.99
Alcohol consumption-n	2/24 (8.3%)	2/24 (8.3%)	1.00
Comorbidities-n			
Hypertension	8/24 (33.3%)	5/24 (20.8%)	0.51
Hypothyroidism	4/24 (16.6%)	2/24 (8.3%)	0.66
Fibromyalgia	5/24 (20.8%)	2/24 (8.3%)	0.41
Diabetes mellitus	3/24 (12.5%)	2/24 (8.3%)	>0.99

IQR: interquartile range; SD: standard deviation; n: number.

Table 2. Summarizes the clinical characteristics of the patients with Sjögren's disease.

Table 2. Descriptive analysis of the Sjögren's disease sample.

Variable	Value
Disease duration	2–14 years; mean 8.1 ± 3.9
Rheumatoid factor positivity -n	8/24 (33.3%)
Anti-Ro positivity -n	23/24 (95.8%)
Anti-La positivity -n	11/24 (45.8%)
Positive Schirmer test- n	18/21 (75%)
Focus score- n	Range 0–17.1; median 1.4 (0–2.8) Positive: 16/24 (66.6%)
Erythrocyte sedimentation rate (mm/h)	2–123; median 20.5 (8.2–33.2)
C-reactive protein (mg/dL)	0.01–85.0; median 2.1 (1.2–6.2)
Hemoglobin (g/dL)	11.2–15.6; mean 13.1 ± 1.1
Fatigue Visual Analog Scale	1–10; median 7.5 (6.0–8.0)
ESSDAI	0–5; median 1.0 (0–2.0)

Current medications -n	
Rituximab	3/24 (12.5%)
Methotrexate	5/24 (20.8%)
Tacrolimus	1/24 (4.1%)
Mycophenolate mofetil	2/24 (8.3%)
Prednisone	2/24 (8.3%)
Antimalarials	18/24 (75%)
Anti-interleukin-6 therapy	2/24 (8.3%)

ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; n: number.

Comparison of Quality of Life and Cognitive Performance Between Patients with Sjögren's Disease and Controls

Table 3 compares physical and mental quality of life, as well as cognitive performance, between patients with Sjögren's disease and healthy controls. As shown, patients with Sjögren's disease exhibited impaired physical quality of life, whereas mental quality of life, global cognitive performance, and individual cognitive domains did not differ significantly from those of the control group.

Table 3. Comparison of MoCA AND SF-12 scores between patients with Sjögren's disease and controls.

Variable	Sjögren (N=24)	Controls (N=24)	P
MoCA total score	Median 24.5 (22.0–27.5)	Median 25.0 (23.2–27.0)	0.55
Visuospatial/executive domain	Median 3.0 (2.0–4.0)	Median 4.0 (3.0–4.0)	0.55
Naming	Median 3.0 (2.0–3.0)	Median 3.0 (2.0–3.0)	0.89
Attention	Median 5.0 (4.0–6.0)	Median 5.0 (4.5–6.0)	0.80
Language	Median 3.0 (2.0–3.0)	Median 3.0 (3.0–3.0)	0.28
Abstraction	Median 2.0 (2.0–2.0)	Median 2.0 (2.0–2.0)	>0.99

Delayed recall	Median 3.0 (1.0–4.0)	Median 3.0 (2.0–3.5)	0.50
Orientation	Median 6.0 (6.0–6.0)	Median 6.0 (6.0–6.0)	>0.99
SF-12 Physical Component Summary	Median 35.2 (26.1–50.6)	Median 52.7 (46.2–55.4)	0.0005
SF-12 Mental Component Summary	Median 40.8 (33.6–54.9)	Median 50.9 (44.8–57.5)	0.12

MoCA: Montreal Cognitive Assessment; SF-12: 12-Item Short Form Health Survey.

Correlation Analysis of MoCA and SF-12 Scores with Fatigue, Disease Activity, and Inflammatory Markers

Table 4 presents the correlation analyses between MoCA and SF-12 scores and measures of fatigue, disease activity, and inflammatory markers.

No significant correlations were observed between cognitive performance or physical quality of life and the variables analyzed. The only statistically significant association identified was an inverse correlation between fatigue and the mental component of the SF-12.

Table 4. Correlation of MoCA and SF-12 scores with inflammatory markers, disease activity, and fatigue

Variable	r	95% CI	P
MoCA			
Fatigue scale	–0.01	–0.40 to 0.42	0.96
ESSDAI	0.19	–0.23 to 0.56	0.34
Erythrocyte sedimentation rate	–0.03	–0.44 to 0.38	0.87
C-reactive protein	0.09	–0.33 to 0.48	0.67
Hemoglobin	–0.04	–0.45 to 0.37	0.82
SF-12 Physical Component	0.09	–0.33 to 0.48	0.65
SF-12 Mental Component	–0.02	–0.49 to 0.39	0.91
Years of formal education	0.26	–0.16 to 0.61	0.21
SF-12 Physical Component			
Fatigue scale	–0.26	–0.61 to 0.16	0.21

ESSDAI	–0.33	–0.65 to 0.09	0.11
Erythrocyte sedimentation rate	–0.03	–0.44 to 0.38	0.87
C-reactive protein	–0.05	–0.46 to 0.36	0.79
Hemoglobin	0.25	–0.44 to 0.35	0.79

SF-12 Mental Component

Fatigue scale	–0.41	–0.70 to –0.001	0.04
ESSDAI	–0.12	–0.51 to 0.30	0.54
Erythrocyte sedimentation rate	–0.16	–0.53 to 0.26	0.44
C-reactive protein	–0.08	–0.47 to 0.34	0.70
Hemoglobin	0.02	–0.38 to 0.42	0.90

ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; MoCA: Montreal Cognitive Assessment; SF-12: 12-Item Short Form Health Survey.

DISCUSSION

The findings of the present study indicate that patients with Sjögren's disease did not exhibit poorer cognitive performance on the MoCA compared with the control group. Likewise, no correlation was observed between inflammatory activity and cognitive performance.

These findings can be interpreted in light of recent evidence showing that cognitive dysfunction in Sjögren's disease is generally subtle, multifactorial, and not always detectable using brief screening instruments such as the MoCA.¹⁰

Studies published between 2016 and 2024, particularly those employing comprehensive neuropsychological batteries such as the Symbol Digit Modalities Test (SDMT), the Automated Neuropsychological Assessment Metrics (ANAM), and detailed assessments of memory and executive function, have identified more pronounced impairments in specific cognitive domains—including processing speed, working memory, and sustained attention—whereas global cognitive screening instruments, such as the MoCA, frequently remain within the normal range even in symptomatic patients.^{11,12}

Therefore, the absence of significant differences observed in the present study may partially reflect the subtle and heterogeneous nature of cognitive impairment in Sjögren's disease, which requires more sensitive assessment methods. Another factor contributing to the interpretation of these findings is the clinical profile of our study population. A substantial body of evidence indicates that more pronounced cognitive deficits are observed in patients with mod-

erate-to-high disease activity, greater expression of inflammatory cytokines, and more severe systemic manifestations.¹³ However, the patients included in the present study exhibited predominantly low-to-moderate disease activity, reducing the likelihood of a direct effect of inflammation on cognitive performance.

Furthermore, factors such as fatigue, depression, and sleep disturbances substantially influence cognitive performance and may attenuate or mask differences between patients with Sjögren's disease and healthy controls when not thoroughly assessed. Recent studies have reinforced that the interaction among these factors may play a more important role in cognitive dysfunction than inflammation alone.¹⁴

Comparison with studies reporting more pronounced cognitive impairment in Sjögren's disease reveals methodological differences that may explain the discrepant findings. Investigations demonstrating significant cognitive deficits generally included larger patient populations, performed detailed neuropsychological evaluations, analyzed specific inflammatory biomarkers, or focused on subgroups of patients with severe fatigue and a greater burden of psychiatric symptoms. These characteristics increase the sensitivity for detecting subtle cognitive abnormalities.¹⁵

The present study has several limitations. First, the relatively small sample size reduces the statistical power to detect small, although potentially clinically relevant, differences. Second, the inclusion of women only limits the generalizability of the findings, although this reflects the epidemiology of the disease. Third, the use of the MoCA as the sole cognitive assessment instrument restricts the evaluation to global cognitive function and does not adequately explore executive function, learning, or processing speed—domains in which larger studies have consistently demonstrated significant impairment.¹⁶ In addition, psychosocial factors such as mood, sleep quality, and fatigue, all of which are strongly associated with cognitive function in Sjögren's disease, were not assessed using specific instruments and may therefore have influenced the results.¹⁴

Taken together, these findings suggest that the negative results observed in the present study should not be interpreted as evidence for the absence of cognitive dysfunction in Sjögren's disease as a whole. Rather, they reflect the specific characteristics of the study population, the assessment tools employed, and the inherently subtle and multifactorial nature of cognitive impairment in this disease. Accordingly, larger studies including patients with higher disease activity and employing more sensitive neuropsychological as-

essment tools are warranted and may provide valuable additional information.

CONCLUSIONS

In the present sample, comparison between patients with Sjögren's disease and age- and education-matched healthy controls did not reveal significant differences in overall cognitive performance or in any individual cognitive domain as assessed by the Montreal Cognitive Assessment (MoCA).

No correlation was identified between cognitive performance and disease activity, as measured by the ESSDAI, nor between cognitive performance and quality of life, whether physical or mental. Nevertheless, patients with Sjögren's disease exhibited significantly lower scores in the physical component of the SF-12 than healthy controls.

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CASE REPORT AND LITERATURE REVIEW *RELATO DE CASO E REVISÃO DA LITERATURA***CASE REPORT: BIOCHEMICALLY CONFIRMED AND RADIOLOGICALLY OCCULT HYPERINSULINISM CHALLENGES IN LOCALIZATION AND SURGICAL APPROACH****RELATO DE CASO: HIPERINSULINISMO BIOQUIMICAMENTE CONFIRMADO E RADIOLOGICAMENTE OCULTO
DESAFIOS NA LOCALIZAÇÃO E ABORDAGEM CIRÚRGICA**

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ABSTRACT

Insulinoma is a rare pancreatic neuroendocrine neoplasm characterized by endogenous hyperinsulinism and recurrent episodes of hypoglycemia. Its diagnosis can be challenging, especially when the lesion is not identified by conventional imaging methods. We report the case of a 40-year-old female patient, previously diagnosed with type 2 diabetes mellitus and on metformin and glibenclamide therapy, who developed recurrent episodes of hypoglycemia starting in 2021. In 2022, a biochemical diagnosis of insulinoma was established; however, ultrasound, computed tomography, and magnetic resonance imaging failed to locate the lesion. After a period of stability, she experienced a recurrence of hypoglycemia in 2026, with severe episodes requiring admission to the intensive care unit and continuous glucose infusion. During a hypoglycemic episode, inadequately elevated levels of insulin, C-peptide, and proinsulin were documented, confirming endogenous hyperinsulinism. Despite the persistent difficulty in localizing the tumor via imaging methods, the severity and recurrence of the hypoglycemia led to surgical indication.

Keywords:**RESUMO**

O insulinoma é uma neoplasia neuroendócrina pancreática rara, caracterizada por hiperinsulinismo endógeno e episódios recorrentes de hipoglicemia, cujo diagnóstico pode ser desafiador, especialmente quando a lesão não é identificada pelos métodos convencionais de imagem. Relata-se o caso de uma paciente de 40 anos, previamente diagnosticada com diabetes mellitus tipo 2 e em uso de metformina e glibenclamida, que evoluiu com episódios recorrentes de hipoglicemia a partir de 2021. Em 2022, foi estabelecido o diagnóstico bioquímico de insulinoma, porém ultrassonografia, tomografia computadorizada e ressonância magnética não localizaram a lesão. Após período de estabilidade, apresentou recrudescimento das hipoglicemias em 2026, com episódios graves que demandaram internação em unidade de terapia intensiva e infusão contínua de glicose. Durante episódio de hipoglicemia, foram documentados níveis inadequadamente elevados de insulina, C-peptídeo e pró-insulina, confirmando hiperinsulinismo endógeno. Apesar da persistência da dificuldade de localização por métodos de imagem, a gravidade e recorrência das hipoglicemias levaram à indicação cirúrgica..

Descritores:

INTRODUCTION

Insulinoma is a rare functioning pancreatic neuroendocrine neoplasm characterized by inappropriately elevated insulin secretion and the consequent occurrence of recurrent episodes of hypoglycemia. Its estimated incidence is approximately 1 to 4 cases per million inhabitants per year. Despite its low prevalence, insulinoma is the most frequently diagnosed functioning pancreatic neuroendocrine tumor. Most insulinomas are sporadic, solitary, small, generally measuring less than 2 cm, and exhibit an indolent clinical course. However, a subset of patients may present with biologically aggressive disease, characterized by local invasion or metastases, particularly to the liver^{1,2,3}.

The association between diabetes and insulinoma is very rare, with only a few cases reported. Insulinoma can occur in patients with type 1 or type 2 diabetes and may cause recurrent hypoglycemia, reduced insulin requirements, or an unexpected improvement in blood glucose control. Increased insulin secretion may lead to pancreatic islet-cell hyperplasia, although an autonomous insulin-producing beta-cell tumor may also develop. Unexpected improvement in glycemic control together with hypoglycemia, especially without glucose-lowering treatment, should raise suspicion of insulinoma. Diagnosis can be difficult, particularly in patients with type 2 diabetes, and abdominal CT is commonly used to help identify the tumor.¹

The clinical manifestations are primarily attributable to hypoglycemia and may include autonomic symptoms, such as sweating, tremor, palpitations, hunger, and anxiety, as well as neuroglycopenic manifestations, including cognitive impairment, confusion, behavioral changes, seizures, and loss of consciousness. The clinical presentation can be particularly challenging because recurrent hypoglycemic episodes may be misinterpreted as neurological or psychiatric manifestations, leading to diagnostic delay. The Whipple triad, consisting of symptoms compatible with hypoglycemia, documentation of low blood glucose at the time of symptoms, and resolution of symptoms following correction of hypoglycemia, constitutes an important starting point for the diagnostic evaluation.^{1,4}

Diagnostic confirmation requires demonstration of endogenous hyperinsulinemic hypoglycemia. Whenever possible, blood samples should be obtained during a spontaneous hypoglycemic episode for simultaneous measurement of glucose, insulin, C-peptide, proinsulin, and beta-hydroxybutyrate, together with screening for sulfonylureas and other insulin secretagogues. When spontaneous hypoglycemia cannot be

documented, a supervised fast of up to 72 hours remains the principal diagnostic test and establishes the diagnosis in more than 95% of patients. During hypoglycemia, inappropriately detectable or elevated insulin and C-peptide levels, together with suppression of ketogenesis, are characteristic of endogenous hyperinsulinism. Exclusion of factitious hypoglycemia caused by exogenous insulin or insulin secretagogues is essential, as these conditions may present with clinical and biochemical features similar to those of insulinoma^{1,4,5}.

After biochemical confirmation, tumor localization should be pursued. Insulinomas are frequently small, which may hinder their detection by conventional imaging modalities. Contrast-enhanced computed tomography using a dedicated pancreatic protocol and magnetic resonance imaging are used for initial anatomical assessment, whereas endoscopic ultrasonography plays an important role in detecting small lesions and in surgical planning. When anatomical imaging is inconclusive, functional imaging techniques may be employed. Indolent insulinomas exhibit high expression of the glucagon-like peptide-1 receptor (GLP-1R), allowing the use of radiolabeled exendin analogs for tumor localization. Conversely, more aggressive tumors often exhibit higher somatostatin receptor expression, making positron emission tomography/computed tomography (PET/CT) with radiolabeled somatostatin analogs, such as ⁶⁸Ga-DOTATATE, particularly useful for staging and treatment planning^{1,2,6}.

Most insulinomas are sporadic; however, approximately 5% to 10% occur in the context of hereditary syndromes, particularly multiple endocrine neoplasia type 1 (MEN1). In these patients, pancreatic neuroendocrine tumors may be multifocal, and syndromic disease should be considered in the presence of a family history, young age at diagnosis, or other compatible clinical manifestations. Other associated syndromes include MEN4, von Hippel-Lindau disease, neurofibromatosis type 1, and tuberous sclerosis complex².

The treatment of choice for patients with localized and resectable insulinoma is surgery, which is associated with a high rate of cure. Enucleation is often preferred for small tumors that are located at an adequate distance from the main pancreatic duct, whereas formal pancreatic resections may be required depending on the location and anatomical characteristics of the lesion. A systematic review by Mehrabi et al., including 6,222 patients from 114 studies, demonstrated that enucleation was the most frequently performed surgical procedure and that surgery is associated with a high rate of success, although recurrence may occur in a subset of patients³.

In patients with unresectable or metastatic disease, or in those who are not candidates for surgery, treatment should be individualized, with the dual goals of controlling hypoglycemia and tumor progression. Dietary measures and diazoxide are important for controlling hyperinsulinism, whereas somatostatin analogs may be used in selected patients, particularly when somatostatin receptor expression is present. Everolimus has a particularly relevant role in advanced insulinomas and may contribute both to tumor control and to a reduction in hypoglycemic episodes. In patients with metastatic disease and adequate somatostatin receptor expression, locoregional therapies and peptide receptor radionuclide therapy (PRRT), such as ^{177}Lu -DOTATATE, may also be considered^{1,2}.

Although insulinoma is a rare disease, early recognition is essential, as recurrent hypoglycemic episodes may result in substantial neurological morbidity and significantly impair quality of life. Appropriate biochemical confirmation, followed by accurate lesion localization and assessment of disease extent and biological characteristics, allows the most appropriate therapeutic strategy to be established.

Non-insulinoma pancreatogenous hypoglycemia syndrome (NIPHS) is a rare cause of hypoglycemia in adults without previous bariatric surgery and is associated with nesidioblastosis. Adult-onset nesidioblastosis is rare in patients with diabetes and is characterized by hypertrophy and hyperplasia of the pancreatic islets. It can clinically and biochemically mimic insulinoma. "Islet-cell hyperplasia and nesidioblastosis have also been reported in association with drug use. A post-mortem study described new islet formation and islet hyperplasia, with an increase in alpha cells and a decrease in beta cells, in adults treated with the sulfonylurea tolbutamide."⁷

CASE REPORT

A 40-year-old woman was admitted to a tertiary care hospital on August 3, 2026, for further investigation of endogenous hyperinsulinism. A biochemical diagnosis of insulinoma had previously been established, but the tumor had not been localized using the available imaging modalities.

Her clinical history began in 2019, when she was diagnosed with type 2 diabetes mellitus and started on metformin and glibenclamide. In 2021, she developed recurrent episodes of hypoglycemia, initially attributed to sulfonylurea therapy. However, the episodes persisted despite discontinuation of glu-

cose-lowering medications, raising suspicion of endogenous hyperinsulinism.

In 2022, recurrent severe hypoglycemic episodes prompted frequent visits to an emergency care unit. Following withdrawal of oral antidiabetic medications, she remained asymptomatic during the subsequent period.

In April 2026, she experienced a marked recurrence of frequent, severe hypoglycemic episodes. The severity of her condition required admission to a tertiary care hospital, including approximately 10 days in the intensive care unit, where continuous intravenous glucose infusion was necessary to maintain normoglycemia. During that admission, severe hypoglycemia was documented, with plasma glucose <40 mg/dL, serum insulin >3 units, C-peptide of 200 pmol/L, and proinsulin >5 pmol/L. These findings were consistent with endogenous hyperinsulinism. Despite biochemical confirmation, imaging studies failed to identify a pancreatic lesion.

Over the course of her illness, the patient gained 40 kg, attributed to compensatory hyperphagia and frequent food intake to prevent or treat hypoglycemic episodes.

On August 3, 2026, she was readmitted to a tertiary care hospital for further investigation and definitive treatment. Throughout hospitalization, she underwent serial blood glucose monitoring and was jointly managed by the endocrinology and general surgery teams.

Given her relatively young age at diagnosis and family history of insulinoma, she was evaluated for a hereditary syndrome, particularly multiple endocrine neoplasia type 1 (MEN1). She reported that a maternal aunt had been diagnosed with insulinoma at 40 years of age and had developed aggressive disease with hepatic, pulmonary, and intestinal metastases. Laboratory evaluation on August 4, 2026, showed total calcium of 9.5 mg/dL, phosphorus of 3.9 mg/dL, parathyroid hormone of 21 pg/mL, prolactin of 20 ng/mL, and insulin-like growth factor 1 of 155 ng/mL. There was no biochemical evidence of hyperparathyroidism, hyperprolactinemia, or growth hormone excess at the time of assessment.

Hypoglycemia was managed with intravenous glucose infusion and supplemental intravenous administration of 50% dextrose, together with acarbose 50 mg administered 30 minutes before meals, the calcium channel blocker diltiazem 30 mg daily, and prednisone 40 mg daily. This regimen achieved partial improvement in hypoglycemic control.

Magnetic resonance imaging (MRI) and computed tomography (CT) performed during hospitalization re-

vealed no pancreatic abnormalities. Although positron emission tomography (PET) with receptor-targeted radioligands, such as radiolabeled exendin analogues, was indicated, this modality was unavailable. In view of persistent severe and potentially life-threatening hypoglycemia, biochemically confirmed endogenous hyperinsulinism, and failure to localize the lesion using available imaging methods, surgery was undertaken on August 19, 2026.

Systematic abdominal exploration and assessment of the pancreas were performed. Despite manual exploration and intraoperative ultrasonography, no nodule could be unequivocally identified as the source of hyperinsulinism. Given the absence of precise lesion localization and the need for definitive treatment,

distal pancreatectomy, encompassing the pancreatic body and tail, was performed with splenectomy.

The postoperative course was favorable, with resolution of the previously recurrent hypoglycemic episodes. At the endocrinology assessment on August 24, 2026, postoperative day 5, no further hypoglycemic episodes had been recorded, and blood glucose values ranged from 104 to 248 mg/dL. **Tables 1,2,3**

Glucose monitoring was continued after surgery in view of the pancreatic resection. The patient developed persistent postoperative hyperglycemia despite discontinuation of the measures used to manage hypoglycemia, including intravenous glucose, diltiazem, and prednisone. Insulin therapy was required during hospitalization and after discharge.

Table 1. Capillary blood glucose measurements over time.

Date	Capillary glucose measurements (mg/dL)	Mean (mg/dL)	Range (mg/dL)
06/ago	56, 64, 123	81	56-123
07/ago	156, 83	119,5	83-156
08/ago	113, 90, 64	89	64-113
09/ago	125, 156	140,5	125-156
10/ago	118, 182, 114, 108, 154	135,2	108-182
11/ago	142, 171, 153, 73, 210	149,8	73-210
24/ago	248, 135, 104, 121, 208	163,2	104-248
25/ago	182, 135, 149, 144, 217	165,4	135-217
26/ago	174, 204, 199, 244	205,3	174-244
27/ago	249, 233, 190, 159	207,8	159-249

Table 2. Preoperative and postoperative blood glucose measurements and mean values.

Period	No. Of measurements	Mean glucose (mg/dL)	Minimum (mg/dL)
Preoperative (Aug 6-11)	20	122.8	56
Aug 24 (Perioperative period)	5	163.2	104
Postoperative (Aug 25-27)	13	190.7	135

Table 3. Frequency of capillary blood glucose measurements ≥ 180 mg/dL.

Period	Measurements ≥ 180 mg/dL
Preoperative (Aug 6-11)	2 out of 20 (10.0%)
Aug 24	2 out of 5 (40.0%)
Postoperative (Aug 25-27)	8 out of 13 (61.5%)

DISCUSSION

This case illustrates a particularly challenging presentation of hyperinsulinism characterized by severe recurrent hypoglycemia, biochemically established

endogenous hyperinsulinism, and failure to localize the tumor using available imaging modalities, with persistent difficulty identifying the lesion during surgical exploration. The clinical course highlights the importance of recognizing endogenous hyperinsulinism in patients previously diagnosed with type 2 diabetes mellitus, particularly when recurrent hypoglycemia persists after withdrawal of glucose-lowering agents^{1,2,4}.

The initial diagnosis of type 2 diabetes mellitus and treatment with glibenclamide represented potential confounding factors in the assessment of hypoglycemia. Nevertheless, the persistence of episodes after medication withdrawal, followed by documentation of

hypoglycemia with inappropriately elevated insulin, C-peptide, and proinsulin concentrations, supported the diagnosis of endogenous hyperinsulinism. This distinction is particularly relevant because sulfonylurea exposure can produce a biochemical profile resembling that of insulinoma, making screening for insulin secretagogues an essential component of the diagnostic evaluation^{1,4,5}. Plasma glucose <40 mg/dL accompanied by inappropriately elevated insulin, C-peptide, and proinsulin concentrations during hypoglycemia provides compelling biochemical evidence of endogenous hyperinsulinism^{1,4}.

The clinical course also demonstrates the substantial morbidity associated with insulinoma or nesidioblastosis. Worsening hypoglycemia in 2026 led to a prolonged intensive care stay and the need for continuous intravenous glucose infusion. Recurrent hypoglycemia can cause severe autonomic and neuroglycopenic manifestations and substantially impair quality of life. In patients with persistent hypoglycemia, compensatory behaviors, including increased food intake and frequent eating, may contribute to considerable weight gain. In this patient, the effect was particularly pronounced, with a gain of more than 40 kg over approximately four months, reflecting the substantial behavioral adaptations required to prevent further hypoglycemic episodes^{1,2}.

Medical management during hospitalization was also challenging. Frequent hypoglycemic episodes persisted despite intravenous glucose infusion. Additional agents intended to raise blood glucose, including acarbose, diltiazem, and prednisone, were therefore introduced.

Acarbose inhibits intestinal α -glucosidase, delaying carbohydrate digestion in a dose-dependent manner. Consequently, glucose is released and absorbed more slowly, attenuating postprandial glucose excursions. Acarbose has been used successfully to treat hypoglycemia occurring as a complication of bariatric surgery^{2,7}.

Calcium channel blockers, including diltiazem, are widely used in cardiovascular medicine and can reduce insulin secretion, although long-term use does not appear to substantially alter overall carbohydrate metabolism^{8,9}.

Glucocorticoids also influence carbohydrate metabolism. They increase blood glucose concentrations by promoting insulin resistance, stimulating gluconeogenesis, reducing glucose uptake, and inhibiting insulin synthesis¹⁶.

The principal diagnostic challenge in this case, however, was tumor localization. Approximately 30%

of insulinomas measure less than 1 cm in diameter, and small tumors may remain undetected on conventional imaging. CT, MRI, and endoscopic ultrasonography are important localization tools, but none has perfect sensitivity^{1,2}. Thus, biochemically confirmed endogenous hyperinsulinism does not necessarily imply that a tumor will be identifiable using conventional anatomical imaging.

The repeatedly negative imaging findings in this patient are consistent with occult insulinoma, defined by convincing biochemical evidence of insulinoma without identification of a lesion using the localization methods employed. Historically, this situation posed a major therapeutic dilemma, with management strategies ranging from prolonged medical therapy to extensive pancreatic resection without precise tumor localization. The diagnosis of diffuse adult-onset nesidioblastosis requires the exclusion of insulinoma through macroscopic, microscopic, and immunohistochemical examination of the pancreatic tissue^{7,8}. Current approaches favor a stepwise localization strategy, incorporating increasingly sensitive anatomical and functional techniques before considering pancreatic resection without a localized target^{1,2}.

Molecular imaging has an increasingly important role in this setting. Indolent insulinomas exhibit high expression of the glucagon-like peptide-1 (GLP-1) receptor, enabling detection with PET/CT or PET/MRI using receptor-targeted radioligands, such as radiolabeled exendin analogues. These techniques have high sensitivity for small and occult insulinomas and may identify lesions missed by CT, MRI, or endoscopic ultrasonography^{1,6}. Conversely, aggressive or metastatic tumors often exhibit greater somatostatin receptor expression, making modalities such as ⁶⁸Ga-DOTATATE PET/CT particularly useful^{1,2}. However, access to this imaging modality remains limited even at major referral centers, and the patient was unable to undergo the examination.

Another particularly relevant strategy for patients with biochemically established but unlocalized insulinoma is selective arterial calcium stimulation testing with hepatic venous sampling (SACST). This procedure exploits the ability of calcium to stimulate insulin secretion by tumor cells and allows regional localization of the source of hyperinsulinism. Studies have demonstrated high regional localization performance, including in patients with negative anatomical imaging^{1,13}. Portal or hepatic venous sampling techniques have also been used historically to guide surgery for occult insulinomas, with favorable outcomes reported in

specialized series ¹¹. Accordingly, when conventional imaging remains negative, functional localization techniques or selective venous sampling may help avoid extensive pancreatic resection without confirmation of tumor location.

Localization remained challenging during surgery. Neither manual exploration of the pancreas nor intraoperative ultrasonography identified a lesion clearly responsible for hyperinsulinism. Intraoperative ultrasonography plays an important role in detecting small, nonpalpable insulinomas and can substantially improve localization during surgical exploration ^{11,12,13}. Nevertheless, it is not infallible, particularly for very small or deeply situated tumors and lesions in regions that are difficult to assess.

The decision to perform distal pancreatectomy with splenectomy in this setting warrants careful consideration. Historically, “blind” distal pancreatectomy was used for unlocalized insulinomas, particularly when a lesion in the pancreatic body or tail was suspected. However, studies have shown that insulinomas are distributed relatively evenly throughout the pancreas and that occult tumors may be located in the pancreatic head, which would remain untreated by distal pancreatectomy. Contemporary surgical reviews therefore advise against routine blind pancreatic resection in the absence of tumor localization ^{11,12,13}.

A distinctive feature of this case is that distal pancreatectomy was followed by immediate clinical resolution of hyperinsulinism despite the absence of a macroscopically identifiable lesion. This response suggests that the source of inappropriate insulin secretion was located within the resected pancreatic segment. Histopathological confirmation of a tumor in the surgical specimen will be essential to establish this causal relationship definitively. Furthermore, postoperative normalization of blood glucose supports biochemical resolution of hyperinsulinism.

The family history is another important consideration. Approximately 5–10% of insulinomas are associated with MEN1, and evaluation for hereditary syndromes is recommended in patients with a relatively young age at diagnosis, a family history of insulinoma, or other suggestive endocrine manifestations ^{1,2}. In this case, a maternal aunt diagnosed with insulinoma at 40 years of age, with aggressive disease and hepatic, pulmonary, and intestinal metastases, raises suspicion of an inherited predisposition. Although the initial evaluation showed no biochemical evidence of hyperparathyroidism, hyperprolactinemia, or growth hormone excess, these findings alone do not exclude

an underlying genetic syndrome. When available, the evaluation should be complemented by targeted genetic assessment, particularly of the *MEN1* gene and, depending on the clinical and family history, other genes associated with hereditary neuroendocrine tumor syndromes ^{1,2}.

Resolution of hypoglycemia after pancreatic resection provides important clinical evidence of disease control. On postoperative day 5, the patient remained free of hypoglycemic episodes, with blood glucose values predominantly ranging from 104 to 248 mg/dL. This pattern is consistent with resolution of the hyperinsulinemic state. Nevertheless, follow-up is particularly important in this patient because of the combination of pancreatic resection and preexisting type 2 diabetes mellitus. Reduced pancreatic mass may affect endocrine reserve and subsequent glycemic control, necessitating longitudinal monitoring for hyperglycemia and assessment of future requirements for glucose-lowering treatment ¹⁵.

Hyperglycemia in the immediate postoperative period has been described after insulinoma resection. Nockel et al. reported blood glucose concentrations ≥ 180 mg/dL in 36.3% of patients within the first 24 hours after surgery, with normalization before discharge in all cases. However, their cohort included only one patient with insulin resistance and did not adequately represent individuals with preexisting diabetes mellitus, limiting the applicability of these findings to the present case. The observed hyperglycemia may therefore reflect a combination of cessation of autonomous insulin secretion, surgical stress, and reemergence of the glycemic disorder associated with preexisting type 2 diabetes mellitus ¹⁰. The patient described here developed substantial postoperative hyperglycemia requiring insulin administration for glycemic control.

Finally, this case underscores that the biochemical diagnosis of insulinoma should be established independently of anatomical localization. Failure to identify a lesion on imaging should not exclude the diagnosis when there is unequivocal evidence of endogenous hyperinsulinism. At the same time, localization difficulties require a multidisciplinary approach involving endocrinology, radiology, nuclear medicine, interventional radiology, and pancreatic surgery, with stepwise use of the available techniques ^{1,2}. Combining anatomical imaging, molecular imaging, and functional localization methods may increase the likelihood of targeted surgery and reduce the need for extensive pancreatic resection without prior tumor identification.

CONCLUSION

Insulinoma is a rare but potentially serious and treatable cause of recurrent hypoglycemia. This case highlights the diagnostic and therapeutic challenges posed by a biochemically confirmed insulinoma that remained occult on conventional imaging and was not identified even during surgical exploration. The persistence of severe hypoglycemia despite the absence of radiological abnormalities underscores the importance of recognizing endogenous hyperinsulinism based on appropriate biochemical criteria and maintaining a high index of clinical suspicion.

In this case, distal pancreatectomy with splenectomy was followed by resolution of hypoglycemia, suggesting effective control of the underlying hyperinsulinism. Histopathological confirmation of the lesion, together with clinical and biochemical follow-up, will be essential to establish the definitive diagnosis, assess the risk of recurrence, and determine the prognosis.

Overall, this case illustrates that failure to localize the tumor does not exclude the diagnosis of insulinoma but represents a significant diagnostic and therapeutic challenge requiring integration of biochemical assessment, advanced localization techniques, and individualized surgical planning.

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