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editorial

# Bridging the knowledge–practice gap in chronic disease management: tele-education in diabetes care training

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The gap between knowledge acquisition and its translation into clinical practice remains a central and persistent challenge in chronic disease management (1). Nowhere is this more evident than in conditions such as diabetes mellitus, where effective care depends not on isolated decisions, but on longitudinal clinical reasoning, continuous therapeutic adjustment, and sustained patient engagement over time. Despite advances in medical education, training environments continue to privilege acute, high-intensity care, often at the expense of competencies required for managing chronic illness (2,3).

In this issue, the study by Lopes and cols. (4) provides timely and relevant insights into this problem by evaluating a tele-education strategy implemented during residency training. Using a widely accessible digital communication platform, the intervention demonstrated a significant improvement in knowledge acquisition among residents. These findings reinforce the growing role of tele-education as a scalable and pragmatic tool to support learning, particularly in resource-constrained or high-demand clinical settings (5,6).

However, the study also highlights a critical limitation that has long been recognized in medical education: the persistence of the knowledge–practice gap. Despite robust cognitive gains, changes in clinical practice, as reflected by insulin management indicators, were modest and did not reach statistical significance (4). This disconnect suggests that clinical behavior is not readily modified by knowledge acquisition alone. Rather, it is shaped by a complex interplay of factors, including established prescribing habits, clinical culture, supervision structures, and system-level constraints (7-9).

Importantly, the absence of short-term improvements in patient-centered outcomes, such as length of hospital stay, should not be interpreted as a failure of the intervention. Instead, it reflects the inherent complexity of healthcare systems, in which outcomes emerge from multiple interacting determinants beyond individual physician knowledge. In this context, the apparent paradox, improved knowledge without measurable outcome change, becomes not only understandable, but expected within complex adaptive systems (10,11).

These findings invite a broader reflection on the limitations of current approaches to competency-based medical education. While knowledge acquisition remains a fundamental component of training, it is insufficient to drive meaningful changes in clinical practice when implemented in isolation. The management of chronic diseases requires competencies that are longitudinal, relational, and context-dependent—dimensions that are often underrepresented in traditional training models, which continue to prioritize immediacy, procedural skills, and acute care efficiency (1,2).

Within this evolving landscape, tele-education represents a promising, yet still under-integrated, strategy. Digital platforms—particularly widely accessible messaging applications—enable new forms of interaction that extend learning beyond formal clinical encounters, supporting case-based discussion,

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asynchronous feedback, and longitudinal mentorship (12,13). As demonstrated by the present study (4), these approaches are effective in enhancing knowledge. However, their capacity to influence clinical practice depends on their integration into structured educational ecosystems that incorporate supervision, audit and feedback mechanisms, and alignment with care processes.

Rather than functioning merely as adjuncts to traditional teaching, tele-education strategies should be understood as catalysts for reconfiguring how learning occurs in clinical environments—continuous, context-sensitive, and embedded within real-world practice. Bridging the knowledge–practice gap will therefore require not only the adoption of innovative educational tools, but also a deliberate effort to align educational strategies with the organizational and cultural realities of healthcare systems (12,13).

Ultimately, the contribution of this study lies in advancing a more nuanced understanding of the relationship between knowledge, practice, and outcomes in medical training. It reinforces a critical message for educators and institutions alike: improving what residents know is necessary, but not sufficient. Transforming clinical practice requires educational approaches that are longitudinal, integrated, and responsive to the complexity of real-world care (14,15).

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editorial

# Relapsed Graves' disease: should continuous low-dose methimazole become a preferred strategy?

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The optimal management of relapsed Graves' disease (GD) remains one of the most debated issues in contemporary thyroidology. Although GD is the most common cause of hyperthyroidism in iodine-sufficient regions, therapeutic decision-making after relapse following an initial course of antithyroid drug (ATD) therapy continues to pose important clinical challenges (1). Traditionally, radioiodine therapy (RAI) and thyroidectomy have been considered definitive treatment strategies. However, accumulating evidence over the past two decades supports prolonged ATD therapy as a safe and effective long-term alternative in selected patients (2-5).

Relapse after discontinuation of ATD therapy occurs in approximately 40%-60% of patients treated for 12-18 months, emphasizing the need for individualized approaches beyond conventional treatment algorithms (2,3). In this context, defining the most appropriate management strategy after recurrence represents a critical step toward optimizing long-term outcomes.

In this issue of the *Archives of Endocrinology and Metabolism*, Carlini and cols. (6) address this clinically relevant dilemma by comparing three therapeutic approaches in patients with relapsed GD: continuous low-dose methimazole, a second course of methimazole until TRAb becomes negative, and RAI followed by levothyroxine replacement. Their study provides valuable real-world evidence that contributes meaningfully to the evolving management paradigm for recurrent disease.

One of the most important observations of the study is the higher proportion of time spent in euthyroidism among patients receiving continuous low-dose methimazole compared with those treated with either RAI or a second ATD course. This finding has clear clinical relevance, since maintaining stable thyroid function represents a central therapeutic objective in GD management. Even mild thyroid dysfunction has been associated with increased cardiovascular risk, including atrial fibrillation, stroke, and mortality, reinforcing the importance of sustained biochemical stability (7).

These results further support the concept that prolonged ATD therapy should no longer be viewed exclusively as a temporary strategy before definitive treatment but rather as a legitimate long-term therapeutic option in appropriately selected patients. Historically, ATDs were mainly considered a bridge to ablation or surgery. However, this perspective has progressively evolved as long-term observational studies and randomized clinical trials demonstrated that extended methimazole therapy is associated with higher remission rates and reduced recurrence risk without relevant safety concerns (4,5,8,9). Importantly, the safety profile of long-term low-dose methimazole has been consistently confirmed in systematic reviews showing that severe adverse effects are rare and occur predominantly during the initial months of treatment and at higher drug doses (4). These findings have contributed to gradual changes in clinical practice patterns, particularly in Europe and Asia, where prolonged ATD therapy is increasingly accepted as a definitive therapeutic strategy in selected patients (2,5).

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Another relevant contribution of the study by Carlini and cols. (6) concerns thyroid eye disease outcomes. The absence of differences among treatment groups is clinically reassuring, especially because concerns regarding orbitopathy progression frequently influence treatment selection after relapse. Current evidence indicates that long-term ATD therapy represents a safe alternative in patients with mild orbitopathy and may be preferable to RAI in selected clinical scenarios (10).

Similarly, the lack of differences in quality of life across treatment strategies highlights an essential aspect of GD management. Quality of life impairment is a major determinant of long-term disease burden and frequently persists despite biochemical control. However, available evidence suggests that quality of life is more closely related to thyroid functional status than to treatment modality itself, reinforcing that maintaining euthyroidism remains the primary therapeutic goal (11).

An additional strength of the study is the long duration of follow-up, exceeding six years in all treatment groups. Such extended observation provides valuable insight into real-world treatment effectiveness and supports the relevance of evaluating long-term biochemical control rather than short-term remission alone. In daily clinical practice, sustained euthyroidism over time is often more meaningful than early treatment response.

The study also raises an important consideration regarding treatment duration after relapse. Patients who achieved remission following a second course of methimazole had longer treatment exposure than those who relapsed, reinforcing previous evidence that remission probability increases with prolonged ATD therapy (8,9). These findings support a more flexible approach in defining treatment duration rather than adhering to fixed therapeutic intervals.

Another noteworthy aspect concerns the role of TRAb measurement in guiding treatment discontinuation. Although TRAb negativity is frequently interpreted as a marker of immunological remission, the present results suggest that it may not reliably predict sustained clinical remission after therapy withdrawal. This observation highlights the complexity of autoimmune regulation in GD and underscores the limitations of relying exclusively on antibody status to guide therapeutic decisions (2,3).

The implications of these findings extend beyond individual patient management. In regions where access to definitive therapies may be limited or delayed, long-term pharmacological treatment represents a particularly valuable strategy. In Latin America, where variability in healthcare access persists across different settings, the demonstration that prolonged low-dose methimazole is both safe and effective supports a more flexible and individualized therapeutic approach adapted to regional realities.

Taken together, the results presented by Carlini and cols. (6) add important evidence supporting continuous low-dose methimazole as a rational long-term strategy for patients with relapsed GD. Rather than representing merely an alternative to definitive therapies, prolonged ATD treatment may in selected cases become a preferred option, particularly for patients seeking to avoid radioiodine exposure or surgery while maintaining stable thyroid function (12). These findings support a progressive shift toward a more individualized and conservative long-term management strategy for patients with relapsed Graves' disease.

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original

# Tele-education to improve residents' knowledge and quality of care in hospital hyperglycemia: a multicenter randomized clinical trial

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## ABSTRACT

**Objective**: To evaluate the impact of a structured tele-education program on hospital hyperglycemia and diabetes, focusing on residents' medical knowledge and inpatient care. **Subjects and methods**: This open-label, multicenter, randomized clinical trial enrolled internal medicine residents from four university hospitals in southern Brazil. Teams were block-randomized to an intervention group that received an online lecture plus 30 days of tele-education via WhatsApp, or to a control group with no intervention. The primary outcome was medical knowledge, assessed with a validated 10-item questionnaire. Secondary outcomes included quality of insulin prescriptions, hypoglycemia and hyperglycemia rates, and hospital length of stay (LOS). Analyses were performed using SPSS v29 (5% significance). **Results**: Fifty residents completed the study. The intervention group achieved higher post-intervention knowledge scores than the control group (median 8 vs. 6 correct answers; $p = 0.005$) and showed significant improvement from pre- to post-test (6 to 8; $p < 0.001$), with consistent gains across centers. Clinical data from 149 hospitalized patients were analyzed (mean age 67.8 years; 55% female); 56% had diabetes, and 44% had hospital-related hyperglycemia. There was a nonsignificant trend toward more appropriate NPH ($p = 0.107$) and regular insulin ($p = 0.203$) prescriptions in the intervention group. Median LOS was longer in the intervention group (19 vs. 13 days; $p = 0.009$). **Conclusion**: The tele-education program improved residents' knowledge of inpatient hyperglycemia. Larger studies are needed to confirm clinical effects and long-term outcomes of tele-education in hospital glycemic management.

**Keywords:** Medical education; hospital hyperglycemia; diabetes mellitus; telemedicine

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## INTRODUCTION

Diabetes is a complex chronic condition that requires continuous medical care, multifactorial risk reduction, and appropriate glycemic control. In the hospital setting, dysglycemia — articularly hospital hyperglycemia — is common and is associated with higher risks of complications and prolonged length of stay (LOS) (1).

In Brazil, data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) — a cohort of

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15,103 civil servants aged 35 to 74 years from six state capitals — reported a diabetes prevalence close to 20%, with approximately half of the cases previously undiagnosed (2).

In the United States, an estimated 22% of hospitalized patients have diabetes, and hospitalizations account for nearly half of the US$174 billion annual medical expenditures related to the disease. Each year, approximately 1.6 million new cases are diagnosed, with an overall prevalence of 23.6 million (7.8% of the population), of which about one quarter are undiagnosed (3).

Hospital readmissions are frequent among people with diabetes, with admission rates ranging from 14% to 20% (4), especially within 30 days of discharge (5). Major risk factors for readmission include lower socioeconomic status, race/ethnicity, comorbid conditions, and recent hospitalization (4).

Although no single protocol universally prevents readmissions, targeted strategies for high-risk patients and transitional care models have demonstrated benefits (6–9). Glycemic disturbances in hospitalized patients — hyperglycemia, hypoglycemia, and glucose variability — have been consistently linked to adverse outcomes, including increased morbidity and mortality (10).

Clinical evidence also underscores the role of physician knowledge and specialized diabetes teams in predicting shorter hospital stays and improved outcomes (11–13).

Scalable educational approaches may help close these gaps. Tele-enabled formats — including remote monitoring, messaging platforms, and decision support — show promise, though most studies focus on outpatients or primary care rather than inpatient settings (14–17).

Given the clinical burden of hospital hyperglycemia, rising costs, and readmission risk, there is a need to evaluate pragmatic educational strategies for clinicians. This study investigates whether a tele-education program — comprising an online lecture and WhatsApp-based microlearning — can improve internal medicine residents' knowledge of inpatient hyperglycemia and influence prescribing practices and the management of glycemic extremes during hospitalization.

## SUBJECTS AND METHODS

This was an open-label, multicenter, randomized clinical trial conducted with internal medicine residents from four teaching hospitals in southern Brazil: the University Hospital of the Federal University of Pelotas (HE-UFPel), the University Hospital Dr. Miguel Riet Corrêa Jr. of the Federal University of Rio Grande (HU-FURG), the University Hospital São Francisco de Paula of the Catholic University of Pelotas (HUSFP-UCPel), and the *Santa Casa de Misericórdia de Porto Alegre*, a teaching hospital affiliated with the Federal University of Health Sciences of Porto Alegre (UFCSPA).

Clinical teams, rather than individual residents, were randomized in blocks to minimize contamination between participants. Randomization was performed using WinPepi software (version 11.65), with stratification by center to ensure balanced allocation across the participating hospitals.

All internal medicine residents from clinical teams at the four institutions were invited to participate. At the time of the study, 51 residents were eligible. Exclusion criteria included being under endocrinological supervision, being on vacation during the intervention period, or declining participation. Of those invited, 50 completed the pre- and post-intervention assessments.

Residents allocated to the intervention group received a live online theoretical lecture, followed by a one-month tele-education program on hospital hyperglycemia delivered via WhatsApp. The program consisted of short videos and supporting materials prepared by three endocrinologists. Videos were shared multiple times, and residents were asked at the end of the intervention to confirm that they had watched the content, ensuring full exposure to the materials. Participants were also able to submit questions during the intervention period. The control group did not receive the lecture or any additional educational support and continued with routine clinical practice.

Medical knowledge regarding inpatient hyperglycemia was assessed in both groups before and after the intervention using a 10-item questionnaire with content validity evidence (13). This baseline assessment served to control for residents' prior knowledge

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levels and to enable within-group comparisons before and after the intervention. Each correct answer was scored as 1 point, for a maximum of 10 points.

In a second phase, clinical data were collected retrospectively from medical records of patients hospitalized under the care of participating residents during the intervention month. Data collection occurred after patient discharge and included demographics, comorbidities, prescriptions, and glycemic outcomes. The educational program was strictly educational and noninterventional in patient care.

The primary outcome was the improvement in residents' knowledge scores. Secondary outcomes were quality of inpatient care indicators, including appropriate prescription of capillary blood glucose monitoring based on nutritional route, use of oral antihyperglycemic agents in the hospital, and insulin therapy practices — particularly the use of NPH and correctional regular insulin. Additional outcomes were the frequency of hyperglycemia (capillary glucose > 180 mg/dL), hypoglycemia (< 70 mg/dL), and hospital LOS. Prescriptions from the last 5 days of hospitalization were reviewed by trained researchers using standardized procedures. Prescriptions were considered appropriate if they followed international recommendations (American Diabetes Association and Endocrine Society) and national guidelines from the Brazilian Diabetes Society on screening and management of hospital hyperglycemia in noncritical patients (18).

Dichotomous variables were described as absolute and relative frequencies (n, %). Continuous variables were presented as mean and standard deviation, or median and interquartile range (IQR) when non-normally distributed. Normality was assessed using the Shapiro–Wilk test. Between-group comparisons were performed using the Student's *t* test for normally distributed data and the Mann–Whitney U test for nonparametric data. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Logistic regression models were used to control for potential confounders and identify independent predictors, including variables with clinical or statistical relevance ($p < 0.20$) in univariate analysis. A significance level of 5% was adopted. Analyses were conducted using IBM SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA).

Sample size estimation was based on previous findings of low baseline knowledge regarding inpatient hyperglycemia, with an average accuracy of 46.4% in a pilot study (13). Assuming an improvement to ~80% correct responses after the intervention, a significance level of 5%, and statistical power of 80%, the minimum required sample size was 62 completed questionnaires.

Participation was voluntary, with written informed consent obtained from all residents. The questionnaire was applied confidentially, and clarifications were provided as needed. Patient data were collected only with institutional authorization, following formal approval for medical record use. Confidentiality and privacy, particularly for sensitive institutional information, were strictly maintained.

The study protocol was approved by the local research ethics committee (approval number 7.393.105) and registered at ClinicalTrials.gov (identifier NCT07108426).

## RESULTS

A total of 51 internal medicine residents were invited to participate: 15 (30%) from UFPel, 7 (14%) from UCPel, 7 (14%) from FURG, and 21 (42%) from UFCSPA. Of these, 50 residents completed both the pre- and post-intervention questionnaires, while one declined participation.

Before the intervention, the median number of correct answers was 5.5 (IQR 5–6) in the control group and 6 (IQR 5–7) in the intervention group ($p = 0.383$). After the intervention, knowledge scores were significantly higher in the intervention group compared with the control group (median 8 [IQR 8–9] vs. 6 [IQR 5–8]; $p = 0.005$). Within-group analysis showed an improvement of 2.5 points in the intervention group from pre- to post-test ($p < 0.001$). The control group also demonstrated a modest but significant improvement (median 5.5 [IQR 5–7] to 6 [IQR 5–8]; $p = 0.009$) (**Figure 1**). Subgroup analysis by center confirmed consistent knowledge gains across all participating hospitals ($p < 0.05$ for each).

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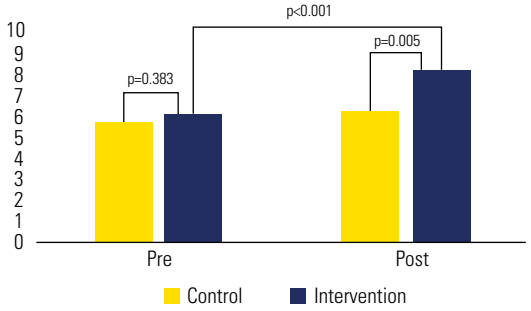



**Figure 1.** Improvement in medical knowledge scores between groups before and after the intervention, evaluated using a validated questionnaire comprising 10 questions on hospital hyperglycemia.

Following the educational intervention, 149 patient medical records were reviewed: 55 (36.9%) from UFPel, 31 (20.8%) from UCPel, 30 (20.1%) from FURG, and 33 (22.1%) from UFCSPA. Patients had a mean age of 67.8 ± 15.6 years, and 82 (55%) were female. Among them, 84 (56%) had a previous diagnosis of diabetes, with a median disease duration of 9.5 years (IQR 2–15 years). The remaining 65 patients (44%) were classified as having hospital-related hyperglycemia. None of the patients had type 1 diabetes. The most common reasons for hospitalization in general were infectious diseases (34.9%), cerebrovascular conditions (16.8%), and neoplasia (14.8%), followed by renal and hepatic diseases (7.4% and 6.7%, respectively). Other less frequent causes accounted for 13.4% of admissions. The most common comorbidity was cerebrovascular disease, present in 103 patients (69.1%). Table 1 summarizes the baseline characteristics of patients by study group.

Analysis of clinical outcomes is presented in Table 2. There was a nonsignificant trend toward more appropriate use of insulin in the intervention group (NPH insulin, p = 0.107; regular insulin prescribed based on isolated capillary glucose, p = 0.203).

**Table 1.** Clinical and demographic characteristics of hospitalized patients, by group (control vs. intervention)

| Variable | Control (n = 73) | Intervention (n = 76) | P value |
|---|---|---|---|
| Age, years | 69.5 ± 13.8 | 66.2 ± 17.2 | 0.2 |
| Male sex, n | 33 (45.2) | 34 (44.7) | 0.954 |
| Diabetes duration, years | 6.5 (2–14) | 10 (6–20) | 0.422 |
| HbA1c | 6.3 (5.75–8.75) | 6.6 (5.9–9.2) | 0.806 |
| Type 2 diabetes, n | 43 (58.9) | 41 (53.9) | 0.542 |

Data are shown as n (%), mean (standard deviation), or median (interquartile range).
HbA1c: glycated hemoglobin.

**Table 2.** Care indicators related to the management of hospital hyperglycemia, by group (control vs. intervention)

| Indicator | Control (n = 73) | Intervention (n = 76) | P value |
|---|---|---|---|
| Blood glucose at admission, n | 48 (65.8) | 51 (67.1) | 0.861 |
| Correct prescription, n | | | |
| Metformin | 7 (9.6) | 8 (10.5) | 0.685 |
| NPH insulin | 48 (65.8) | 59 (77.6) | 0.107 |
| Regular insulin | 55 (75.3) | 59 (77.6) | 0.742 |
| Sliding scale insulin | 51 (69.9) | 60 (78.9) | 0.203 |
| HbA1c at admission, n | 24 (32.9) | 27 (35.5) | 0.733 |
| Appropriate glucose monitoring, n | | | |
| Oral diet | 46 (63) | 48 (63.2) | 0.985 |
| Enteral tube feeding | 13 (17.8) | 6 (7.9) | 0.07 |
| Total parenteral nutrition | 0 | 1 (1.3) | 0.325 |
| Nothing by mouth | 1 (1.4) | 0 | 0.306 |
| Hyperglycemia, n | 56 (76.7) | 52 (68.4) | 0.257 |
| Hypoglycemia, n | 4 (5.5) | 7 (9.2) | 0.534 |
| Hospital stay, days | 13 (7–25) | 19 (11–39) | 0.009 |

Data are shown as n (%) or median (interquartile range).

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However, median hospital LOS was longer in the intervention group compared with the control group (19 days [IQR 11–39 days] vs. 13 days [IQR 7–25 days], respectively; p = 0.009). In exploratory analyses, the only factor significantly associated with prolonged LOS was the study center. The longest stays were observed at UFCSPA (median 26 days [IQR 14.5–46.5 days]) and UFPel (median 24 days [IQR 13–37 days]), while UCPel (median 13 days [IQR 6.5–18 days]) and FURG (median 7 days [IQR 6–11 days]) had shorter stays (p < 0.001).

## DISCUSSION

This multicenter randomized trial demonstrated that a structured educational intervention on hospital hyperglycemia, delivered via tele-education, significantly improved internal medicine residents' knowledge across four teaching hospitals. These results reinforce the importance of continuous medical education strategies aimed at enhancing physicians' understanding of inpatient glycemic management. The remote design — an online lecture combined with 1 month of support via WhatsApp, a widely available and free platform — illustrates the feasibility of implementing scalable, low-cost educational programs across diverse training contexts.

The consistent improvement observed across all participating hospitals suggests that such interventions may be applicable to different institutional settings. This finding is supported by previous evidence. A meta-analysis of randomized clinical trials reported that telemedicine interventions, particularly those involving feedback and two-way communication, were effective in improving glycemic control, resulting in significant reductions in HbA1c levels. (15) Our study adds to this body of knowledge by demonstrating similar benefits in the inpatient context, targeting the education of residents rather than direct patient management.

High adherence, with only one resident declining participation, underscores the acceptability and practicality of WhatsApp as an educational tool. This is particularly relevant for health systems facing resource constraints or geographic barriers, where accessible platforms are critical to ensure continuity of medical education.

Our previous observational study on medical education in hospital hyperglycemia found comparable improvements, though without the methodological rigor of randomization (13). Similarly, Desimone and cols. (17) conducted a randomized trial with residents and reported significant knowledge gains in the management of corticosteroid-induced and perioperative hyperglycemia, though without clear improvements in clinical outcomes. Together, these findings suggest that while education reliably enhances physician competence, translation into measurable patient outcomes likely requires additional strategies. Moreover, translating individual knowledge into effective clinical practice is inherently complex. Factors such as institutional culture, competing clinical priorities, and limited feedback mechanisms may hinder behavior change, despite educational improvement.

Analysis of patient records in our study indicated a nonsignificant trend toward more appropriate use of NPH insulin and correctional regular insulin prescriptions in the intervention group. This suggests a potential influence of the educational program on prescribing practices, although knowledge gains did not fully translate into consistent behavior change. Similar challenges have been described in multicenter studies of resident physicians, which identified gaps in confidence and familiarity with insulin use and institutional protocols (14). These data highlight the importance of structured ongoing education combined with supervised clinical support to achieve more robust improvements in care quality and patient safety.

An unexpected finding was the longer hospital LOS observed in the intervention group. One possible explanation is a learning-curve effect, in which residents exposed to the intervention sought to optimize glycemic control more rigorously, inadvertently prolonging hospitalization. Another contributing factor may be institutional heterogeneity, as substantial variation in LOS was observed across centers, likely reflecting differences in workflows, case complexity, and resource availability. In addition, the high prevalence of cerebrovascular disease among patients may have contributed to these differences, as this comorbidity is often associated with longer hospitalizations and greater clinical complexity. Although our sample size

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was limited, this factor likely reflects the real-world profile of the inpatient population across centers. Previous studies have shown that patients with diabetes are more prone to prolonged or repeated hospitalizations, not only due to disease severity but also because of systemic factors in care coordination (6). Future research should examine whether the longer LOS is transient and improves as residents gain experience and institutional systems adapt.

This study has limitations. The sample size of patients was modest, follow-up was short, and knowledge retention over time was not assessed. Verification of residents' exposure to educational materials relied on self-report, and the internal consistency of the short knowledge questionnaire was moderate. Qualitative evaluation of participants' perceptions was not performed, and cost-effectiveness or feasibility analyses were not included. In addition, standardized severity indices were not collected across centers, which limits further adjustment for baseline clinical complexity. Nevertheless, residents' prior knowledge was controlled through the pre-test and post-test design, ensuring comparable baseline levels between groups. Despite these limitations, strengths include the randomized multicenter design — rare in medical education studies focused on inpatient glycemic control — and the use of a pragmatic, low-cost tele-education platform. By evaluating both knowledge and clinical outcomes, this trial provides a more integrated assessment of educational interventions in the hospital setting.

In conclusion, the structured tele-education intervention significantly improved internal medicine residents' knowledge on the management of hospital hyperglycemia, with consistent effects across multiple teaching hospitals. While trends toward better prescribing practices were observed, further research with larger samples and longer follow-up is required to determine whether such educational strategies translate into sustained improvements in clinical outcomes.

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**Data availability:** datasets related to this article will be available upon request to the corresponding author.

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original

# Treatment outcomes in patients with relapsed Graves' disease

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## ABSTRACT

**Objective:** The objective of this study was to assess the outcomes of various treatment strategies in patients with relapsed Graves' disease (GD) after standard antithyroid drug therapy (ATD). The three treatment strategies evaluated were radioiodine therapy (RAI), continuous low-dose methimazole (MMI), and a second course of MMI. **Subjects and methods:** This was a retrospective cohort study of 330 patients with GD who received initial MMI therapy for 12 to 24 months, of whom 159 subsequently relapsed. The cohort was categorized into three groups: 39 patients received RAI therapy plus levothyroxine, 46 patients received continuous low-dose MMI, and 74 patients received a second course of MMI. The analysis included thyroid function monitoring, progression of thyroid eye disease (TED), quality-of-life measures, and changes in body weight during follow-up. **Results:** The group receiving continuous low-dose MMI had a longer period of euthyroidism compared with the other groups. The TED outcomes and body weight changes were similar across all groups. Quality of life did not differ significantly across groups. Notably, 45% of patients in the group receiving a second MMI course relapsed when MMI was discontinued after a mean treatment duration of approximately 42 months. **Conclusion:** The administration of continuous low-dose MMI is an alternative for managing patients with relapsed GD, particularly for those who prefer to avoid definitive treatments.

**Keywords:** Graves' disease; hyperthyroidism; thyroid eye disease

## INTRODUCTION

Graves' disease (GD) is the most prevalent cause of hyperthyroidism in iodine-sufficient regions, with an annual incidence of 20 to 50 cases per 100,000 (1). It can manifest at any age; however, the peak incidence typically occurs in patients aged 30 to 60 years (1), with a notably higher incidence among African Americans (2) and occurring more frequently in women (3).

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Antithyroid drugs (ATDs) are the first-line treatment for GD worldwide (1,4-10); they inhibit thyroid hormone synthesis and may exert both direct and indirect effects on the immune system (10). The most frequent side effects are mild and include skin rash, arthralgia, and urticaria, occurring in 1%–5% of cases. Rare but severe side effects occur in less than 0.5% of cases and include agranulocytosis, fulminant hepatitis, and cholestatic jaundice (10,11); these are typically associated with high ATD doses and occur within the first 6 months of treatment (10).

According to the latest guidelines (12,13), the recommended duration of initial ATD therapy is 12–18 months, with a maximum remission rate of 50%–60% observed within this timeframe. Patients exhibiting persistently positive TSH receptor antibodies (TRAb) after 12–18 months may continue ATD therapy;

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otherwise, options include radioiodine (RAI) therapy or thyroidectomy. A meta-analysis assessing the use of ATD over a mean period of 5 years indicated that long-term treatment is safe, particularly at low doses in adults, suggesting it should be considered as an alternative for GD treatment; this approach is associated with minor complication rates, ranging from 2%–36%, while more serious complications are associated with higher doses, the use of propylthiouracil, and treatment in pediatric populations (14).

The long-term use of low ATD doses appears to be a viable option for patients who prefer to avoid RAI or thyroidectomy because of radiation exposure and potential postoperative complications, respectively (15,16). Some authors propose low ATD doses in GD as an alternative treatment for selected groups, analogous to other chronic diseases (17).

The primary objective of this study was to evaluate the effectiveness of the following treatment modalities in patients with GD who received standard ATD therapy for 12–24 months: (1) continuous use of a low MMI dose (MMIc), (2) a second course of ATD therapy (MMIs) after relapse, continued until TRAb became negative, and (3) RAI plus levothyroxine replacement. Notably, none of the patients in this study underwent surgery for GD treatment. Secondary study objectives include evaluating the safety of the three treatments, comparing the three groups in terms of thyroid eye disease (TED) outcomes, and assessing the patients' quality of life and changes in body weight. Predictors of remission were also analyzed in the MMIs group.

## SUBJECTS AND METHODS

### Patients with Graves' disease

This was a retrospective study conducted at a Brazilian university hospital center in accordance with the ethical principles of Good Clinical Practice, the Declaration of Helsinki, and the International Council for Harmonisation (ICH) E6 guidelines. The study was approved by the institution's Ethics Committee under the approval number CAAE 39062520.8.0000.5481. The study complied with the countries' legal requirements and received approval from an institutional review board, with all patients providing signed informed consent. In total, 330 patients diagnosed with GD who underwent treatment with methimazole (MMI) for 12–24 months were evaluated.

The diagnosis of GD was based on the presence of a diffuse goiter on physical examination and confirmed by thyroid ultrasound, together with decreased TSH and elevated free T4 levels. The etiology of hyperthyroidism was established through positive TRAb levels or diffuse iodine uptake on thyroid scintigraphy. Relapse was defined as the reappearance of clinical symptoms of hyperthyroidism after discontinuation of MMI, confirmed by laboratory tests (TSH suppression and elevated free T4), after the end of treatment, with positive TRAb. Remission was defined as persistent clinical and laboratory euthyroidism for more than 1 year after MMI interruption. Exclusion criteria included age below 18 years, use of propylthiouracil for hyperthyroidism management, pregnancy or lactation, and thyroidectomy or RAI as primary treatment.

### Study design

Data on 330 consecutive patients with GD were retrospectively reviewed. The patients were treated with MMI for 12–24 months, and the drug was discontinued in those who tested negative for TRAb. A total of 159 patients experienced relapse after ATD interruption. These patients were offered three treatment options: (1) RAI therapy (RAI group) plus levothyroxine replacement, (2) reintroduction of MMI in continuous low dose uninterruptedly, using the lowest MMI dose to maintain euthyroidism during follow-up (MMIc group), and (3) a second MMI course or at least 24 months or until TRAb became negative (MMIs group). Using a standardized algorithm, D.V. or R.B.S. (both authors and physicians) discussed the advantages and disadvantages of each treatment with the patients, allowing the patients to make the final decision.

In the RAI group (39 patients), individuals were informed of the potential side effects of RAI. A fixed RAI dose of 15 mCi was used, and levothyroxine replacement was initiated when TSH levels exceeded 5.0 mIU/L. All patients developed hypothyroidism after 6 months. The MMIc group (46 patients) received MMI in continuous low doses to maintain euthyroidism. The MMIs group (74 patients) received a second MMI course for at least 24 months or until testing

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negative for TRAb. Patients in both MMI groups were informed of the potential side effects of MMI and symptoms of thyrotoxicosis.

All patients underwent clinical and laboratory evaluations during the first year, every 3 months, and subsequently every 6 months during routine consultations. Data collected included smoking status, symptoms of hyperthyroidism, and other comorbidities (diabetes, hypertension, arrhythmia, cardiovascular events, and other autoimmune diseases). Body weight, TED, and side effects associated with the medication were assessed for those receiving treatment. Laboratory tests, including TSH, free T4, white blood cell count, serum aspartate aminotransferase, serum alanine aminotransferase, gamma-glutamyl transferase, fasting glucose, and creatinine, were conducted at each visit. All patients were encouraged to adhere to and maintain their treatments throughout the follow-up period.

## Weight evaluation

Patient weight was measured in routine medical appointments using certified scales. Prior to weighing, patients were instructed to remove shoes, coats, and light jackets.

## Thyroid eye disease evaluation

Assessment of TED was performed using the Clinical Activity Score (CAS) as described by Morrits and cols. (18). This scoring system evaluates seven TED manifestations: eyelid edema, conjunctival edema (chemosis), caruncle edema, conjunctival hyperemia, eyelid erythema, spontaneous retrobulbar pain, and pain due to eye movement. One point is assigned for each manifestation, ranging from zero (no activity) to seven (very high activity). Palpebral fissure, visual acuity, and intraocular pressure were measured. Proptosis was assessed using a Hertel exophthalmometer, and diplopia was classified as constant, inconstant, and intermittent. Worsening of CAS was defined as an increase of at least 2 points from baseline, and inflammation was considered active when CAS was > 3. Patients in the RAI group with CAS > 3 were treated with prednisone at a dose of 0.5 mg/kg/day, followed by tapering over 30–40 days until discontinuation (19).

## Quality-of-life assessment

Quality of life was assessed at the end of the study using ThyPRO-39 (20), a questionnaire specific to thyroid diseases. This questionnaire consists of 39 items that evaluate signs and symptoms of thyroid disease across multiple domains, including hyperthyroidism, hypothyroidism, goiter, TED, fatigue, cognitive decline, anxiety, depression, social impairment, daily and sexual life, and aesthetic concerns. Each item is rated on a 5-point Likert scale (0 = not at all; 1 = a little; 2 = moderately; 3 = quite a bit; 4 = very much). Domain and overall scores are then linearly transformed to a 0–100 scale, with higher scores indicating greater symptom burden and poorer quality of life. The questionnaire was administered exclusively to patients with euthyroidism.

## Thyroid dysfunction and TRAb evaluations

Thyroid function was evaluated during routine medical appointments by measuring TSH and free T4 levels, which were monitored throughout follow-up. Thyroid function status was categorized as euthyroidism (TSH and free T4 levels within reference values), overt hyperthyroidism (TSH levels below and free T4 elevated above reference values), subclinical hyperthyroidism (TSH levels below and free T4 within reference values), overt hypothyroidism (elevated TSH levels and free T4 below reference values), and subclinical hypothyroidism (elevated TSH levels with free T4 within reference values). Thyroid function tests were performed every 3 months during the first year, then every 6 months thereafter. The percentage of periods in euthyroidism was calculated by dividing the number of times thyroid function was within the euthyroid range by the total number of thyroid function assessments performed during the study period. In the MMIs and MMIc groups, TRAb levels were measured yearly until they became negative.

## Laboratory analysis

Reference values for serum measurements included TSH (0.4–4.5 mIU/L) and free T4 (0.8–1.9 ng/dL or 10.3–24.5 pmol/L), as measured using the Elecsys system (Roche Diagnostics, Rotkreuz, Switzerland). Serum TRAb levels were considered positive when

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> 10 IU/mL, as assessed using a radioreceptor assay (RSR Limited, Cardiff, United Kingdom). Thyroid size was evaluated by ultrasound (GE Healthcare, Chicago, IL, USA) and was considered normal when measuring 6–15 mL. Thyroid 99mTc-pertechnetate uptake (Siemens Healthineers, Erlangen, Germany) was considered normal within the reference range of 0.35%–1.7%. Thyroid peroxidase antibodies (TPOAb; positive when > 35 IU/mL) and antithyroglobulin antibodies (TgAb; positive when > 40 IU/mL) were measured routinely at baseline using a chemiluminescence assay (Elecsys system, Roche Diagnostics). Plasma glucose levels were measured using the oxidase method, and serum creatinine and liver enzymes were measured using colorimetric and enzymatic assays, respectively (Roche Diagnostics).

### Statistical analysis

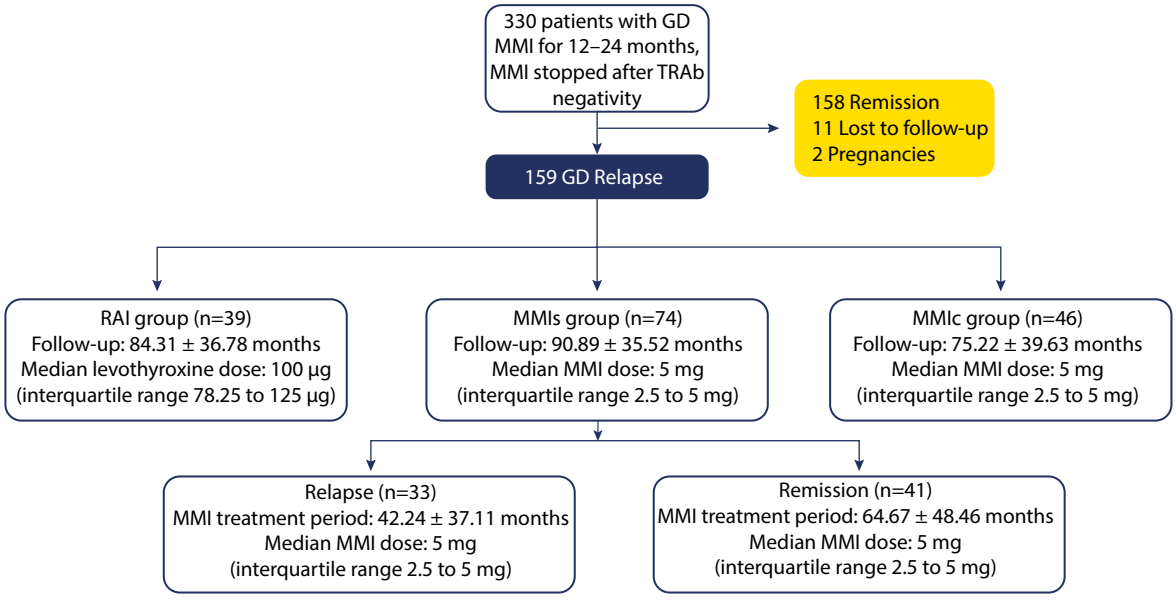
A descriptive analysis presents frequency tables for categorical variables and measures of central tendency and dispersion for numerical variables. The Mann-Whitney test was applied for comparisons between two groups, whereas the Kruskal-Wallis test was used for comparisons among three groups, followed by Dunn's *post hoc* test for multiple comparisons when appropriate. The chi-square test or Fisher's exact test was employed as required. Analysis of variance (ANOVA) was performed to compare weight change across age groups. A significance level of 5% was adopted for all statistical tests. Analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

## RESULTS

The study design and patient distribution by treatment are shown in **Figure 1**.

**Table 1** describes the baseline characteristics of the patients at relapse, after an initial course of ATD. No significant differences among groups were observed regarding age, sex, smoking status, thyroid function, positive thyroid antibodies, TED evaluated by the CAS scoring system, and body weight. Measurement of TRAb, obtained when patients relapsed after the initial ATD course, showed positive results in 97.4% of patients in the RAI group, 95.7% in the MMIc group, and 100% in the MMIs group (p = NS). Three patients with negative TRAb had relapsed disease, one in the RAI group and two in the MMIc group.

The mean follow-up durations in the MMIc, MMIs, and RAI groups were 75.2 ± 39.6 months, 90.9 ± 35.5




Mean values are presented with standard deviation, and median values are presented with interquartile range. GD: Graves' disease; RAI: radioiodine therapy; MMIc: continuous low-dose methimazole; MMIs: second course of methimazole after relapse continued until TRAb became negative.

**Figure 1.** Study design and distribution of patients with Graves' disease according to treatment.

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**Table 1.** Initial baseline characteristics of the three groups

| Parameters | RAI group | MMIc group | MMIs group | *P value* |
|---|---|---|---|---|
| Number, n | 39 | 46 | 74 | |
| Age, years | 45.10 ± 14.00 | 46.02 ± 13.34 | 46.04 ± 13.16 | |
| Sex (F/M ratio), n | 34/5 | 35/11 | 65/9 | NS |
| Smokers, % | 33% | 28.3% | 31.1% | NS |
| TSH level, mIU/L | 0.024 ± 0.084 | 0.021 ± 0.046 | 0.015 ± 0.031 | NS |
| Free T4, ng/dL | 3.87 ± 1.87 | 3.88 ± 2.26 | 3.96 ± 2.37 | NS |
| Positive TRAb, % | 97.4% | 95.7% | 100% | NS |
| Positive TPOAb, % | 68.8% | 58.5% | 70.6% | NS |
| Positive TgAb, % | 46.7% | 40% | 41.2% | NS |
| Thyroid US, mL | 37.40 ± 48.13 | 20.17 ± 12.39 | 21.55 ± 12.56 | NS |
| CAS | 0.62 ± 1.04 | 0.76 ± 1.21 | 0.47 ± 1.06 | NS |
| CAS 0, n | 27 | 30 | 56 | |
| CAS 1, n | 4 | 5 | 10 | |
| CAS 2, n | 6 | 5 | 5 | |
| CAS 3, n | 1 | 4 | 0 | |
| CAS 4, n | 1 | 2 | 1 | |
| CAS 5, n | 0 | 0 | 2 | |
| CAS 6, n | 0 | 0 | 0 | |
| CAS 7, n | 0 | 0 | 0 | |
| Weight, kg | 59.08 ± 14.68 | 67.20 ± 13.55 | 65.82 ± 12.40 | NS |
| Follow-up, months | 84.31 ± 36.78 | 75.2 ± 39.6 | 90.89 ± 35.52 | |

Data are shown as mean ± standard deviation or percentage (%).F: female; M: male; RAI: radioiodine therapy; MMIc: continuous low-dose methimazole; MMIs: second course of methimazole after relapse until TRAb became negative; TSH: thyrotropin; FT4: free thyroxine; TRAb: thyroid receptor antibody; TPOAb: thyroperoxidase antibody; TgAb: thyroglobulin antibody; US: ultrasound measurement of thyroid volume; CAS: clinical active score; kg: kilogram; NS: not significant.

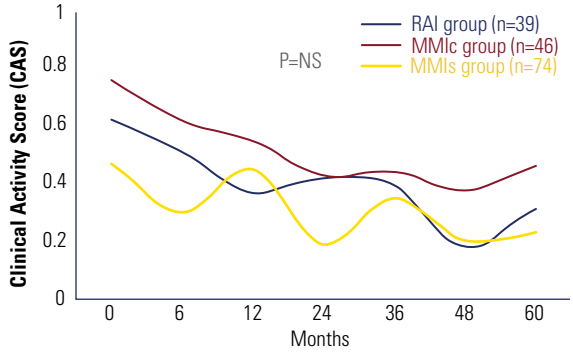
months, and 84.3 ± 36.8 months, respectively (p = NS). The median MMI dose in the MMIc and MMIs groups was 5 mg/day (interquartile range [IQR] 2.5–5 mg), and the median levothyroxine replacement dose in the RAI group was 100 μg/day (IQR 78.25–125 μg) (**Figure 1**).

Thyroid function was evaluated in the three groups every 3 months during the first year and every 6 months thereafter. The percentage of periods in euthyroidism was calculated by dividing the number of times thyroid function was within the euthyroid range by the total number of thyroid function assessments performed during the study period in each group. Euthyroidism was more frequent in the MMIc group (78.7%) compared with the RAI group (51.2%, which notably received levothyroxine replacement), and the MMIs group (55.4%) (p < 0.05). No side effects were observed with prolonged MMI use or with RAI treatment.

No significant differences in mean CAS values were observed among the three groups at baseline or during follow-up (**Figure 2**). Quality of life — evaluated in euthyroid patients at the end of the study using the ThyPRO-39 questionnaire — was similar across all three groups. **Figure 3** illustrates the percentage weight variation across the three groups during the study. All groups experienced weight gain during treatment of hyperthyroidism, but no significant differences were observed among them during follow-up. Additionally, weight gain was evaluated in relation to participants' age to assess its influence. The ANOVA test showed no statistically significant differences among the three groups across all periods.

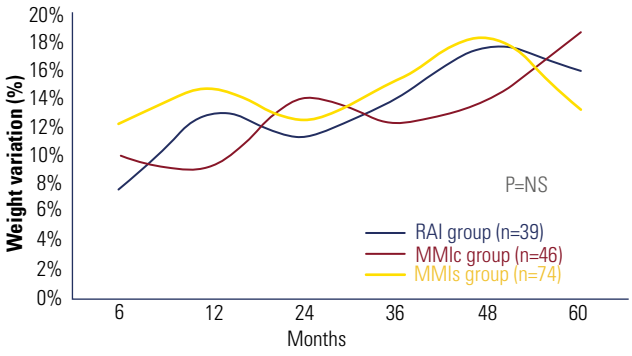
Regarding cardiovascular disease, there were no significant differences between the groups. Acute coronary syndrome was present in two patients in the MMs and RAI group and in one patient in the MMI group. Atrial fibrillation occurred in one case in the RAI and MMIc groups. The three groups had two patients with ischemic stroke. No side effects related to MMI use were observed in the MMIc and MMIs groups.

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RAI: radioiodine therapy; MMIc: continuous low-dose methimazole; MMIs: second course of methimazole after relapse continued until TRAb became negative; NS: not significant.

**Figure 2.** Progression of the Clinical Activity Score (CAS) during follow-up.




RAI: radioiodine therapy; MMIc: continuous low-dose methimazole; MMIs: second course of methimazole after relapse continued until TRAb became negative; NS: not significant.

**Figure 3.** Percentage of weight variation during follow-up in the three groups.

### Remission rates in the MMIs group

All patients in the MMIs group were euthyroid and tested negative for TRAb when ATD was discontinued after the second treatment course at a mean treatment duration of 54.39 ± 44.77 months. Among the 74 patients in this group, 33 (44.6%) relapsed at a mean of 12.36 ± 10.87 months after MMI interruption. Table 2 outlines the characteristics of these patients at cessation of the second MMI course; no significant differences were observed between the remission and relapse groups with respect to sex, age, smoking status, thyroid antibodies, or TED evaluated by CAS. However, treatment duration in the remission group (64.67 ± 48.46 months) was longer than in the relapse group (42.24 ± 37.11 months; $p < 0.01$). The mean follow-up duration after MMI interruption in the remission group was 36.5 ± 6.29 months. Notably, quality-of-life was evaluated in the MMIs group in patients who were euthyroid, those with remission, and those who relapsed and resumed MMI therapy.

## DISCUSSION

The management of GD has long posed a clinical challenge. Recent guidelines from both the American Thyroid Association (13) and the European Thyroid Association (12) support the consideration of a second course or prolonged ATD use in patients who experience disease relapse. Moreover, a recent international survey indicated that over 90% of respondents favored ATD therapy as the initial treatment choice for a typical case of uncomplicated GD (21). This survey (21) further investigated treatment preferences in cases of GD relapse, revealing that 60% of clinicians selected ATDs as the first-line approach, followed by RAI at 37%, and surgery at just 3%. Nevertheless, the evidence base for these preferences remains limited. Few studies have directly compared ATD and RAI therapies in relapsed GD patients, and the optimal duration of a subsequent ATD course, as well as its impact on long-term remission rates, has yet to be established. The present study aimed to address this clinically important and underexplored issue.

A real-world study conducted in Sweden, with a long-term follow-up of ATD treatment for GD, reported relapse rates of 59.7% over a follow-up period of 6–10 years (22). These findings are comparable to those of the present study, in which 159 out of an initial cohort of 330 patients experienced relapses, corresponding to a 48% relapse rate. Notably, similar relapse rates were observed in patients who used MMI in the first and second cycles. The MMIs group, after a mean duration of MMI use of approximately 6 years, had a 45% relapse rate.

In contrast, Azizi and cols. (23) reported a significantly lower relapse rate in a group of patients treated with long-term ATD (8 years; relapse rate 15%) compared with another group treated with short-term ATD (approximately 2 years; relapse rate 53%) during a mean follow-up of 4 years. A relationship between ATD therapy duration and remission rates has also been reported in a Korean study (24), which found remission rates of 57% for 12 months of ATD treatment and 81% for 72 months.

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**Table 2.** Clinical characteristics of patients who experienced relapse versus remission upon cessation of methimazole (MMI)

| Parameters | Relapse | Remission | *P value* |
|---|---|---|---|
| Number, n | 33 | 41 | |
| Age, years | 44.97 ± 14.71 | 46.90 ± 11.88 | NS |
| Sex (F/M ratio), n | 30/3 | 35/6 | NS |
| Smokers, % | 24.2% | 36.6% | NS |
| Positive TRAb, % | 100% | 100% | NS |
| Positive TPOAb, % | 62.1% | 76.9% | NS |
| Positive TgAb, % | 42.9% | 40% | NS |
| Thyroid US, mL | 21.76 ± 12.37 | 21.39 ± 12.88 | NS |
| CAS | 0.30 ± 0.77 | 0.60 ± 1.24 | NS |
| CAS 0, n | 26 | 29 | |
| CAS 1, n | 6 | 4 | |
| CAS 2, n | 0 | 5 | |
| CAS 3, n | 0 | 0 | |
| CAS 4, n | 1 | 0 | |
| CAS 5, n | 0 | 2 | |
| CAS 6, n | 0 | 0 | |
| CAS 7, n | 0 | 0 | |
| MMI use, months | 42.24 ± 37.11 | 64.67 ± 48.46 | 0.0065 |
| Weight, kg | 67.51 ± 14.15 | 64.74 ± 11.31 | NS |
| Follow-up, months | 93.38 ± 38.64 | 88.95 ± 33.24 | |

Data are shown as mean ± standard deviation or percentage (%).F: female; M: male; TRAb: thyroid receptor antibody; TPOAb: thyroperoxidase antibody; TgAb: thyroglobulin antibody; US: ultrasound measurement of thyroid volume; CAS: clinical active score; kg: kilogram; NS: not significant.

The discrepancies between these findings may stem from differences in genetic predisposition or environmental factors among the study populations. Additionally, the patients in the present study experienced relapse after initial treatment, whereas those in the study by Azizi and cols. (23) and the Korean study received continuous ATD therapy for over 5 years. It is possible that ongoing ATD treatment, while maintaining euthyroidism, positively influences the autoimmune system.

The ultimate goal of GD treatment is to control thyroid function, as thyroid dysfunction is associated with deleterious effects on quality of life, morbidity, and mortality, even in subclinical forms (25-27). The current study demonstrated that euthyroidism was more frequent in the MMIc group (78%) than in the RAI group (plus levothyroxine replacement; 51%) and the MMIs group (55%) over a follow-up period exceeding 6 years. Several studies have evaluated thyroid function control in patients with hypothyroidism from various etiologies, reporting euthyroidism rates of 60%–65% (28-30), highlighting the challenges in managing patients with hypothyroidism. Other cohorts describe lower euthyroidism rates with the use of RAI and levothyroxine replacement when compared with ATD (5). Why thyroid function is better controlled with MMI (continuous, low-dose) than with levothyroxine remains an intriguing question.

The results in the MMIs group can be viewed as a "glass-half-full/glass-half-empty" dilemma. On the one hand, nearly half of the patients achieved remission and no longer required ATD; on the other hand, a substantial proportion relapsed after stopping ATD, exposing them to the adverse effects of hyperthyroidism, necessitating the reintroduction of ATD. Remission rates in this group were 55%, and longer treatment duration was identified as the sole variable distinguishing patients who relapsed from those who remained in remission. Notably, TRAb was negative in both groups when MMI was discontinued and was not a reliable prognostic marker. Although no direct linear relationship exists, the data suggest that increased

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treatment duration correlates with higher remission rates (31). Wiersinga and cols. recently proposed that long-term treatment with ATDs (*i.e.*, 5–10 years) is feasible and associated with fewer recurrences (15%) compared with short-term treatment (*i.e.*, 12–18 months) (32).

Analysis of TED across the three groups revealed no differences in progression or resolution during the follow-up period. Several key aspects warrant emphasis: most patients had either no TED or only mild TED; patients in the RAI group with risk factors for deteriorating TED post-RAI received glucocorticoid prophylaxis in accordance with established guidelines (33). The results of the present study reinforce the safety and potential benefits of long-term ATD treatment for TED.

Quality of life and body weight gain are two critical concerns for patients with thyroid dysfunction. Analysis using the ThyPRO-39 questionnaire indicated no significant difference in quality of life among the groups. To avoid confounding variables, data were analyzed exclusively in patients with euthyroidism. Furthermore, body weight increased in all three groups, with no significant differences among them. Previous studies have investigated body weight changes associated with GD treatments (5,34). Treatment with RAI appears to be associated with significantly greater weight gain than ATD, likely influenced by thyroid function control (35). Ultimately, the present study reinforces the safety of prolonged MMI use, consistent with findings from recent meta-analyses (15).

This study has some limitations. As this was a retrospective study, it may be subject to bias, and the findings should be interpreted with caution. Additionally, the quality-of-life questionnaire was administered only at the end of the study, rather than at baseline, which affected data interpretation. Furthermore, due to the small number of patients with moderate-to-severe TED, we were unable to evaluate how patients with more severe forms of TED would respond to the three treatment options.

This study aimed to address an essential question in clinical practice: Do patients who relapse from GD and restart ATD require a second course of ATD (to aim for remission) or should they remain on a continuous low-dose of ATD? To our knowledge, this is the first study comparing a second course of MMI with continuous MMI and RAI treatments in patients with relapsed GD. This represents a real-world dilemma for clinicians and patients when deciding on subsequent treatment strategies following GD relapse.

In conclusion, our study demonstrated that continuous long-term use of low-dose MMI may be an alternative treatment option for patients who relapse after initial ATD treatment for 12–18 months, and is associated with improved thyroid function control and no differences in TED outcomes, quality of life, or body weight changes compared with a second course of MMI or RAI therapy. Additionally, if a second course of ATD is chosen after ATD is interrupted and TRAb is negative, it is recommended to extend treatment duration to longer periods (> 5 years), as a shorter second MMI course may be associated with relapse rates similar to those of the initial course.

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original

# Individualized aerobic session modulates key metabolic genes in liver and muscle of male offspring from obese dams

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## ABSTRACT

**Objective:** Exercise interventions can improve parameters in offspring predisposed to metabolic issues. In this study, we investigate whether acute aerobic exercise in offspring can improve metabolism via miRNA modulation in mice programmed by maternal obesity. **Materials and methods:** Female Swiss mice fed either a standard chow (C) or a high-fat diet (HF) during gestation and lactation were mated with C male mice. Offspring fed the C diet underwent swimming exercise protocols, consisting of water adaptation (14 days), a lactate minimum test, and an aerobic intensity exercise session or no exercise at 84 days of age. **Results:** Offspring of obese dams (OHF) exhibited increased hepatic glycogen and triglyceride compared to offspring of control dams (OC). However, in offspring of obese dams subjected to an individualized aerobic session (OHF-E), these parameters did not differed from the other groups. Hepatic gene expression analysis showed that *miR-122* was upregulated in OHF-E, inversely to *Agpat* levels. Additionally, OHF exhibited higher *miR-370* and lower *Cpt1a* levels; exercise restored *miR-370* and elevated *Cpt1a* levels in OHF-E. Regarding muscle tissue, exercise reduced *Ptp1b* expression in OHF-E and increased *Hif1a* and *Pparg*, despite no changes observed in *miR-206* levels. **Conclusion:** A single session of exercise significantly affected miRNA and transcript levels related to hepatic lipid and muscle glucose metabolism, suggesting that even one bout of exercise can benefit offspring in the context of maternal metabolic programming. This highlights tissue responsiveness and adaptive capacity, warranting further investigation into its potential as a long-term, non-pharmacological intervention.

**Keywords:** Aerobic exercise; microRNAs; maternal obesity; DOHaD

## INTRODUCTION

The impact of the perinatal environment on offspring's health is part of the Developmental origins of health and disease (DOHaD) research field, which has been extensively studied for a better understanding of chronic noncommunicable disease genesis (1). Maternal obesity during critical developmental periods is related to predisposition to obesity, hypercholesterolemia, hypertension, body weight gain, insulin resistance, and fatty liver in offspring (2). Investigations in animal models have demonstrated an association between these alterations and epigenetic modulations in metabolically active tissues during pregnancy and lactation (3).

Among all recognized epigenetic mechanisms, changes in the expression of microRNAs (miRNAs) have been extensively studied due to their capacity to regulate gene expression at the post-transcriptional level (4). Specific types of miRNAs, including myo-miRNAs like *miR-206* and liver-specific miRNAs such as *miR-122* and *miR-370*, are highly abundant in skeletal muscle and liver tissue, respectively, and play essential roles in various biological processes, including muscle regeneration (5) and lipid metabolism (6).

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The literature shows that miRNAs can also be modulated by physical exercise (6-8). Concomitantly, while not addressed here, the release of trained muscle exosomes carrying a specific miRNA signature could explain, at least in part, the communication between muscle and target tissues during exercise (9). Evidence suggests that maternal exercise has a protective effect on metabolic programming and may lead to the reprogramming of the offspring, resulting in long-term benefits such as reduced liver fat accumulation and improved glucose tolerance (10). Few studies have shown that early post-weaning training interventions in offspring can improve metabolic parameters impaired by maternal obesity, regardless of nutritional modifications (11). However, to our knowledge, there is a gap in the literature regarding whether physical exercise can modify the miRNA expression in offspring promoted by an obesogenic environment during development, potentially representing an early event in the glycemic homeostasis regulation.

Thus, this study aimed to investigate whether a single aerobic session in adult offspring could modulate the expression of miRNAs and their target genes related to lipid metabolism and insulin sensitivity in the liver and muscle, which may be programmed by maternal obesity.

## MATERIALS AND METHODS

### Animals and diet

In total, 10 five-week-old female Swiss mice were randomly chosen to receive either a standard chow (C, 3.5 kcal/g) or a high-fat diet (HF, F45%, 4.6 kcal/g) (**Supplementary Table 1**) *ad libitum* for an adaptation period of 21 days, and were then mated for 3 days with male mice fed only C (2 females and 1 male per cage). Animals were housed in polypropylene micro-isolators at 22°C ± 1°C with lights on from 06:00 to 18:00 h. The HF diet was prepared as described elsewhere (3). Females were fed the same diet as during the adaptation period (C or HF) throughout gestation and lactation (**Figure 1A**).

On the delivery day, litters were adjusted to eight (n = 8) pups per dam. Offspring were weaned at d18 and fed C. On d68, all mice underwent a water adaptation protocol, and by d82, they were subjected to the lactate minimum test (LMT) to determine individual maximal aerobic capacity. Then, two pups from each litter were randomized into exercised and non-exercised groups, giving rise to four groups: non-exercised offspring (OC, n = 5 and OHF, n = 5) and exercised offspring (OC-E, n = 5 and OHF-E, n = 5). A single exercise session was performed at d84. In this case, OC-E and OHF-E were subjected to physical effort at 70% of the LM intensity for 1 hour. After the exercise, the animals were fasted for 4 hours, followed by anesthetic deepening (139.2 mg/bw ketamine, 18.4 mg/bw xylazine, and 4 mg/bw diazepam) and then euthanized by decapitation for sample collection. Maternal data were collected after weaning. Dams underwent 8h of fasting before euthanasia, when the epigonadal white adipose tissue was weighed to measure adiposity, and blood was collected to measure fasting blood glucose and insulin. All procedures complied with the ethical standards of the Brazilian national guidelines on the care and use of laboratory animals. The study was approved by the Research Ethics Committee on the Use of Animals (Protocol Number 3913-1). Metabolic assessments and molecular evaluations were conducted independently and in a blinded manner to reduce bias.

### Adaptation to water

Individual adaptation to the aquatic environment began at d68 and lasted 14 days to minimize stress, as described elsewhere (12). This involved the progressive exposure of all mice from each group to water temperatures of 31±1°C in polyvinyl chloride tanks (30 cm diameter × 100 cm depth) with smooth surfaces to prevent mice from resting on the bottom of the tank. Briefly, mice were placed in shallow water (3 cm deep) for 15 min/day over the first three days. By the fourth day, they transitioned to swimming in deep water (100 cm deep) for 2 min, with an increment of 2 min/day until the eighth day of the procedure. From the ninth day, mice swam in deep water with a progressively increasing load (3%–15% bw) tied to the back of each animal, for periods varying from 5 minutes to 30 seconds over the course of six days, always followed by another 5-min session without load. Since the adaptation protocol was performed individually, while one group of animals underwent the adaptation protocol or exercise, the remaining groups were housed in their usual cages with food *ad libitum*.

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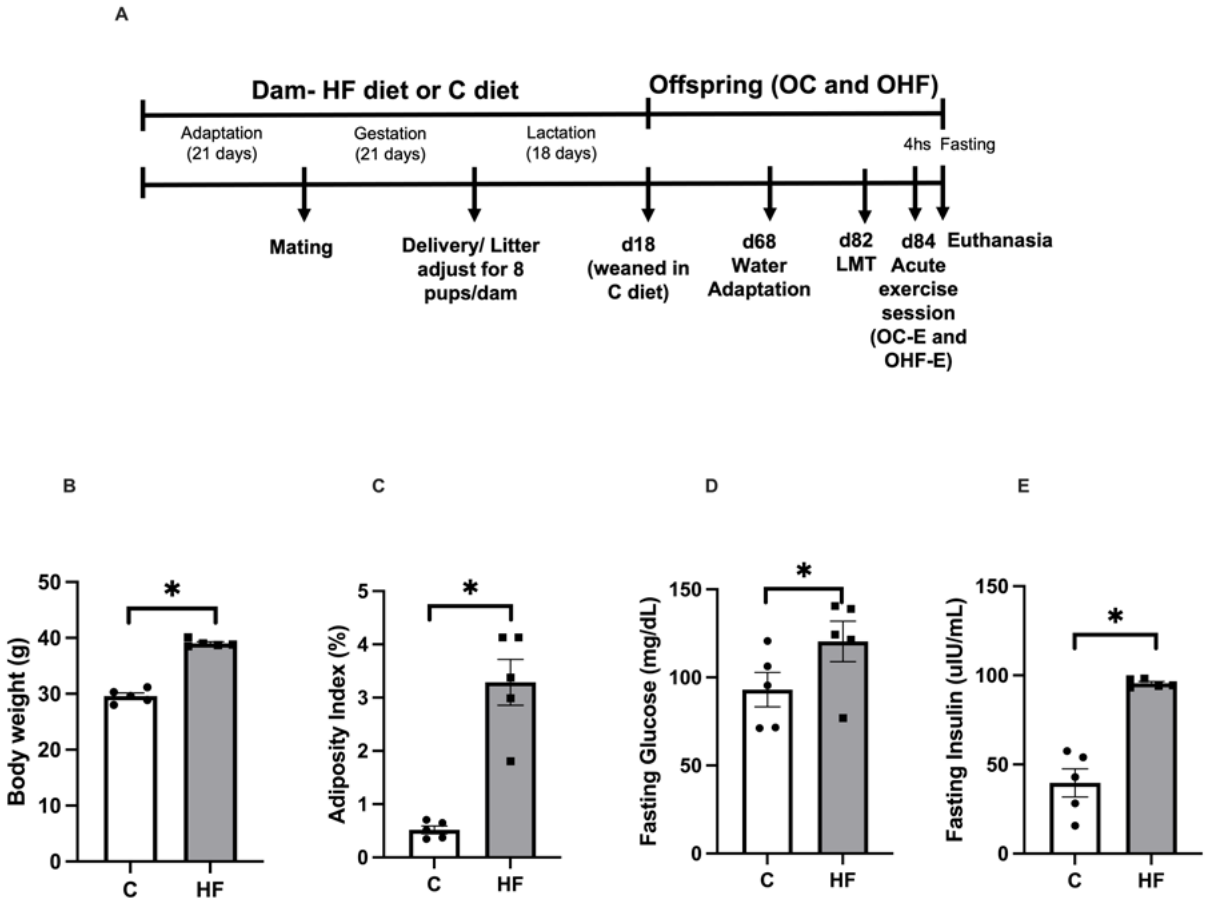



**Figure 1.** Maternal murinometric and metabolic parameters of HF and C groups. Experimental Design (**A**), maternal body weight (**B**), adiposity index (**C**), fasting glucose (**D**), and insulin (**E**) after weaning. n = 5/group. Student's t-test. n=5/group. *p<0.05.

## Lactate minimum test (LMT) and individualized exercise session in offspring

The maximal aerobic capacity for each animal in all groups was determined by performing the LMT, as previously described (12). Briefly, LMT was conducted in three steps: i) induction of hyperlactatemia, performed by a 30-second swimming effort with a 13% body weight (bw) load, followed by 30 seconds of rest, and then a second effort with 13% bw load until exhaustion; ii) a 9-min passive recovery period to allow the accumulation of lactate in the bloodstream; and iii) an incremental phase, in which 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0% bw loads were carried for 5 min, with a 30-second interval between efforts, and blood samples collected to determine lactatemia during the incremental protocol. The maximal aerobic capacity (i.e., anaerobic threshold intensity [AnT]) determined by LMT was individually obtained as the intensity relative to a point derived from a second order polynomial curve ($R^2$ > 0.8). An example of the curve from OC and OHF mice is shown in **Supplementary Figure 1A-B**, respectively.

The acute exercise session was conducted in the morning of d84. It consisted of one hour of swimming, with a load corresponding to 70% of the lactate minimum intensity previously determined by the LMT. Due to the high bias of the LMT in estimating individual aerobic capacity for female mice (12), only male offspring were evaluated.

## Murinometric and biochemical analysis

Body weight and food consumption were measured weekly. Following euthanasia, the epididymal and retroperitoneal white adipose tissue depots were carefully dissected and weighed separately. The adiposity index was estimated as the ratio of the combined

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weight of these fat pads to the final body weight. Fasting glucose was determined using an Accu-Chek Performa glucometer (Roche Diagnostics). Hepatic total lipids were measured by the Folch method, as previously described in Panzarin and cols. (13), and hepatic glycogen was evaluated as described in Fante and cols. (14).

### *In silico* analysis of miRNAs predicted targets

*In silico* analysis was performed to identify predicted target genes in *Mus musculus* corresponding to miRNAs previously identified as altered in the offspring of obese dams, specifically *miR-370*, *miR-122*, and *miR-206* (15). The prediction of miRNA/mRNA targets was conducted using the TargetScan platform, version 8.0 (https://www.targetscan.org/vert_80/).

### mRNA and microRNA analysis

Total RNA and miRNA from the liver and soleus muscle were extracted from about 150 mg of tissue using RNAzol RT (MRC, US), following the manufacturer's recommendations. cDNA was obtained as described elsewhere (3). Relative miRNA or gene expression was determined using the Taqman detection system and primers for *miR-122-5p* (ID 002245), *miR-370-3p* (ID 002275), *miR-206* (ID 000510), *Agpat1* (Mm00479699_g1), *Acadvl* (Mm00444293_m1), *Cpt1a* (Mm01231183_m1), *Hif1a* (Mm00468869_m1), *Pparg* (Mm00440940_m1), and *Ptp1b* (Mm00448427_m1). *U6snRNA* (ID 001973) or β*actin* (Mm02619580_g1) were used as endogenous controls (Thermo Fisher Scientific, US). qRT-PCR was performed on the ABI Prism 7500 Fast, and data were expressed as relative values determined by the comparative threshold cycle (Ct) method ($2^{-\Delta\Delta Ct}$).

### Statistical analysis

Results are expressed as mean ± standard error. The GraphPad Prism (Version 10.0.0, GraphPad Software Inc., San Diego, CA, US) was used for data analysis. To determine outliers, the Grubbs' method was applied. The normality of the data was tested using the Shapiro-Wilk test. Student's *t*-test was used to compare two groups, while two-way analysis of variance (ANOVA) was used for multiple comparisons A post hoc test (Šidák) was applied only when an interaction with a p-value < 0.05 was detected to determine a significance level of $p < 0.05$. The significance indicators of each graph were specified in the respective figure legend.

## RESULTS

### The aerobic session improved some metabolic parameters from offspring of obese dams

The maternal obesity phenotype of HF dams was confirmed by increased body weight, adiposity, fasting glucose, and insulin measurements compared to C (**Figure 1B-E**).

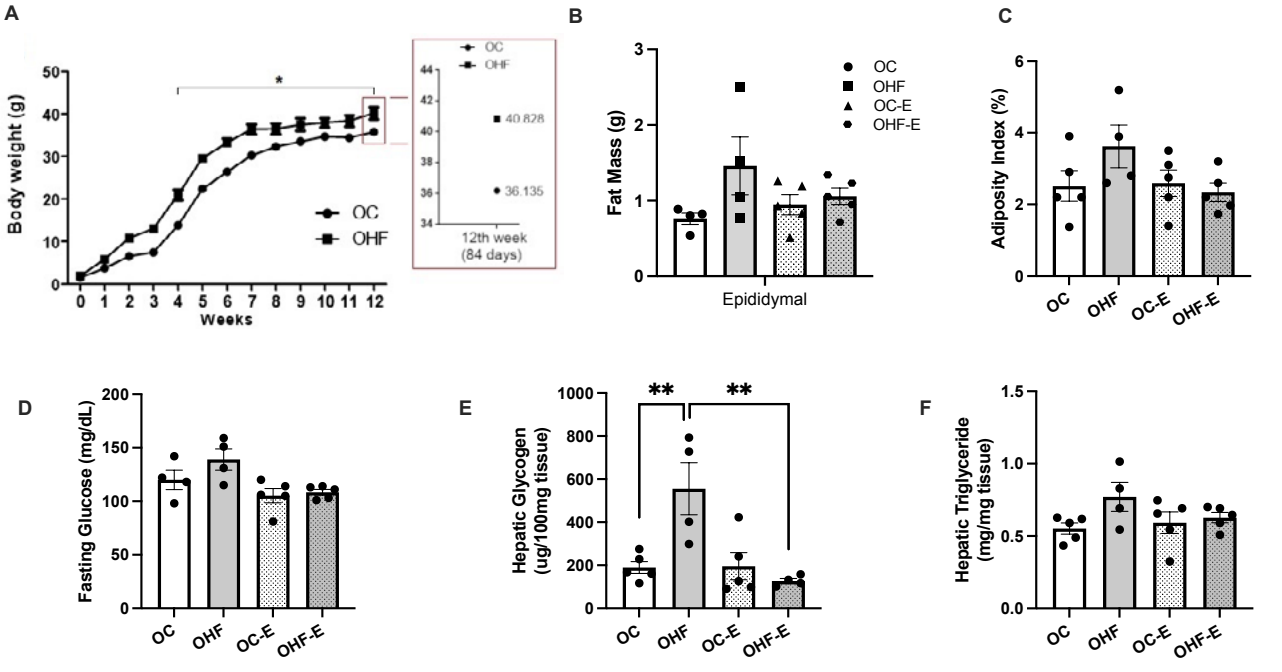
Offspring of obese dams (OHF) had higher body weight (1.13-fold) and increased hepatic glycogen (2.6-fold) compared to offspring of control dams (OC). Maternal high fat diet (HF) also seemed to impact fat mass ($p = 0.0596$) and hepatic triglyceride content ($p = 0.0659$) at d84, although no differences were observed in food intake (25.1 ± 2.075, OHF vs 24 ± 1.25 kcal/day, OC) (**Figure 2A-F**). Additionally, aerobic capacity did not differed between mice from the OC and OHF groups, including those that were randomized for exercise (OC-E and OHF-E) (**Supplementary Figure 1C-D**).

Conversely, fasting glucose was affected by exercise although without interference from the maternal diet (**Figure 2D**). OHF-E presented lower hepatic glycogen than OHF (4.37-fold), while fat mass, adiposity index, and triglycerides presented no significant changes (**Figures 2B-C, E-F**).

### Aerobic exercise modulated key hepatic and muscle microRNAs in the offspring of obese dams

Previous studies have demonstrated that offspring exposed to maternal obesity during gestation and/or lactation experience alterations in hepatic microRNAs that disrupt energy and lipid homeostasis, notably with the upregulation of *miR-370* and downregulation of *miR-122* (3,16). Moreover, studies reported that *miR-206* levels can significantly change after a single exercise session, with effects lasting up to 24 hours (17).

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G

| Two-way ANOVA | | | |
|---|---|---|---|
| **Variable** | **Effect of Maternal diet** | **Effect of Exercise** | **Interaction** |
| Fat mass (g) | F(1, 14) = 4.203, p = 0.0596* | F(1, 14) = 0.3057, p = 0.5890 | F(1, 14) = 2.206, p = 0.1597 |
| Adiposity Index (%) | F(1, 15) = 1.092 , p = 0.3126 | F(1, 15) = 2.160 , p = 0.1623 | F(1, 15) = 2.740 , p = 0.1187 |
| Fasting glucose (mg/dL) | F(1, 14) = 2.406, p = 0.1432 | F(1, 14) = 10.06, p = 0.0068* | F(1, 14) = 0.94, p = 0.2882 |
| Hepatic glycogen (ug/100 mg tissue) | F(1, 14) = 5.050, p = 0.0413* | F(1, 14) = 10.20, p = 0.0065* | F(1, 14) = 10.81, p = 0.0054# |
| Hepatic triglyceride (ug/100 mg tissue) | F(1, 15) = 3.936, p = 0.0659* | F(1, 15) = 0.6413, p = 0.4358 | F(1, 15) = 2.061, p = 0.1716 |

**Figure 2.** The acute aerobic exercise session ameliorates metabolic parameters in offspring. Body weight (**A**), fat mass (**B**), adiposity index (**C**), fasting glucose (**D**), hepatic glycogen (**E**), and triglyceride (**F**) of adult non-exercised offspring from control (OC) and obese (OHF) dams, and adult exercised offspring from control (OC-E) and obese (OHF-E) dams. ANOVA table results (**G**). post hoc test (Sidák). n=5/group. *p<0.05; **p<0.01.

Our analysis revealed that exercise have a significant impact on *miR-122* expression (p = 0.0471) (Figure 3A) in the liver. Nonetheless, OHF showed elevated *miR-370* levels (3.0-fold) compared to OC, and these levels were normalized following an exercise session in OHF-E (p = 0.0011) (**Figure 3C**). In muscle tissue, *miR-206* was not different between groups (**Figure 4A**).

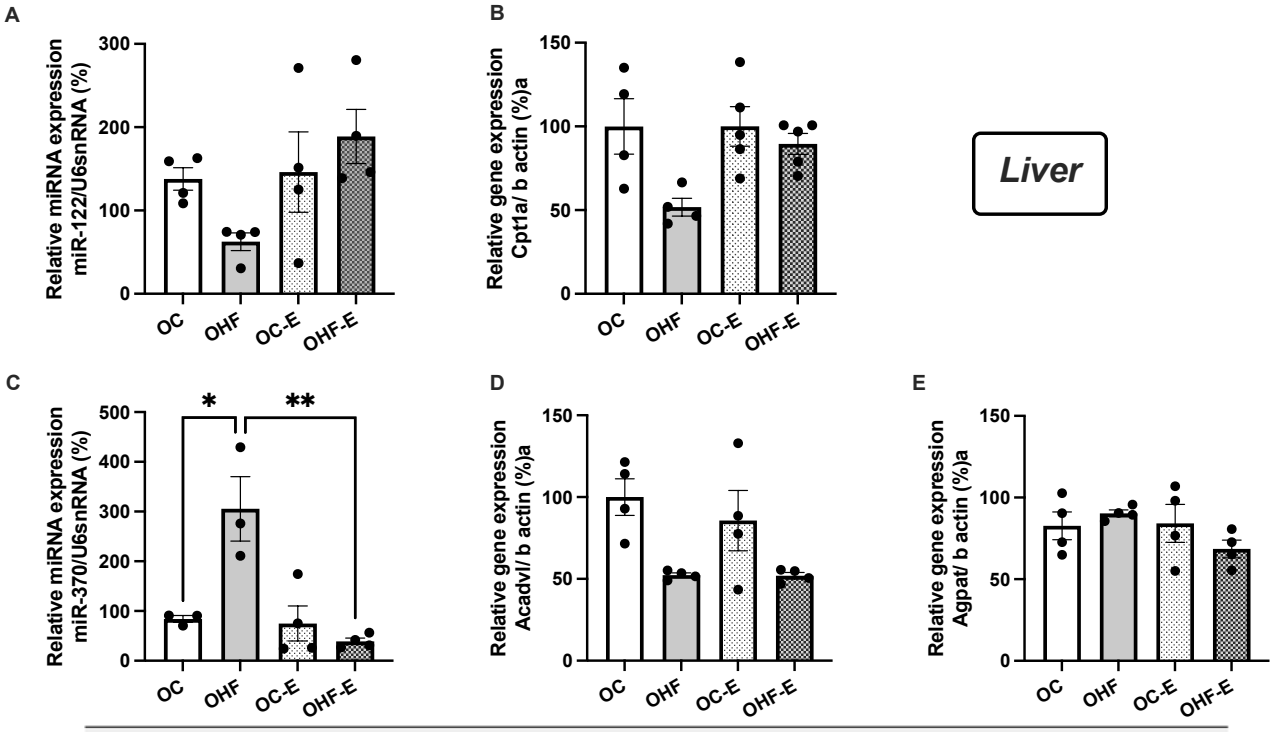
*In silico* analysis identified predicted targets for *miR-122*, *miR-370*, and *miR-206* in *Mus musculus*. While hepatic miRNAs (*miR-122* and *miR-370*) may target genes important for lipid metabolism, muscle *miR-206* may influence genes related to insulin sensitivity and mitochondrial biogenesis (**Table 1**).

Specifically, *miR-122-5p* is predicted to target 1-acylglycerol-3-phosphate O-acyltransferase 1 (*Agpat*), which encodes an enzyme crucial for triglyceride synthesis in the liver, at three different sites on the mature mRNA sequence. Concurrently, *miR-370-3p* is predicted to target the *Cpt1a* mRNA, encoding carnitine palmitoyl transferase, a protein essential for mitochondrial fatty acid transport and oxidation (**Table 1**).

Bioinformatic analysis also revealed two mRNA pairing sequences for miR-206: one corresponds to the gene encoding the alpha subunit of the transcription factor hypoxia-inducible factor-1 (HIF-1), a critical regulator of cellular responses to hypoxia, and the other targets the protein-tyrosine phosphatase PTP1B, which negatively regulates insulin signaling (**Table 1**).

The modulation of hepatic and muscle microRNAs in the offspring of obese dams by acute aerobic exercise resulted in changes to their predicted mRNA targets.

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F

| Two-way ANOVA | | | |
|---|---|---|---|
| **Variable** | **Effect of Maternal diet** | **Effect of Exercise** | **Interaction** |
| Relative miRNA expression **miR-122** | F(1, 12) = 0.2896, p = 0.6003 | F(1, 12) = 4.894, p = 0.0471* | F(1, 12) = 3.777, p = 0.0758 |
| Relative gene expression **Cpt1a** | F(1, 14) = 7.457, p = 0.0162* | F(1, 14) = 3.094, p = 0.1004 | F(1, 14) = 3.094, p = 0.1004 |
| Relative miRNA expression **miR-370** | F(1, 10) = 7.233, p = 0.0227* | F(1, 10) = 15.93, p = 0.0026* | F(1, 10) = 13.90, p = 0.0039# |
| Relative gene expression **Acadvl** | F(1, 12) = 13.98, p = 0.0028* | F(1, 12) = 0.4605, p = 0.5103 | F(1, 12) = 0.4136, p = 0.5322 |
| Relative gene expression **Agpat** | F(1, 12) = 0.2691, p = 0.6134 | F(1, 12) = 1.707, p = 0.2158 | F(1, 12) = 2.243, p = 0.1601 |

**Figure 3.** Key hepatic miRNAs and genes related to lipid metabolism modulated by the acute aerobic exercise sessions in offspring. qRT-PCR of hepatic *miR-122* (A), *Cpt1a* (B), *miR-370* (C), *Acadvl* (D), and *Agpat* (E) of adult non-exercised offspring from control (OC) and obese (OHF) dams, and adult exercised offspring from control (OC-E) and obese (OHF-E) dams. ANOVA table results (F). post hoc test (Sidák). n=3-5/group. *p<0.05; **p<0.01.

The bioinformatic analysis was validated using qPCR with specific primers for predicted target genes, as well as those identified in the literature as potential targets.

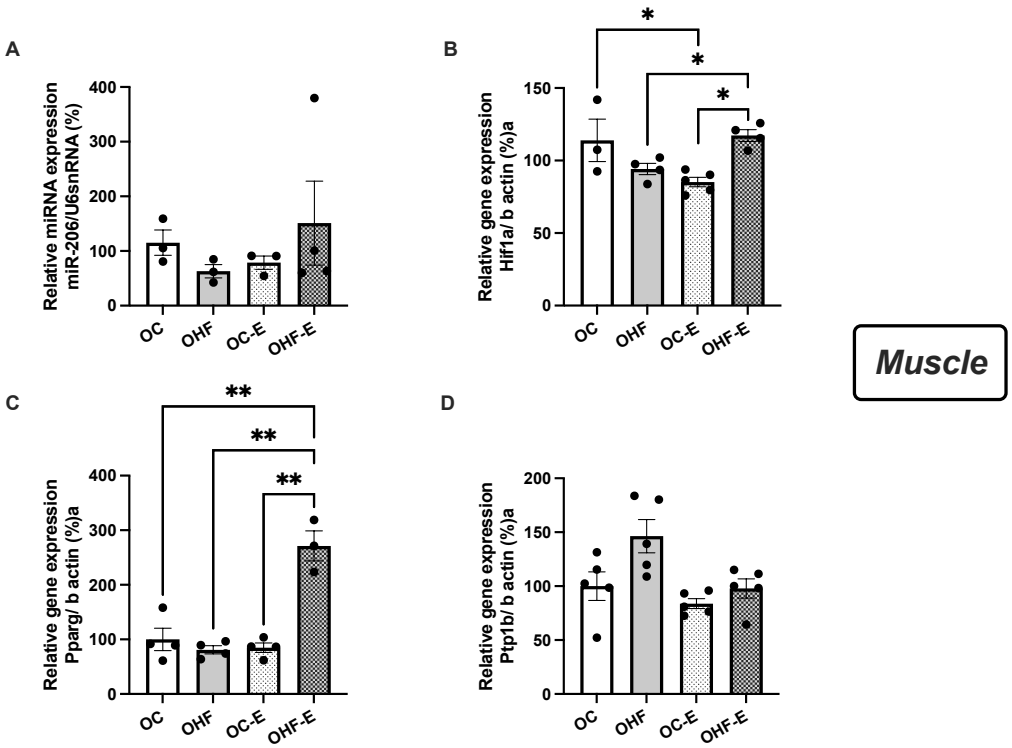
Liver data indicated an effect of maternal HF diet on *Cpt1a* levels (p = 0.0162) and *Acadvl* (p = 0.0028), paralleling the increase in *miR-370* (**Fig. 3B-D**). Although *Acadvl* (Acyl-CoA Dehydrogenase Very Long Chain) is not a predicted target of *miR-370-3p*, previous findings show an inverse relationship between hepatic *miR-370* and *Acadvl* expression in offspring of obese dams (3,13,16), which justified its inclusion in the evaluation.

In muscle tissue, the exercise session induced an increase in *Hif1a* expression (28%) in the OHF-E group compared to OHF and OC-E (**Figure 4B**). Moreover, the exercise promoted an increase in *Pparg* levels (2.7-fold) compared to both non-exercised OC and OHF and OC-E (**Figure 4C**). Additionally, *Ptp1b*, a predicted target of *miR-206*, presented an effect of both maternal HF diet (p = 0.0169) and exercise (p = 0.0114), although no interaction was observed between them (**Figure 4D**).

## DISCUSSION

The influence of physiological and environmental conditions during pregnancy and early postnatal development on lifelong health trajectories and disease susceptibility has been well-established in scientific literature (18). Recent findings emphasize that even brief exercise interventions can produce lasting

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E

| Two-way ANOVA | | | |
|---|---|---|---|
| **Variable** | **Effect of Maternal diet** | **Effect of Exercise** | **Interaction** |
| Relative miRNA expression **miR-206** | F(1, 9) = 0.03884, p = 0.8482 | F(1, 9) = 0.2514, p = 0.6281 | F(1, 9) = 1.473, p = 0.2558 |
| Relative gene expression **Hif1a** | F(1, 12) = 0.9363, p = 0.3523 | F(1, 12) = 0.2033, p = 0.6601 | F(1, 12) = 16.53, p = 0.0016# |
| Relative gene expression **Pparg** | F(1, 11) = 25.53, p = 0.0004* | F(1, 11) = 27.86, p = 0.0003* | F(1, 11) = 38.44, p = 0.0001# |
| Relative gene expression **Ptp1b** | F(1, 16) = 7.111, p = 0.0169* | F(1, 16) = 8.1666, p = 0.0114* | F(1, 16) = 2.036, p = 0.1728 |

**Figure 4.** Key muscular miRNA and genes related to insulin sensitivity and mitochondrial biogenesis modulated by the acute aerobic exercise sessions in offspring. qRT-PCR of muscular *miR-206* (A), *Hif1a* (B), *Pparg* (C), and *Ptp1b* (D) of adult non-exercised offspring from control (OC) and obese (OHF) dams, and adult exercised offspring from control (OC-E) and obese (OHF-E) dams. ANOVA table results (E). ost hoc test (Sidák). n=3-5/group. *p<0.05; **p<0.01.

**Table 1.** Bioinformatic analysis by searching for mouse *miR-122-5p*, *miR-370-3p*, and *miR-206*, and their putative mRNA targets.

| microRNA | Target mRNA | Pairing sequence* | | References (PMID)** |
|---|---|---|---|---|
| **miR-122-5p** | **Agpat** | Position 134-140 of AGPAT1 3′ UTR<br>mmu-miR-122-5p | 5′ ...UUCUGAAGUGAAUGUCACUCCAU...<br>3′ GUUUGUGGUAACAGUGUGAGGU | |
| | | Position 190-196 of AGPAT1 3′ UTR<br>mmu-miR-122-5p | 5′ ...CGUGGGUGCAGUCUCCACUCCAA...<br>3′ GUUUGUGGUAACAGUGUGAGGU | 22820288<br>35048271<br>28239403 |
| | | Position 461-467 of AGPAT1 3′ UTR<br>mmu-miR-122-5p | 5′ ...UGGAAGCUGCACCUGACACUCCU...<br>3′ GUUUGUGGUAACAGUGUGAGGU | |
| **miR-370-3p** | **Cpt1a** | Position 552-558 of CPT1A 3′ UTR<br>mmu-miR-370-3p | 5′ ...UCCACAUUUCCUGGA-AGCAGGAU...<br>3′ UGGUCCAAGGUGGGGUCGUCCG | 20124555<br>24666709<br>35048271<br>28239403 |
| **miR-206** | **Hif1a** | Position 3466-3472 of HIF1A 3′ UTR<br>hsa-miR-206 | 5′ ...UAACCUCACGAUUAUCAUUCCAA...<br>3′ GGUGUGUGAAGGAAUGUAAGGU | 23628900<br>30250188<br>31880296 |
| | **Ptp1b** | Position 1172-1178 of PTPN1 3′ UTR<br>hsa-miR-206 | 5′ ...GGGAUCAGCCUCCGCCAUUCCAA...<br>3′ GGUGUGUGAAGGAAUGUAAGGU | 31048362<br>31695578<br>31894853 |

*According to TargetScan release 8.0 (https://www.targetscan.org/vert_80/). **PubMed Identifier.

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metabolic benefits (19). This is particularly relevant in maternal obesity, which is linked to adverse metabolic programming outcomes in offspring.

Our investigation focused on whether an aerobic exercise session in adulthood could modulate the expression of key hepatic and muscle genes involved in lipid metabolism and insulin sensitivity in offspring of obese dams. Remarkably, we found that even a single session of exercise can modulate microRNA expression, impacting target genes within these tissues and leading to improvements in metabolic parameters in offspring (**Supplementary Figure 2**).

Consistent with prior research, our results confirmed that maternal obesity correlates with increased body weight in offspring from early life (20). The pronounced weight gain observed in offspring of obese dams (OHF) from the fourth week underscores the propensity for early excessive weight gain driven by maternal obesity.

While existing studies demonstrated that interventions during pregnancy or lactation, such as nutritional or exercise modifications, can improve offspring outcomes (21), there is limited investigation into postnatal interventions after the establishment of a programmed phenotype. Our findings suggest that even a single session of swimming may transiently counteract some metabolic disturbances associated with maternal obesity, highlighting its potential as a modifiable stimulus.

In contrast, a study by Kasch and cols. (22) reported that voluntary wheel running was ineffective in reducing weight gain or reversing impaired glucose metabolism gene expression in the skeletal muscle of offspring from high-fat diet (HF)-fed dams, indicating a potential limitation in training efficacy. However, in our study, a session of swimming exercise provided significant benefits to offspring of obese dams (OHF-E), including improved biochemical parameters such as hepatic glycogen, bringing them in line with control groups (OC and OC-E). Similar improvements after acute resistance or aerobic exercise have been reported by others (23,24). This highlights that targeted exercise can reduce, at least temporarily, metabolic dysfunctions caused by maternal obesity.

The association of maternal obesity with an increased risk of metabolic dysfunction-associated steatotic liver disease (MASLD) and its progression to metabolic dysfunction-associated steatohepatitis (MASH) in offspring is especially concerning (25,26). In the liver, alterations in the expression of *miR-122* — the most abundant miRNA — and *miR-370*, highlighted in our findings, are associated with changes in hepatic lipid metabolism and are known to be implicated in MASLD and MASH (3). Through *in silico* predictions and qPCR validations, we identified mRNA targets influenced by exercise-modulated miRNAs that are closely associated with pathways driving triglyceride synthesis and hepatic fatty acid metabolism (27,28). Previously, our group demonstrated that offspring of obese dams displayed increased hepatic lipid accumulation due to the upregulation of *miR-370* and lipogenic genes like *Agpat*, alongside the downregulation of *miR-122* and oxidative genes, such as *Acadvl* and *Cpt1a* (3). Here, we demonstrate that exercise induces upregulation of hepatic miR-122. Additionally, the elevated hepatic miR-370 observed in OHF was normalized following exercise in OHF-E. Although Cpt1a, which was downregulated by maternal diet, was not influenced by exercise and direct measurements of lipid oxidation were not conducted, it is plausible that the exercise-induced changes in hepatic microRNA expression may contribute to the restoration of lipid oxidation capacity.

Likewise, prolonged swimming exercise, combined or not with intermittent fasting, has been shown to improve blood glucose and insulin levels and effectively combat MASLD in diet-induced obesity models, modulating *miR-122-5p* and subsequently reducing the expression of lipid synthesis-related genes (*Srebp-1c*, *Fasn*, and *Acc1*) while enhancing genes related to fatty acid oxidation (*Cpt1a*) (29). Although our intervention was conducted in a distinct animal model and did not constitute a long-term training protocol, the acute exercise response illustrates potential similarities with chronic responses. Adjustments in both intervention duration and frequency could reveal more significant benefits in offspring hepatic lipid metabolism.

Exercise is also known to influence microRNA profiles in skeletal muscle, leading to improved glucose metabolism and insulin sensitivity (8). In skeletal muscle, *miR-206* has emerged as a biomarker and

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potential therapeutic target (7). *miR-206*, a highly conserved muscle-specific microRNA essential for myogenic differentiation (28), is upregulated after acute aerobic exercise (15,17) and a single session of resistance training, with changes detectable for up to 24 hours, including significant alterations within the first hour post-exercise (15). Conversely, high-fat diet intake suppresses *miR-206*, a change linked to impaired vascular reactivity and muscle function (7). Moreover, its overexpression has been shown to inhibit lipogenesis and triglyceride secretion (8). In our study, although *miR-206* levels did not differ in offspring of obese dams, bioinformatic predictions indicated that it influences genes related to insulin sensitivity and mitochondrial biogenesis.

*miR-206* is known to target *Hif1a* (30), a key regulator of glycolysis, as well as *Ptp1b* (31), a negative modulator of insulin signaling. Furthermore, it appears to have a positive correlation with *Pparg* (32), which plays a significant role in increasing insulin sensitivity and promoting endogenous adiponectin production, thereby exerting a protective effect against diet-induced insulin resistance. Our findings indicate that a single exercise session significantly increased *Hif1a* expression in offspring of obese dams subjected to exercise (OHF-E) compared to non-exercised OHF. Moreover, we observed elevated *Pparg* levels in OHF-E relative to both non-exercised offspring (OC and OHF) and exercised control (OC-E). Notably, *Ptp1b* was impacted by both maternal HF diet and exercise. The concurrent upregulation of *Hif1a* and *Pparg* post-exercise, alongside the effects on *Ptp1b* levels, highlights the diverse metabolic health benefits conferred by acute physical activity.

The absence of differences in the reciprocal modulation of miRNA and some predicted targets may be due to the low sample size, which is a limitation of the study. Although miR-206 levels remained unchanged, exercise modulated its target genes, *Ptp1b*, *Pparg*, and *Hif1a*, illustrating the dynamic, time-sensitive nature of microRNA regulation. Conversely, the relationship between *miR-122* and *Agpat* exhibited the opposite pattern. Target mRNAs can respond quickly via translational repression or destabilization, whereas mature miRNAs are relatively stable, creating a temporal mismatch between gene and miRNA expression changes (33).

Fante and cols. (14) reported that offspring of obese dams displayed pronounced insulin resistance in the soleus skeletal muscle, evidenced by elevated levels of PEPCK and PTP1B along with reduced p-IRS1 and p-AKT in response to insulin stimulation. Although our study did not assess the long-term effects of exercise, the acute modulation observed was sufficient to reduce *Ptp1b* gene expression and decrease fasting glucose. Given the critical roles of myokines and other signaling pathways activated during physical activity, a comprehensive analysis of these factors, in conjunction with epigenetic modifications, will be essential for developing effective strategies to combat obesity and related metabolic disorders, with implications that may extend across generations.

In summary, our findings suggest that a single session of swimming may transiently counteract some metabolic alterations associated with maternal obesity, highlighting its potential as an initial modulating stimulus. However, it is unlikely that these changes observed after a single exercise session persist for an extended period unless reinforced by repeated exercise stimuli. Nevertheless, they may represent a molecular signature that signals the tissue's responsiveness to exercise and its capacity for metabolic adaptation. The long-term benefits of such interventions require further investigation, including assessments of the gain and loss of function of miRNA modulated by exercise. Since outcomes may differ between sexes, future studies should include female subjects. Although one limitation is that a group of mice was kept fed in their cages while others underwent training, we believe this had minimal impact on the results, as exercise sessions occurred in the morning, a period typically characterized by reduced food intake.

Further studies should focus on elucidating the sustainability of these molecular changes and uncovering the underlying mechanisms of exercise-induced reprogramming, with the goal of establishing robust, lifelong strategies against metabolic disorders arising from maternal dietary influences.

**Author contributions:** AST, LAPS, FBMG and PBV carried out the study concept and design, data interpretation, and manu-

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script writing. TRG, TF and NAR conducted the data acquisition and analyses. AST, MAT did the critical review and provided funding for the study. All authors have approved the final version of the article.

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**Ethical statements:** all experimental procedures were conducted complying to the ethical standards established by the "Brazilian College for Animal Experimentation" (COBEA) and were approved by the "Ethical Committee for Animal Use" (CEUA #3913-1), State University of Campinas – Unicamp (Brazil).

**Disclosure:** no potential conflict of interest relevant to this article was reported.

**Data availability:** datasets related to this article will be available upon request to the corresponding author.

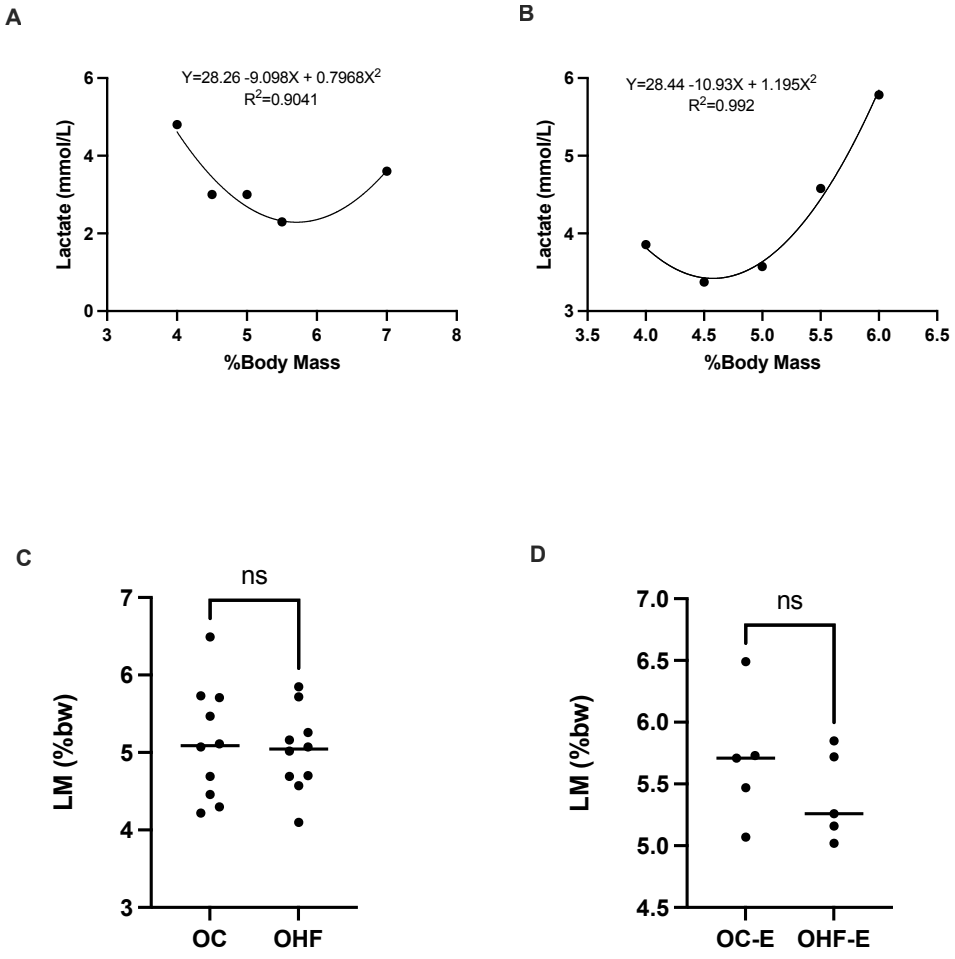
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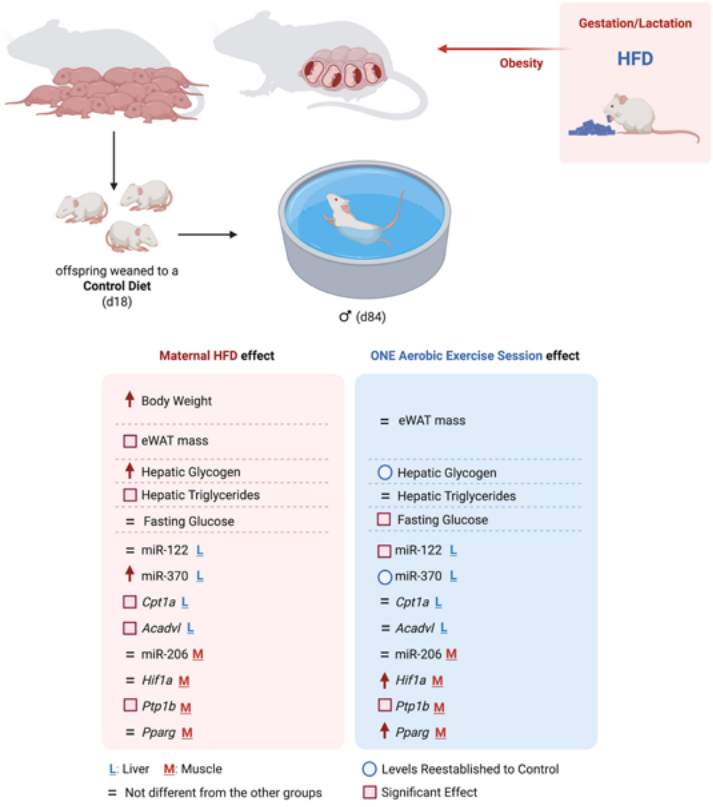
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## SUPPLEMENTARY MATERIALS




**Supplementary Figure 1.** Comparison of the intensity of lactate minimum (LM) during incremental phase. Example curve of blood lactate concentration (mmol·L$^{-1}$) versus intensity (percentage of body mass (% bm)) during the stages (5 min) of the incremental phase of OC mice (**A**), Example curve of blood lactate concentration (mmol·L$^{-1}$) versus intensity (percentage of body mass (% bm)) during the stages (5 min) of the incremental phase of OHF mice (**B**). Intensity of LM of all mice from OC and OHF groups (**C**), Intensity of LM of mice from groups randomized to exercise -OC-E and OHF-E (**D**). Intensity of LM (%bw) expressed as mean. ns, not statistically significant.

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A single individualized aerobic exercise session can ameliorate metabolic parameters, microRNAs and target-genes expression in liver and soleus muscle of offspring programmed by maternal obesity.

**Supplementary Figure 2.** Graphical abstract.

**Supplementary Table 1.** Nutritional composition of the experimental diets

| | Chow Diet * | HFD# (45% kcal from fat) |
|---|---|---|
| Net Protein (g %) | 20.0 | 23.3# |
| Fat content (g %) | 4.0* | 24.0*# |
| Carbohydrates (g %) | 65.95* | 42.1# |
| Fiber (g %) | 5.0 | 5.55 |
| Mineral Mix (g %) | 3.5 | 3.5 |
| Vitamin Mix (g %) | 1.0 | 1.0 |
| Choline | 0.25 | 0.25 |
| Cystine | 0.3 | 0.3 |
| Total | 100.0 | 100.0 |
| Energy (kj/g) | 14.6 | 19.3 |

*NUVILAB® Cr-1; Nuvital .
#Based on AIN-93G (34) – AIN-93G was modified to contain a higher fat content (45%) than control diet and a higher net protein than AIN-93M. It is indicated during pregnancy, lactation and growth period in rodents.
*Soy oil and #lard.
*#Starch and sucrose.

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original

# Are serum MOTS-c levels and *MOTS-c* m.1382A>C polymorphism related to polycystic ovary syndrome?

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## ABSTRACT

**Objective:** MOTS-c is a mitochondria-derived peptide associated with reduced insulin resistance and obesity. The m.1382A>C polymorphism of the *MOTS-c* gene is linked to an increased risk of type 2 diabetes in men. However, no studies have explored the relationship between this polymorphism and MOTS-c levels in adolescents with polycystic ovary syndrome (PCOS). This study aimed to investigate the differences in MOTS-c levels between adolescents diagnosed with PCOS and those without PCOS, as well as the associations with metabolic parameters. The association between the *MOTS-c* gene polymorphism and serum MOTS-c levels in adolescents with PCOS was also evaluated. **Subjects and methods:** Adolescents aged 12–18 diagnosed with PCOS were recruited based on irregular menstrual cycles and clinical/biochemical hyperandrogenism, excluding other conditions. The control group consisted of adolescents with regular menstruation. Serum MOTS-c levels were measured using ELISA, and the m.1382A>C polymorphism was analyzed by sequencing. **Results:** The study included 121 adolescents with PCOS and 125 healthy controls. The mean serum MOTS-c levels in the PCOS group were higher than in the control group; however, this difference did not reach statistical significance ($p = 0.059$). There was no significant association between MOTS-c levels and anthropometric or metabolic parameters within the PCOS group ($p > 0.05$). All participants had the wild-type (A/A) genotype for the m.1382A>C polymorphism. **Conclusion:** Results indicate that the *MOTS-c* gene (m.1382A>C) polymorphism shows no significant association with PCOS, and serum MOTS-c levels are comparable between individuals with PCOS and healthy controls, suggesting that MOTS-c may have a minor involvement in the pathophysiology of PCOS.

**Keywords:** MOTS-c; mitochondrial DNA; polymorphism; polycystic ovary syndrome; adolescent

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder that typically manifests during adolescence. PCOS is thought to affect from 5% to 10% of women of reproductive age worldwide (1). This is strongly associated with metabolic abnormalities such as insulin resistance, hyperinsulinemia, type 2 diabetes, hypertension, dyslipidemia, and infertility (2). Obesity in individuals with PCOS further exacerbates the risk of these metabolic comorbidities. Insulin resistance and hyperandrogenism are believed to be interrelated and may serve as central contributors to PCOS pathogenesis (3). Although the exact etiology of PCOS remains unclear, familial aggregation suggests a genetic component. The underlying etiology of PCOS is still being elucidated. An autosomal dominant pattern was initially proposed, based on the high prevalence of PCOS among first-degree relatives of affected individuals (4).

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However, twin studies emphasize the X-linked or polygenic inheritance (5,6). Thus, PCOS is now considered a complex and highly heritable disorder (7).

Mitochondria play a fundamental role in essential cellular processes, including metabolism, growth, and apoptosis, which are governed not only by nuclear DNA but also by signals encoded within mitochondrial DNA (mtDNA) (8). The interplay between nuclear and mitochondrial genomes, referred to as mitonuclear communication, has been increasingly recognized as a key regulatory mechanism. Among the molecules encoded by mtDNA are mitochondrial-derived peptides (MDPs), including humanin, MOTS-c (mitochondrial open reading frame of the 12S rRNA-c), and small humanin-like peptides (SHLPs). MOTS-c is a 16-amino acid peptide encoded within the 12S rRNA region, and it plays a critical role in maintaining insulin sensitivity and metabolic homeostasis, primarily via activation of the AMP-activated protein kinase (AMPK) pathway (9). MOTS-c is expressed in tissues such as muscle, brain, and liver and is also detectable in plasma (10). In mice studies, exogenous MOTS-c administration has been shown to improve insulin resistance, reduce adiposity, and mitigate obesity-related phenotypes (9,11). However, human studies investigating the metabolic effects of MOTS-c remain limited and show inconsistent findings (10,12,13). To date, only one study has explored the relationship between PCOS and MOTS-c, focusing on its response to intralipid and insulin infusion (14). Beyond circulating levels, genetic variation in the MOTS-c-encoding region (m.1382A>C; rs111033358) has been linked to altered metabolic risk in specific populations, potentially by modifying the peptide sequence and bioactivity (15,16). Given the metabolic disturbances characteristic of PCOS—including hyperinsulinemia, insulin resistance, obesity, and dyslipidemia—and the limited literature on MOTS-c, it was hypothesized that the m.1382A>C polymorphism and circulating MOTS-c levels may contribute to the pathogenesis of PCOS.

Therefore, this study aimed to compare the *MOTS-c* m.1382A>C polymorphism and serum MOTS-c levels in adolescents with and without PCOS and to evaluate the association of serum MOTS-c levels with metabolic and anthropometric parameters.

## SUBJECTS AND METHODS

A case-control study involving adolescent girls diagnosed with PCOS and a control group of adolescents with regular menstrual cycles was conducted. The local ethics committee approved the study (Date: 05.02.2020, Number: 2). Written informed consent was obtained from all participants and their legal guardians.

### Inclusion criteria

Adolescent girls aged 12 to 18 years diagnosed with PCOS, based on the existence of menstrual irregularity (oligomenorrhea) or anovulatory (secondary amenorrhea) cycles, and clinical or biochemical hyperandrogenism at least two years after menarche were enrolled in the PCOS group (17). The control group consisted of healthy volunteers with regular menstrual cycles for at least two years following menarche and without clinical signs of hyperandrogenism.

#### *Diagnosis of PCOS in adolescents*

Diagnosis of PCOS in adolescents was based on the following two criteria:

**A. Menstrual irregularity (oligomenorrhea) or anovulatory (secondary amenorrhea) cycles**

**B. Clinical or biochemical hyperandrogenism**

**Clinical hyperandrogenism**

- Hirsutism was evaluated using the modified Ferriman–Gallwey score (FGS), with a score ≥ 8 considered as diagnostic for clinical hyperandrogenism (1).

**Biochemical hyperandrogenism**

- Biochemical evaluation was performed in the 2$^{nd}$ and 5$^{th}$ days of the follicular phase between 08:00 h and 09:00 h. In cases without regular periods, a progesterone withdrawal test was used for timing of blood sampling (18).

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), 17-hydroxy-progesterone (17-OH-PG), dehydroepiandrosterone sulfate ($DHEA_{SO4}$), sex hormone binding globuline (SHBG), and total testosterone levels were measured in all cases with PCOS.

Due to the inability to measure free testosterone levels in the laboratory where the study was conducted, free androgen index [(total testosterone/SHBG)

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> 4.5] and total testosterone levels (> 55 ng/dL) were used as biochemical criteria for diagnosing hyperandrogenemia in patients with PCOS (1).

### *Pelvic ultrasonography (USG)*

All participants underwent pelvic ultrasound between days two and five of the follicular phase, conducted by a single radiologist to exclude other pathologies that could mimic PCOS.

## Exclusion criteria

Exclusion criteria included girls under 12 or over 18 years; individuals less than two years post-menarche; those with chronic diseases (cardiovascular, gastrointestinal, respiratory, oncological, etc.); regular medication use, or endocrine conditions that could result in oligo/amenorrhea (e.g., thyroid disorders, pregnancy, primary ovarian failure, congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, or hyperprolactinemia).

## Anthropometric assessment

Anthropometric measurements were conducted using a Harpenden stadiometer (Crosswell, Crymych, Pembs., SA41 3UF, UK), with a height measurement accuracy of 0.1 cm, and a SECA scale (Hammer Steindamm 3-25, 22089 Hamburg, Germany) with an accuracy of 0.1 kg for weight assessment. Participants were assessed wearing only light underwear to ensure accurate measurements. The Body Mass Index (BMI) was calculated by dividing weight (kg) by the squared height (m), and then converted into a standard deviation score (SDS) using national BMI reference data (19).

## Body fat analysis (bioimpedance analysis)

The bioelectrical impedance analysis method evaluated body fat mass (kg) and body fat percentage (Tanita BC-418, Tokyo, Japan).

## Blood pressure

Systolic (SBP) and diastolic blood pressures (DBP) were measured twice from the right arm using a calibrated sphygmomanometer after a 10-minute rest in the supine position. Blood pressure values higher than the 95$^{th}$ centile for height, age, and gender were defined as hypertension (20).

## Metabolic syndrome (MS)

MS is defined by the International Diabetes Federation criteria for adolescents aged 10–16 years (21). It consists of abdominal or central obesity (90$^{th}$ centile of waist circumference or adult cut-off if lower) plus at least two of the following features:

- Fasting plasma triglyceride (TG) ≥ 150 mg/dL;
- High-density lipoprotein cholesterol (HDL-C) < 40 mg/dL;
- Systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg;
- Fasting plasma glucose ≥ 100 mg/dL, or known T2DM.

## Pubertal staging

Pubertal development was assessed by Tanner's staging (22). Therefore, breast development Stage II or above is defined as puberty.

## Genetic analysis

Blood samples were taken from all subjects in an EDTA tube to study the *MOTS-c* gene variant. First, DNA was isolated using a commercial kit (DNA Isolation Kit for Mammalian Blood Roche) according to the manufacturer's instructions. Genomic DNA quantity and purity were assessed by spectrophotometry (A260/A280 ≈ 1.8–2.0) and integrity was verified by agarose gel electrophoresis. Only samples meeting these thresholds were subjected to polymerase chain reaction (PCR) test. Then, the spectrophotometer and gel electrophoresis determined the purity and the quality of the isolated genomic DNA samples.

## Study protocol for determination of genotypes

SNP numbered *rs111033358* in the *MOTS-c* gene was evaluated. NCBI and Ensembl databases were used to access the gene sequence to examine the human *MOTS-c* gene. It was tested whether the forward and reverse primer sequences designed for the PCR reaction to be applied to this gene sequence were correctly seated. To identify the binding sites of the designed forward and reverse primer sequences on the genome and to assess their specificity, potential matches in the human genome were analyzed using the Primer-BLAST program capable of scanning the entire genome.

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Conventional PCR testing was conducted with primers specific to the target gene region used in our study. Amplification success and specificity were confirmed by agarose gel electrophoresis, ensuring a single band of the expected amplicon size for each sample before sequencing. As a result, PCR products contain the SNP gene region numbered *rs111033358* in a large amount of the *MOTS-c* gene of each case.

DNAse and RNAse-free sterile distilled water were added to the lyophilized forward and reversed primers and the final concentration was adjusted to 100 pmol/μL. After the primer stocks were aliquoted, they were diluted at 1:10 with distilled water to be used in the PCR testing. While preparing the PCR reaction, all procedures were carried out on the cold block. A mixture containing *MOTS-c* gene-specific primer pairs was used for PCR reaction of genomic DNA whose purity and amount were determined. Then, the master mix was added to the isolated DNAs and placed in the thermal cycler. After the PCR program information was selected in the device, the obtained PCR products were checked by the gel electrophoresis method. Then, sequence analysis was performed with service procurement.

All PCR products were subjected to bidirectional Sanger sequencing at an accredited commercial facility (Macrogen, Seoul, South Korea). Raw electropherograms were independently inspected by two researchers using Chromas with manual verification of peak morphology and signal-to-noise at the polymorphic site (m.1382; rs111033358). Base calls were accepted only when forward and reverse reads were concordant and of adequate quality; discrepant or low-quality sites were reexamined and recalled after joint review.

The sequences containing the *rs111033358* SNP gene region in the *MOTS-c* gene were read both ways by Macrogen® (Seoul, South Korea) with forward and reverse primers. The data obtained were shared with our laboratory as DNA sequences by the company. The shared data were evaluated individually in the Chromas program as a graphic (**Figure 1**).

## Biochemical analysis

Venous blood samples were collected into plain blood collection tubes (BCT)s (BD Vacutainer® SST II Advance Tube, 5 mL, 13 x 100 mm, USA). Serum samples were separated from cellular fragments by centrifugation for 10 minutes at 1.500 g within one hour after blood sampling. Serum samples were aliquoted and stored at −80 °C until further analysis.

Fasting serum glucose, total cholesterol (TC), TG, low-density lipoprotein-cholesterol (LDL-C), and HDL-C and alanine transaminase (ALT) levels were measured in Beckman AU5800 (Beckman Coulter, Brea, CA, USA). Serum free thyroxine (fT4), thyroid stimulating hormone (TSH), total testosterone, insulin, prolactin, FSH, LH, E2, and $DHEA_{SO4}$ levels in DxI 800 immunoassay systems (Beckman Coulter, Brea, CA, USA) by using original in vitro diagnostic reagents. 17-OH-PG levels were analyzed using a RIA kit (17α-hydroxyprogesterone-RIA-CT; DIAsource ImmunoAssays S.A, Belgium) by following manufacturer's instructions. The reference range for total testosterone was 8.4–48.1 ng/dL.

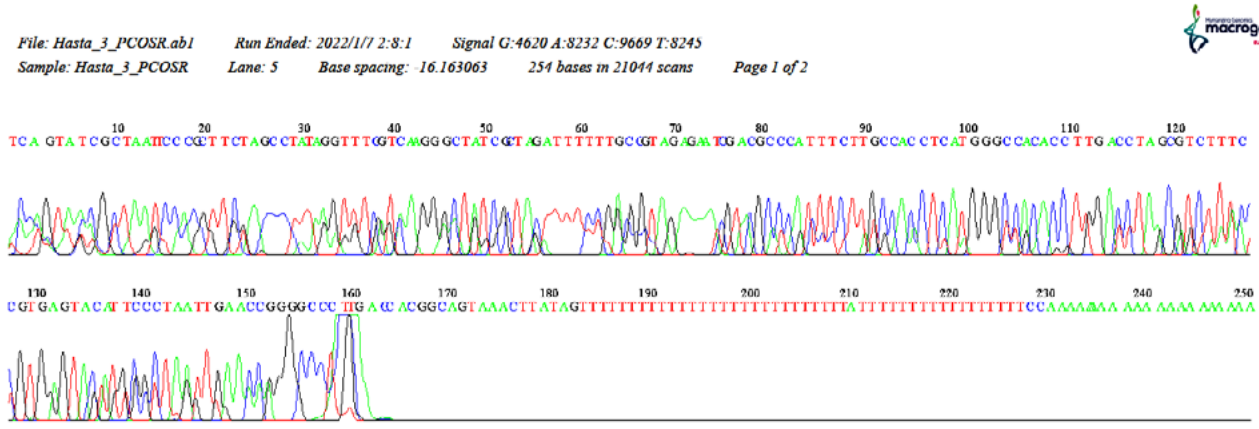



**Figure 1.** Sample image of DNA sequences in the Chromas program.

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Serum MOTS-c concentrations were determined by a commercial kit employing the quantitative ELISA technique (Cloud-Clone Corp., TX, USA) (Catalog No: CEX132Hu, Lot No: L210719103). The analysis was carried out according to the manufacturer's instructions. All ELISA measurements were run in duplicate; duplicate agreement met the pre-specified criterion (≤ 15% CV) for all samples. The kit's intra- and inter-assay coefficient variations (CVs) were < 10% and <12%, respectively. The test's detection limit (LOD) value was less than 0.97 ng/mL. The measurement range of the assay was 2.47 to 200 ng per mL.

The cut-off points used to define dyslipidemia (> 95$^{th}$ percentile values in healthy children for TC, TG, LDL-C, and < 5$^{th}$ percentile value for HDL-C) were: TC > 200 mg/dL; TG > 150 mg/dL; LDL-C >130 mg/dL; HDL-C < 40 mg/dL (23).

The pathological criterion of impaired fasting glucose >100 mg/dL was used (24). Moreover, the "Homeostasis model assessment-insulin resistance" (HOMA-IR) index was used to evaluate insulin resistance. HOMA-IR was calculated as fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405. A HOMA-IR value ≥ 4 was accepted as insulin resistance since all participants included in the study were pubertal (25). Pathological cut-off values for some biochemical parameters were as follows: TSH > 5 IU/L, ALT > 25 IU/L, 17-OH-PG > 2 ng/mL, total testosterone > 55 ng/dL (26), and prolactin > 25 ng/mL (27).

### Sample size

The G*Power 3.1 program was used to calculate the sample size with a power >80% at a 5% significance level. The calculation was based on the study by Ramanjaneya and cols. (14), which assessed plasma MOTS-c levels in adult women with PCOS. According to the results of the power analysis, a minimum of 204 participants (102 PCOS, 102 controls) were required to detect a 10% difference in the frequency of the *MOTS-c rs111033358* polymorphism between the PCOS and control groups and to observe a mean difference of 86.37 ng/mL in serum MOTS-c levels.

### Statistical analysis

All statistical analyses were conducted using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Before comparisons, data distribution was assessed using the Kolmogorov–Smirnov test, Q–Q plots, histograms, and evaluation of skewness and kurtosis. Descriptive statistics summarized demographic and clinical characteristics. Group proportions were compared with the Chi-squared test. For continuous variables, normally distributed data were analyzed with the Student's *t*-test, whereas non-normally distributed data were evaluated using the Mann–Whitney *U* test. Differences in serum MOTS-c levels across subgroups (PCOS with obesity, PCOS without obesity, controls with obesity, and controls without obesity) were assessed using one-way analysis of variance (ANOVA). Owing to non-homogeneous distributions, correlations between serum MOTS-c and anthropometric or metabolic parameters were evaluated with Spearman's correlation (ρ). Normally distributed variables are expressed as mean ± standard deviation (SD), and non-normally distributed variables as median [interquartile range (IQR)]. In addition to *p*-values, effect sizes were calculated to better convey the magnitude of group differences and associations: for *t*-tests, Cohen's *d* was computed; for Mann–Whitney *U* tests, the effect size *r* was calculated as $Z/\sqrt{N}$. For correlation analyses, 95% confidence intervals (CIs) were estimated via bias-corrected and accelerated (BCa) bootstrapping with 1,000 resamples. Effect sizes were interpreted according to Cohen's criteria ($r < 0.10$ negligible, 0.10–0.29 small, 0.30–0.49 medium, ≥ 0.50 large). A two-sided $p < 0.05$ was considered statistically significant.

## RESULTS

### Demographic parameters

A total of 121 adolescent girls with PCOS (median age: 16.2 years) and 125 healthy controls (median age: 15.7 years) were included in the study. The median birth weights were 3,300 g in the PCOS group and 3,350 g in the control group, with gestational ages of 40.0 and 39.0 weeks, respectively.

Among the PCOS participants, 68% (n = 85) had menstrual irregularities, 25.6% (n = 32) with hirsutism, and 6.4% (n = 8) for other complaints. Hirsutism was observed in 61.6% (n = 77) of the PCOS group, whereas no hirsutism was identified in the control group.

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Obesity was observed in 41.6% (n = 52) of the PCOS group and 14.9% (n = 18) of the controls; small for gestational age (SGA) births were recorded in 12% (n = 12) of the PCOS and 6.6% (n = 8) of the control group. A positive family history of PCOS was reported in 20.8% (n = 26) of the PCOS group and 9.9% (n = 12) of the control group.

## Anthropometric, metabolic, and radiological parameters

Anthropometric data of adolescents with PCOS, BMI, BMI-SDS, waist circumference, fat mass, fat percentage, and SBP values were significantly higher than the control group (**Table 1**).

The median serum MOTS-c level in the PCOS group was higher than in the control group (93.1 vs. 66.2 ng/mL), with a trend toward significance (p = 0.059) (**Table 1** and **Figure 2**). Among the PCOS group, there was no significant difference in the median serum MOTS-c levels of the adolescents with (n = 30) and without insulin resistance (n = 95) [84.1 (205.4) ng/mL & 93.0 (140.5) ng/mL, respectively; p = 0.892] (**Figure 3**). Participants were stratified into four subgroups: (1) adolescents with obesity in the PCOS group (n = 51), (2) adolescents without obesity in the PCOS group (n = 74), (3) adolescents with obesity in the control group (n = 19), and (4) adolescents without obesity in the control group (n = 102). One-way ANOVA revealed no significant difference in serum MOTS-c levels between these subgroups (p = 0.268).

The prevalence of impaired fasting glucose, insulin resistance, and subclinical hypothyroidism were 2.4%, 24%, and 4.8%, respectively, in the PCOS group. The frequencies of elevated TG, TC, LDL-C, ALT, and reduced HDL-C were 16.8%, 18.4%, 12%, 15.2%, and 16%, respectively. Total testosterone was elevated in 73.6% of adolescents with PCOS.

**Table 1.** Demographic and anthropometric characteristics and serum MOTS-c levels of the PCOS and control groups

| | PCOS group (n = 121) | Control group (n = 125) | p | Effect size *r* |
|---|---|---|---|---|
| Age (years) | 16.2 (1.6) | 15.6 (2.3) | **0.026**[a] | **0.14** |
| BMI (kg/m$^2$) | 25.6 (8.2) | 21.3 (6.1) | **<0.001**[a] | **0.37** |
| BMI-SDS | 1.43 (2.02) | 0.05 (2.38) | **<0.001**[a] | **0.35** |
| Waist circumference (cm) | 79 (18.2) | 67.0 (13.0) | **<0.001**[a] | **0.43** |
| Fat percentage (%) | 32.9 ± 8.3 | 26.7 ± 8.5 | **<0.001**[b] | **0.35** |
| Fat mass (kg) | 23.3 (14.8) | 13.9 (13.1) | **<0.001**[a] | **0.37** |
| SBP (mm/Hg) | 110 (10.0) | 110 (5) | **0.001**[a] | **0.23** |
| DBP (mm/Hg) | 70 (10.0) | 70 (10.0) | 0.960[a] | < 0.1 |
| Serum MOTS-c (ng/mL) | 93.1 (146.6) | 66.2 (115.3) | 0.059[a] | 0.12 |

[a]Mann-Whitney U test; [b]Student's t-test.
Data are presented as mean ± SD or median (IQR).
BMI: body mass index; SDS: standard deviation score; SBP: systolic blood pressure; DBP: diastolic blood pressure.

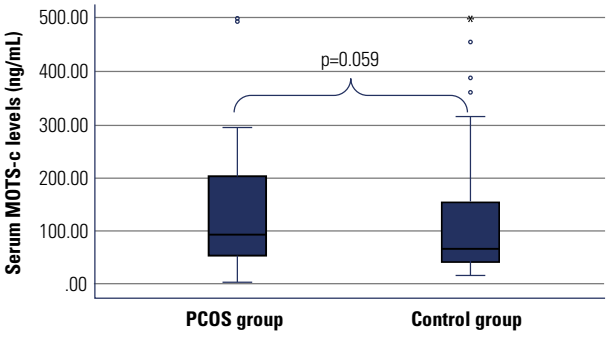



**Figure 2.** Serum MOTS-c levels of PCOS and control groups.

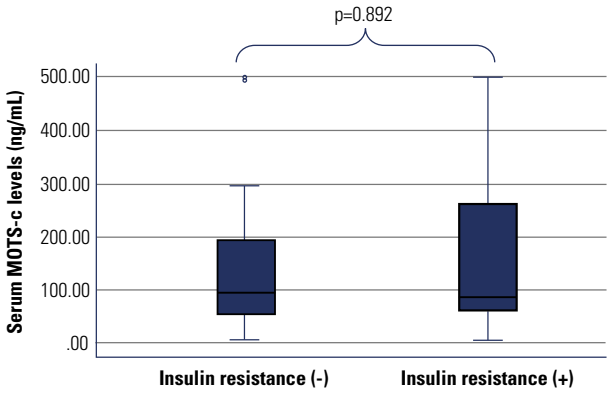



**Figure 3.** Serum MOTS-c levels according to insulin resistance in patients with PCOS.

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PCOS group was compared regarding metabolic parameters according to the presence of obesity: fasting insulin, HOMA-IR, ALT, TG, TC, and LDL-C levels were significantly higher in adolescents with obesity in the PCOS group. However, no significant difference in serum MOTS-c levels was observed between adolescents with and without obesity in the PCOS group (101.0 vs. 80.9 ng/mL, p = 0.160) (**Table 2**).

No significant differences in LH, FSH, LH/FSH ratio, E2, total testosterone, progesterone, $DHEA_{SO4}$, or 17-OH-PG were observed between adolescents with and without obesity in the PCOS groups, except for progesterone levels.

Metabolic syndrome was present in 11 (21.5%) adolescents with obesity in the PCOS group. Serum MOTS-c levels were comparable between adolescents with and without metabolic syndrome in the PCOS group [98.7 (439) vs. 101.8 (191.7) ng/mL; p = 0.889].

No significant correlation was observed between the PCOS group's serum MOTS-c level and any of the anthropometric or metabolic parameters. Furthermore, after correcting for age, BMI, and BMI-SDS with partial correlation analysis, no correlation between serum MOTS-C level and anthropometric or metabolic parameters was found (**Table 3**).

### *MOTS-c* gene polymorphism

All participants in both groups had the wild-type genotype (A/A) for the m.1382A>C (rs111033358) SNP.

## DISCUSSION

Most women with PCOS are either overweight or obese. Studies report that about 80% of women with PCOS in the United States and 30%–50% in other countries are overweight or obese, 44%–70% have insulin resistance, and 21.4%–37% show impaired glucose tolerance (28,29). Dyslipidemia is characterized by elevated TC, TG, LDL-C, and, along with reduced HDL-C levels, it is highly prevalent, affecting up to 70% of women with PCOS (30). Moreover, biochemical hyperandrogenism is present in 60-80% of women with PCOS (31). In our adolescent cohort, we observed obesity in 42% of participants with PCOS, impaired fasting glucose in 2.4%, insulin resistance in 24% and biochemical hyperandrogenism in 76.6%. There were elevated TG, TC, and LDL-C levels in 16.8%, 18.4%, and 12% of patients with PCOS, respectively and 16% had low HDL-C levels.

Insulin resistance, chronic low-grade inflammation, and oxidative stress are well- established characteristics of PCOS (32), with elevated oxidative markers consistently reported in affected individuals compared to healthy controls (33). However, the precise role of mitochondrial dysfunction in the pathophysiology of PCOS remains unclear. This study investigated the differences in MOTS-c levels, one of the MDPs, between adolescents diagnosed with PCOS and the control group. We investigated whether

**Table 2.** Metabolic parameters of the PCOS group according to obesity

| | PCOS group | | p | Effect size *r* |
|---|---|---|---|---|
| | Adolescents with obesity (n = 52) | Adolescents without obesity (n = 73) | | |
| Fasting glucose (mg/dL) | 87.0 (9.0) | 87.0 (9.0) | 0.950[a] | 0.01 |
| Fasting insulin (mU/L) | 17.6 (11.1) | 10.4 (7.0) | **<0.001[a]** | **0.44** |
| HOMA-IR | 3.9 (1.9) | 2.3 (1.5) | **<0.001[a]** | **0.45** |
| ALT (IU/L) | 17.0 (9.75) | 14.0 (9.0) | **0.009[a]** | **0.23** |
| TG (mg/dL) | 112.5 (72.0) | 74.0 (28.5) | **<0.001[a]** | **0.45** |
| TC (mg/dL) | 186.9 ± 32.5 | 157.5 ± 33.1 | **<0.001[b]** | **0.41** |
| LDL-C (mg/dL) | 113.6 ± 29.6 | 90.0 ± 25.0 | **<0.001[b]** | **0.40** |
| HDL-C (mg/dL) | 47.0 (11.0) | 49 (15) | 0.096[a] | 0.15 |
| Serum MOTS-c (ng/mL) | 101.0 (187.5) | 80.9 (151.0) | 0.160[a] | 0.13 |

[a]Mann-Whitney U test; [b]Student's *t*-test.

Data are presented as mean ± SD or median (IQR).

HOMA-IR: homeostasis model assessment-insulin resistance; ALT: alanine transaminase; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol.

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**Table 3.** The relation of serum MOTS-c level with anthropometric and metabolic parameters in the PCOS group

| | PCOS group (n = 125) | | |
|---|---|---|---|
| | Spearman's correlation (ρ) | *p | *95% CI (BCa) |
| Age (years) | 0.001 | 0.987 | [-0.20, 0.19] |
| BMI (kg/m$^2$) | 0.089 | 0.325 | [-0.06, 0.29] |
| BMI-SDS | 0.086 | 0.338 | [-0.06, 0.29] |
| Waist circumference (cm) | 0.057 | 0.537 | [-0.11, 0.26] |
| Fat percentage (%) | 0.058 | 0.542 | [-0.12, 0.23] |
| Fat mass (kg) | 0.074 | 0.435 | [-0.10, 0.25] |
| Fasting glucose (mg/dL) | -0.175 | 0.051 | [-0.34, 0.00] |
| Fasting insulin (mIU/L) | 0.103 | 0.253 | [-0.05, 0.29] |
| HOMA-IR | 0.078 | 0.389 | [-0.08, 0.27] |
| TG (mg/dL) | 0.073 | 0.416 | [-0.08, 0.27] |
| TC (mg/dL) | 0.109 | 0.227 | [-0.04, 0.30] |
| LDL-C (mg/dL) | 0.105 | 0.246 | [-0.05, .030] |
| HDL-C (mg/dL) | 0.024 | 0.789 | [-0.16, 0.21] |
| ALT (IU/L) | 0.068 | 0.448 | [-0.12, 0.22] |
| LH (mIU/L) | 0.043 | 0.631 | [-0.15, 0.22] |
| FSH (mIU/L) | -0.078 | 0.386 | [-0.28, 0.09] |
| E2 (pg/mL) | -0.025 | 0.781 | [-0.23, 0.16] |
| 17-OH PG (ng/mL) | -0.039 | 0.667 | [-0.27, 0.13] |
| Progesterone (ng/mL) | -0.138 | 0.125 | [-0.38, 0.02] |
| Total Testosterone (ng/mL) | 0.137 | 0.127 | [-0.07, 0.33] |

*Spearman correlations with BCa-bootstrapped 95% CIs (1,000 samples).
BMI: body mass index; SDS: standard deviation score; HOMA-IR: homeostasis model assessment-insulin resistance; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol; ALT: alanine transaminase; LH: luteinizing hormone; FSH: follicle stimulating hormone; E2: estradiol; 17-OH PG: 17-hydroxy progesterone.

serum MOTS-c levels, one of the MDPs involved in metabolic regulation, differed between adolescents with PCOS and healthy controls. Although serum MOTS-c levels tended to be higher in the PCOS group compared with controls, the difference did not reach conventional levels of statistical significance. To date, only one human study has examined the relationship between MOTS-c and PCOS. That study focused on adult women and examined changes in MOTS-c levels following intralipid and insulin infusion (14). At baseline, MOTS-c levels did not significantly differ between women with PCOS and healthy controls. Intralipid infusion increased MOTS-c levels significantly, whereas insulin blunted this response. Moderate exercise over eight weeks had no impact on circulating MOTS-c levels (14). Our findings are in line with those reported in adults, suggesting that PCOS may not have a substantial impact on baseline MOTS-c levels during adolescence. On the other hand, a borderline difference in serum MOTS-c levels between PCOS and controls was observed (p = 0.059). Although the study was adequately powered to detect moderate effect sizes based on prior data (14), smaller intergroup differences may not have been detected due to limited statistical power. Therefore, this trend should be interpreted cautiously.

Animal studies have demonstrated promising therapeutic effects of MOTS-c, including the prevention of diet-induced obesity, insulin resistance, and fat accumulation (9,11). These benefits resemble those observed with metformin treatment (34). MOTS-c administration has improved insulin sensitivity and glucose metabolism in various experimental models, including gestational diabetes (35), autoimmune diabetes (36), and ovariectomy-induced obesity (37). Despite the robust evidence in animal models, human studies yield inconsistent results, likely due to variations in age, comorbidities, and methodological approaches. For

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example, Du and cols. (38) reported reduced MOTS-c levels in boys with obesity, but not girls, and a negative correlation with obesity markers (BMI, HOMA-IR, hemoglobin A1c (HbA1c), and fasting insulin). In contrast, Cataldo and cols. (13) found no difference between adults with obesity and control group but reported a positive correlation between MOTS-c levels and insulin sensitivity individuals with normal weight.

Conflicting associations between MOTS-c levels and metabolic parameters such as BMI, HOMA-IR, and lipid profile suggest a complex, context-dependent role of MOTS-c in humans (10,39). Some evidence supports the hypothesis that MOTS-c levels rise during the early stages of metabolic imbalance as a compensatory mechanism (39), whereas overt metabolic disease (e.g., type 2 diabetes) is associated with decreased MOTS-c levels (40). In our study, neither obesity, insulin resistance, nor metabolic syndrome significantly affected serum MOTS-c levels in adolescents with PCOS. Furthermore, no correlation was observed between MOTS-c and metabolic or hormonal parameters, including BMI, glucose, insulin, lipid profile, or androgen levels. Moreover, subgroup analyses according to obesity, insulin resistance, and metabolic syndrome status were exploratory in nature and aimed to further characterize the metabolic heterogeneity of PCOS. Since these comparisons involved secondary analyses rather than independent hypotheses, no formal correction for multiple testing was applied. Therefore, these findings should be interpreted with caution, and future studies with larger cohorts are needed to confirm whether the observed trends represent true biological differences.

Interestingly, MOTS-c levels have been shown to fluctuate based on physiological states. For example, there were elevated levels in pregnant women with obesity and in cases of metabolic syndrome without diabetes (39,41), whereas levels decreased in type 1 and type 2 diabetes (10,36) and in individuals with coronary endothelial dysfunction (42). Our adolescent cohort may represent an early stage of metabolic disturbance, in which compensatory increases in MOTS-c are still ongoing. Longitudinal studies capturing different disease stages may help to clarify these dynamic patterns. Demonstrating this pattern would provide a clearer insight into the relationship between PCOS and MOTS-c, which is closely linked to obesity, insulin resistance, and metabolic syndrome, which could also help to clarify the pathophysiology of PCOS. Furthermore, MOTS-c levels could serve as a marker for PCOS stages or for monitoring its metabolic effects.

We also explored the potential role of the m.1382A>C (rs111033358) polymorphism in the *MOTS-c* gene, previously associated with type 2 diabetes risk in males from East Asia (15,16). This SNP, which was found predominantly in haplogroup D4b2, alters the amino acid sequence of *MOTS-c* (K14Q), potentially impairing its biological function. Interestingly, haplogroup D4b2 was associated with longevity (43,44). However, a larger study reported similar frequencies of the m.1382A>C polymorphism between centenarians and controls, suggesting that this variant is unlikely to contribute substantially to longevity (16). In the same study, Japanese male individuals with the m.1382A>C allele variant in the *MOTS-c* gene who are sedentary have an increased incidence of T2DM compared to men with polymorphism who engage in physical activities (16). With these results, it has been suggested that the combination of a sedentary lifestyle and the m.1382A> C polymorphism may contribute to an increased risk of type 2 DM (16). Moreover, the K14Q MOTS-c treatment of high-fat-fed mice failed to provide the metabolic benefits associated with natural MOTS-c administration, suggesting that the m.1382A>C variant results in inactive endogenous MOTS-c (11,16). Furthermore, this polymorphism may serve as a biomarker for sex-specific susceptibility to metabolic complications (45). Given that the metabolic variability of MOTS-c levels differed by gender in some studies, along with observed gender-specific differences in the m.1382A>C polymorphism, we hypothesized that the *MOTS-c* polymorphism may play a role in PCOS, which is a condition unique to women and characterized by metabolic disturbances. However, in this study, m.1382A>C polymorphism was detected as wild-type (A/A) in all adolescents. The allele frequency of this variant was very low in the Korean population, with C = 0.0520 (https://www.ncbi.nlm.nih.gov/snp/rs111033358). The absence of

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this variant in our study may be related to the low allele frequency. To the best of our knowledge, this variant was not investigated in patients with PCOS before, so, with this study, we may conclude that the m.1382A>C polymorphism in the *MOTS-c* gene is not a risk factor for developing PCOS. Conversely, the absence of this variant in our study cohort suggests that it may be a population-specific variant. As this polymorphism was originally identified in populations from East Asia, ethnic differences may influence its distribution, and future studies conducted in women with PCOS from this demographic could yield different results.

Another critical finding about MOTS-c studies is that the results can show gender-specific differences. For instance, only male mice were used in the study in which MOTS-c treatment reduced obesity and insulin resistance (11). While MOTS-c had no apparent metabolic effect in non-ovariectomized female mice, it prevented metabolic imbalance in ovariectomized mice, suggesting that MOTS-c treatment may prevent postmenopausal obesity and insulin resistance (37). In the study previously mentioned, circulating MOTS-c levels were lower in boys and in adolescents with obesity, whereas no difference was observed between girls with and without obesity (38). Moreover, the effect of m.1382A>C variant on T2DM is prominent only in male individuals (16). These results led to the following claims: the resistance of mitochondrial functions to oxidative stress and antioxidant responses may be related to gender; estrogen activates mitochondrial biogenesis; and ovarian hormones may affect MOTS-c (16,38). Consistent with previous reports, serum MOTS-c levels were comparable between adolescents with PCOS and healthy peers in our study. Moreover, for the first time, we investigated the relationship between serum MOTS-c concentrations and gonadotropin, estradiol, progesterone, and total testosterone levels in patients with PCOS. No significant associations were observed between MOTS-c and these hormones. These findings suggest that sex-related differences in MOTS-c levels are unlikely to be primarily driven by ovarian or gonadotropic hormones. Large-scale studies involving adolescents, women, and men from diverse ethnic backgrounds are needed to further elucidate these associations.

Several limitations should be considered when interpreting our findings. The main limitations of this study include the absence of metabolic and hormonal profiling in the control group and the evaluation of only a single polymorphism (rs111033358, m.1382A>C) among the *MOTS-c* gene variants. The metabolic and hormonal parameters of the control group were not assessed due to budgetary constraints and ethical considerations regarding extensive blood sampling in adolescents. Although these parameters were presumed to be within the normal range, any unrecognized metabolic or hormonal alterations in controls could have influenced the observed associations. Furthermore, the study was conducted within a specific ethnic population attending a single center. As the prevalence of biochemical or clinical hyperandrogenemia and PCOS differs among ethnic groups, caution is needed when generalizing these findings. Considering that the *MOTS-c* m.1382A>C (rs111033358) polymorphism was first identified in populations from East Asia, further research is needed to evaluate its frequency and potential association with PCOS in diverse ethnic cohorts. Future studies should also include comprehensive metabolic and hormonal profiling of control groups and involve larger, multiethnic populations to enhance the interpretability and generalizability of MOTS-c–related findings.

In conclusion, serum MOTS-c levels were comparable between adolescents with PCOS and healthy controls. Additionally, obesity, insulin resistance, dyslipidemia, and hyperandrogenism did not seem to influence MOTS-c concentrations in adolescents with PCOS. The *MOTS-c* gene m.1382A>C polymorphism was detected as wild-type (A/A) in all participants, suggesting that this variant could not play a role in the etiopathogenesis of PCOS. Taken together, these findings indicate that, in this dataset, we found no evidence of differences in circulating MOTS-c or presence of the m.1382A>C variant related to PCOS. Larger studies in diverse populations are needed to further investigate potential metabolic relevance.

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**Ethical approval:** the local ethics committee of Tepecik Training and Research Hospital for Interventional Clinical Studies approved the study (Date: 05.02.2020, Number: 2).

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**Data availability:** datasets related to this article will be available upon request to the corresponding author.

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original

# Impact of early diagnosis of overt diabetes on pregnancy outcomes: a retrospective cohort study among Brazilian women

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## ABSTRACT

**Objective:** Early diagnosis of overt diabetes has not been extensively explored. We compared pregnancy outcomes of women diagnosed with overt diabetes in the first trimester (up to the 13 completed weeks) to those with a later diagnosis. **Subjects and methods:** We evaluated women with overt diabetes and a type 2 diabetes phenotype. Pregnancy evolution (gestational weight gain, HbA1c, inpatient glycemic control) and outcomes (preeclampsia, neonatal hypoglycemia, and admission to the neonatal intensive care unit) were compared using multivariable analyses (linear regression or Poisson regression with robust variances). All models computed the early diagnosis and other specific clinically relevant outcome-related variables. Results are b (linear coefficient) or adjusted relative risk (RR) with 95% CI. **Results:** Of the 217 study participants, 127 (58.5%) were diagnosed in the first trimester (early diagnosis group); those with a late diagnosis presented a higher 3rd-trimester HbA1c (n = 169; b = 1.29; 0.04; 0.55, p = 0.026). Maternal inpatient care was associated with later diagnosis (n = 195; RR 1.29; 1.02; 1.63, p = 0.033) and higher initial HbA1c (n = 195; RR 1.25; 1.16; 1.35, p < 0.001). Aside from a better maternal metabolic control, early diagnosis of overt diabetes did not impact any other pregnancy outcome. **Conclusions:** In the present cohort of women with overt diabetes, diagnosis before the 14 gestational week modestly improved a few maternal metabolic aspects that did not translate into better pregnancy outcomes.

**Keywords:** Type 2 diabetes; overt diabetes; diabetes in pregnancy; pregnancy outcomes

## INTRODUCTION

Overt diabetes is hyperglycemia that meets the diagnostic criteria for diabetes and is first detected at any time during pregnancy (1). Any case of diabetes can thus present as overt diabetes, probably representing a previously undiagnosed hyperglycemia, associated with the global rise in obesity and type 2 diabetes, now affecting women at progressively younger ages (2). Like gestational diabetes mellitus (GDM), overt diabetes can resolve after pregnancy, since only ~34% of women presented postpartum hyperglycemia in a meta-analysis (3); those diagnosed in the first trimester exhibited a higher prevalence of postpartum hyperglycemia (4).

During normal pregnancy, the pattern of glucose and insulin interrelation undergoes adaptive changes (5). Beginning at 12 to 14 gestational weeks, insulin sensitivity decreases, followed by an increase in insulin resistance with advancing gestation, which has been associated with placental hormone production (5). In pregnant women with type 2 diabetes, preexisting

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insulin resistance antagonizes the compensatory effect of the increased insulin sensitivity (6), unveiling hyperglycemia already in the early first trimester. For many authors, these findings at early pregnancy extend to mid-pregnancy, ~20 weeks of gestation (7). Since these metabolic alterations begin early in pregnancy, we chose the 14th gestational week, which represents the end of the first trimester in the Brazilian prenatal chart (8), as the cutoff point to divide our sample into early and late diagnosis groups.

Emerging evidence suggests that women with overt diabetes exhibit prevalence and risk of adverse perinatal outcomes comparable to those with pregestational type 2 diabetes (7,9), and generally greater than those associated with GDM (10). Early diagnosis and treatment of GDM, particularly before the 20th week, were associated with modest improvements in selected outcomes, including reduced incidence of neonatal respiratory distress, without affecting most outcomes (11). Few investigations have specifically addressed the impact of early versus late diagnosis of overt diabetes on pregnancy outcomes (7,12,13). Although overt diabetes should be identified by universal screening in the first trimester, as recommended by the World Health Organization (1), many cases are detected only later, when GDM screening traditionally occurs (14,15). Notably, an early diagnosis did not always translate into immediate treatment (10).

This study compared pregnancy outcomes according to the timing of overt diabetes diagnosis in a cohort of Brazilian women.

## SUBJECTS AND METHODS

### Participants and ethics statement

A secondary analysis was conducted using a retrospective cohort of participants with overt diabetes identified among 646 women with a clinical phenotype of type 2 diabetes (9). All women were enrolled between 2015 and 2021 at the two busiest public specialized prenatal care units in the Sistema Único de Saúde (SUS, the Brazilian public health system) in southern Brazil. The ethics committee of both hospitals approved the study protocol on July 28, 2016 (project number 2016-0331, *Hospital de Clínicas de Porto Alegre*). The study was registered in Plataforma Brasil under the national ethics appreciation certificate (CAAE) number 57365016.3.0000.5327, and the authors signed a data use agreement to ensure data confidentiality.

### Study variables

Overt diabetes was diagnosed at any time during pregnancy as per the 2013 World Health Organization guideline (fasting plasma ≥ 126 mg/dL or 2 h glucose after a 75 g load ≥ 200 mg/dL) (1) and/or according to the American Diabetes Association recommendation of glycated hemoglobin (HbA1c) ≥ 6.5% (16).

Since working with a complete dataset, we did not calculate the sample size; instead, we calculated the study power (type II error) for the main pregnancy outcomes. Target sample size and study power were calculated using the PSS-Health tool (17). We set a more conservative relative risk (RR) of 2.0, based on the relative risk of delivering large for gestational age babies (RR = 2.3) described by Jayasinghe and cols. (12) in a study with 58 participants, which compared risks between overt diabetes diagnosed in the first trimester to the other trimesters.

Data were retrieved from the electronic medical records. Demographic characteristics included age, schooling years, and skin color. Presence of diabetes complications (retinopathy, nephropathy, and/or neuropathy) and other baseline information, such as family history of diabetes or hypertension, smoking, chronic hypertension, prior gestational diabetes or macrosomia, and maternal admission for glycemic control, were collected as recorded in the hospital charts. Pre-pregnancy weight was self-reported, and height was measured at the first prenatal appointment. Weight was measured at each visit. We calculated the pregestational body mass index (BMI) as the informed pregestational weight in kilograms divided by the square of height, in meters, classifying women as normal pregestational BMI (BMI < 25.0 kg/m$^2$), overweight (BMI ≥ 25.0 kg/m$^2$ to BMI < 30.0 kg/m$^2$), or obesity (BMI ≥ 30 kg/m$^2$) (18). Gestational weight gain was calculated as the weight measured after the 35 weeks gestation minus the pregestational weight. Gestational weight gain adequacy for each BMI category was classified as insufficient, adequate, or

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excessive according to the 2009 National Academy of Medicine recommendation (18). Preeclampsia was defined according to current recommendations (19). HbA1c was measured at booking, regardless of gestational age, and was collected at least once more ~ the 28th week. Assays were conducted with high-performance liquid chromatography (Variant II Turbo HbA1c; BioRad, Hercules, CA, USA) in line with the National Glycohemoglobin Standardization Program guidelines (http://www.ngsp.org/index. asp).

Birth weight categories were classified according to the World Health Organization chart (20) as small for gestational age (SGA, below the 10th percentile), large for gestational age (LGA, above the 90th percentile), and adequate (AGA, between the 10th and 90th percentiles). Macrosomia was defined as a birthweight ≥ 4,000 g. We defined congenital anomalies according to the Q chapter of the 10th International Classification of Diseases. Preterm birth refers to delivery before the 37+0 gestational weeks. Neonatal intensive care unit (NICU) admission and hypoglycemia diagnoses were accepted as determined by the neonatal assistant team. We excluded twin pregnancies and babies with genetic anomalies from the analyses of the neonatal outcomes.

Primary outcomes included maternal inpatient care for glycemic control, pre-eclampsia, NICU admission, and neonatal hypoglycemia. Gestational weight gain and 3rd-trimester HbA1c constituted the secondary outcomes.

### Statistical analysis

To explore the influence of the timing of overt diabetes diagnosis on pregnancy outcomes, we divided the sample into an early (up to 14 weeks gestation) and a late group (≥ 14 weeks) (5). We performed univariable analyses of the primary outcomes (maternal inpatient glycemic control, preeclampsia, neonatal hypoglycemia, and NICU admission) and secondary outcomes (total gestational weight gain and 3rd-trimester HbA1c) to identify relevant variables that were then entered into the multivariable analyses.

First, we calculated the crude risk for each outcome, testing early overt diabetes diagnosis against each of the selected outcomes. Then, we built multivariable models including potential confounders for early overt diagnosis and the variables found as relevant for each outcome by the univariable analyses. Baseline maternal characteristics (potential confounders) were included in the models only when a causal association was deemed probable. We tested the correlation between potentially related variables.

Continuous variables were described as mean and standard deviation or median and interquartile range, according to the profile of normal distribution analyzed by the Shapiro-Wilk test. Categorical variables were reported as absolute and relative frequencies. We used Student's *t*-test or linear regression for continuous variables and the chi-square test for dichotomous variables to compare characteristics between groups.

Multivariable analyses were performed using Poisson regression with robust variances (21) for dichotomous outcomes, and the results were expressed as crude and adjusted relative risks (RR) and 95% confidence intervals (95% CI), p-value. We used linear models for continuous variables, and the results were presented as b coefficient (95% CI) and the corresponding p-value. We used ChatGPT 4o to adapt the English writing to that of a native speaker.

We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (22) to write the manuscript.

## RESULTS

Of the 646 participants with clinical characteristics of type 2 diabetes, 217 presented overt diabetes (33.6%, 95% CI 30.0%-37.4%); 127 (58.5%) were diagnosed before 14 gestational weeks and 90 (41.5%) after. In the **Supplementary Table 1**, we present the univariable analyses. Variables associated with maternal inpatient care included center of enrollment, gestational age, and weight gain at booking, and baseline HbA1c. Preeclampsia was associated with the center of enrollment, although two important clinical risk factors for this outcome, chronic hypertension and obesity, were included in the multivariable analyses. Maternal smoking, preeclampsia, gestational age at birth, male gender, and macrosomia were factors associated with neonatal hypoglycemia; smoking, preeclampsia,

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3rd-trimester HbA1c, and gestational age at birth were associated with NICU admission. Gestational weight gain models included previous macrosomia, obesity, booking weight gain, and gestational age at delivery. The 3rd-trimester HbA1c models included previous macrosomia, booking weight gain, and booking HbA1c. Due to their clinical relevance as risk factors for the outcomes, these variables were included in the multivariable analyses along with late diagnosis of overt diabetes. Gestational age at booking was significant in univariable analyses of some outcomes. However, a moderate correlation (r = 0.645, p < 0.001) (23) between gestational age at booking and gestational age at overt diabetes diagnosis hindered the inclusion of the first variable in any multivariable analysis.

In **Supplementary Table 2**, we present the study power for the main outcomes using data from other studies and their prevalence in the current sample. Study power for different outcomes ranged from 23.2% (congenital anomaly) to 100% (NICU admission), considering a 5.0% significance level. Prevalence of outcomes in our study varied from 13.6% (congenital anomaly) to 38.6% (NICU admission). Although congenital anomalies exhibited an unexpected high frequency, we lacked an adequate sample size to evaluate the influence of early diagnosis on this outcome.

In **Table 1**, we summarize the participants' baseline characteristics according to the timing of diagnosis, up to or after 14 gestational weeks. Fasting plasma glucose, alone or combined with HbA1c, was the most employed diagnostic tool for early diagnosis, whereas 2-h glucose was mostly used in the later period. Of the total sample, 94 women (78.3%) in the early group and 57 (65.5%) in the late group already presented a diagnostic fasting plasma glucose ≥ 126 mg/dL. Participants with early diagnosis more frequently had a family history of hypertension (56.7% vs. 36.7%, p = 0.006) and of previous hypertension (23.6% vs. 7.8%, p = 0.004), arrived earlier at the prenatal care facility (20.3 ± 7.7 weeks vs. 29.5 ± 5.5 weeks, p < 0.001) and required fewer hospital admissions for glycemic control during pregnancy (50.0% vs. 68.3%, p = 0.014). Total gestational weight gain was lower (6.5 kg ± 7.3 kg vs. 10.8 kg ± 10.5 kg, p = 0.002), and excessive weight gain was less frequent (26.1% vs. 44.3%, p = 0.004). They also reached a lower 3rd-trimester HbA1c (6.1% ± 0.9 % vs. 6.6% ± 1.2%, p = 0.02). Preeclampsia and neonatal (NICU admission and hypoglycemia) outcomes were similar between groups, and male gender was more common in early diagnosis pregnancies (67.6% vs. 46.8%, p = 0.007).

In **Table 2**, we present the crude and adjusted risks for each outcome. The risk of maternal inpatient care for glycemic control increased by 37% (RR 1.37, 95% CI 1.08-1.72, n=204 women) with a late diagnosis. In multivariable analyses (n=195), this risk decreased to 29% (RR 1.29, 95% CI 1.02-1.63, p = 0.033) when adjusted for other associated factors (center of enrollment and baseline HbA1c).

In the preeclampsia models (n = 201), the center of enrollment was associated with a greater than two-fold risk (RR: 2.41, 95% CI 1.48-3.91, p < 0.001). Neither early diagnosis nor other relevant risk factors (chronic hypertension and obesity) were significant in the multivariable analysis.

Early diagnosis showed no effects on the neonatal outcomes. Several expected risk factors related to hypoglycemia (n = 186 women) were associated in adjusted models: gestational age at delivery (RR 0.86, 95% CI 0.75-0.99, p = 0.032), macrosomia (RR 1.99, 95% CI 1.16-3.43, p = 0.013), and smoking (RR 1.86, 95% CI 1.08-3.18, p = 0.025). Smoking (RR 2.22, 95% CI 1.47-3.36, p < 0.001) and a higher maternal 3rd-trimester HbA1c (RR 1.39, 95% CI 1.14-1.68, p < 0.001) were associated with an increased risk for NICU admission (n = 152 neonates), whereas a higher gestational age at delivery (RR 0.85, 95% CI 0.77-0.93, p < 0.001) was protective.

Gestational weight gain and 3rd-trimester HbA1c were associated with an early diagnosis of diabetes in non-adjusted models. In adjusted models, a late diagnosis was associated with an increased 3rd-trimester HbA1c value (b = 0.29, 95% CI 0.04-0.55, p = 0.026).

## DISCUSSION

In this cohort of 217 women with diabetes first diagnosed during pregnancy, mostly before 14 weeks gestation, early diagnosis did not improve main pregnancy outcomes, even when adjusted for clinically relevant variables. A late diagnosis was associated with

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**Table 1.** Characteristics and outcomes of overt diabetes diagnosed before or after 14 gestational weeks in a cohort of pregnant Brazilian participants

| Characteristic/outcome | Timing of overt diabetes diagnosis | | p |
|---|---|---|---|
| | Up to 14 weeks n = 127 (58.5) | ≥ 14 weeks n = 90 (41.5) | |
| **Maternal** | | | |
| Center | | | 0.957 |
| Center 1 | 59 (46.5) | 43 (47.8) | |
| Center 2 | 68 (53.5) | 47 (52.2) | |
| Maternal age (years) | 32.3 ± 5.8 | 31.1 ± 6.5 | 0.153 |
| White skin color | 86 (67.7) | 68 (75.6) | 0.271 |
| ≤ 11 years of schooling | 121 (95.3) | 87 (96.7) | 0.872 |
| Smoking | 16 (12.6) | 10 (11.1) | 0.904 |
| Family history of diabetes | 80 (63.0) | 53 (58.9) | 0.638 |
| Previous gestational diabetes | 46 (36.2) | 24 (26.7) | 0.182 |
| Family history of hypertension | 72 (56.7) | 33 (36.7) | 0.006 |
| Chronic hypertension | 30 (23.6) | 7 (7.8) | 0.004 |
| Diabetes complications | 2 (1.6) | 0 (0.0) | 0.635 |
| Diabetes diagnosis by | | | <0.001 |
| FPG | 31 (24.4) | 7 (7.8) | |
| HbA1c | 14 (11.0) | 5 (5.6) | |
| FPG + HbA1c | 48 (37.8) | 25 (27.8) | |
| 2-h glucose | 12 (9.4) | 18 (20.0) | |
| 2-h glucose + HbA1c | 1 (0.8) | 6 (6.7) | |
| FPG + 2-h glucose | 7 (5.5) | 8 (8.9) | |
| All | 14 (11.0) | 21 (23.3) | |
| FPG ≥ 126 mg/dL | 94 (78.3) | 57 (65.5) | 0.059 |
| | 120 | 87 | |
| Gestational age at diagnosis (weeks) | 8.9 ± 3.0 | 22.4 ± 6.5 | |
| Gestational age at booking (weeks) | 20.3 ± 7.7 | 29.5 ± 5.5 | <0.001 |
| Folate use 1st trimester | 22 (17.3) | 9 (10.0) | 0.186 |
| Obesity | 95 (76.0) | 56 (67.5) | 0.233 |
| | 125 | 83 | |
| Weight gain at booking (kg) | 3.4 ± 5.6 | 7.9 ± 9.8 | <0.001 |
| | 125 | 84 | |
| HbA1c at booking (%) | 7.0 ± 1.3 | 7.0 ± 1.3 | 0.692 |
| | 126 | 90 | |
| HbA1c < 6.5% 1st trimester | 12 (22.6) | 1 (100) | 0.540 |
| | 53 | 1 | |
| First HbA1c ≥ 7.0% | 60 (47.6) | 42 (46.7) | >0.999 |
| | 126 | 90 | |
| Inpatient glycemic control | 61 (50.0) | 56 (68.3) | 0.014 |
| | 122 | 82 | |
| Gestational weight gain | | | |
| From booking to delivery (kg) | 3.1 ± 4.3 | 2.5 ± 4.7 | 0.380 |
| | 121 | 80 | |
| Total weight gain (kg) | 6.5 ± 7.3 | 10.8 ± 10.5 | 0.002 |
| Weight gain adequacy | | | 0.005 |
| Insufficient | 51 (42.9) | 33 (41.8) | |
| Adequate | 37 (31.1) | 11 (13.9) | |
| Excessive | 31 (26.1) | 35 (44.3) | |
| | 119 | 79 | |
| Insulin treatment during pregnancy | 101 (82.1) | 74 (83.1) | 0.990 |
| | 123 | 89 | |
| HbA1c 3rd-trimester (%) | 6.1 ± 0.9 | 6.6 ± 1.0 | 0.005 |
| | 105 | 65 | |
| HbA1c 3rd-trimester < 6.5% | 73 (69.5) | 30 (46.2) | 0.004 |
| | 105 | 65 | |
| Preeclampsia | 38 (31.1) | 28 (31.8) | >0.999 |
| | 122 | 88 | |

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| Characteristic/outcome | Timing of overt diabetes diagnosis | | p |
|---|---|---|---|
| | Up to 14 weeks n = 127 (58.5) | ≥ 14 weeks n = 90 (41.5) | |
| **Neonatal** | | | |
| Preterm birth | 26 (23.4) | 13 (16.5) | 0.322 |
| | 111 | 79 | |
| Male gender | 75 (67.6) | 37 (46.8) | 0.007 |
| | 111 | 79 | |
| Birth weight categories* | | | 0.512 |
| SGA | 7 (6.3) | 4 (5.1) | |
| AGA | 64 (57.7) | 40 (50.6) | |
| LGA | 40 (36.0) | 35 (44.3) | |
| | 111 | 79 | |
| Birth weight ≥ 4,000 g | 18 (16.2) | 12 (15.2) | >0.999 |
| | 111 | 79 | |
| Congenital anomaly | 14 (12.5) | 6 (7.6) | 0.395 |
| | 112 | 79 | |
| NICU admission | 42 (38.9) | 28 (35.4) | 0.743 |
| | 108 | 79 | |
| Neonatal hypoglycemia | 26 (24.1) | 14 (17.7) | 0.387 |
| | 108 | 79 | |
| Neonatal death | 1 (0.9) | 0 (0.0) | >0.999 |
| | 108 | 79 | |

FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; SGA: small for gestational age; AGA: adequate for gestational age; LGA: large for gestational age; NICU: neonatal intensive care admission.

*Birth weight categories classified according to the World Health Organization chart.

Data are presented as mean ± standard deviation or n (%).

Statistical tests: Student *t*-test for continuous variables and chi-square test for categorical variables.

a higher risk of maternal inpatient care for glycemic control and a higher 3rd-trimester HbA1c level.

The clinical implications of the timing of overt diabetes diagnosis remain insufficiently investigated. In our cohort, the physiological alterations of pregnancy, further enhanced by an already increased insulin resistance due to a previous undiagnosed hyperglycemia (plus the effects of obesity), could explain the differences observed in the comparisons between the first and later trimesters of pregnancy. We posit that insulin sensitivity was affected earlier in these pregnancies due to pregestational insulin resistance associated with obesity. Among studies with more than 100 participants, one reported no significant differences in pregnancy outcomes when comparing diagnoses made before and after the 20th gestational week (7). When applying this cutoff to our sample, baseline maternal characteristics and pregnancy outcomes were similar, limiting further relevant analyses. Metabolic changes related to placental secretion of hormones that compete with insulin are enhanced from the second trimester onwards, partially justifying the similarity found between the groups. Moreover, the behavior of metabolic changes during pregnancy can differ between overt diabetes and GDM, as insulin resistance is probably more pronounced at the beginning of pregnancy in overt diabetes (6).

Other studies on the timing of overt diabetes diagnosis are available. In a recent study from China, the authors found an association between early diagnosis and increased risks of macrosomia and large-for-gestational-age (LGA) neonates (13). Smaller studies have suggested increased risk of LGA (12) and postpartum diabetes associated with first-trimester overt diabetes (4). Our findings revealed an association between early diagnosis of overt diabetes and some maternal benefits, such as reduced hospitalization for glycemic control; however, this improvement did not translate into better maternal and neonatal outcomes. Delayed access to specialized prenatal care in Brazil can partially explain this finding. Although some initial treatment steps could have been implemented in the primary care setting, these measures are not standardized in the general prenatal care. Even women diagnosed in the first trimester arrived at the specialized care after ~20 gestational weeks, despite diabetes in pregnancy being a referral priority in the Brazilian public health system (24). In a study of 319 women, only ~33% reported arrival before 15 days

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**Table 2.** Risk factors for selected pregnancy outcomes in overt diabetes: comparing late with early diagnosis in a cohort of Brazilian participants

| Maternal outcomes | | | | | | |
|---|---|---|---|---|---|---|
| **Inpatient glycemic control** | | | | | | |
| **Risk factor** | **n** | **Crude RR (95% CI)** | **p** | **n** | **Adjusted RR (95% CI)** | **p** |
| Late diagnosis | 204 | 1.37 (1.08; 1.72) | 0.008 | 195 | 1.29 (1.02; 1.63) | 0.033 |
| Center 2 | 204 | 1.44 (1.11; 1.86) | 0.007 | | 1.28 (1.01; 1.63) | 0.046 |
| Weight gain at booking | 196 | 1.02 (1.01; 1.03) | <0.001 | | 1.01 (1.00; 1.02) | 0.096 |
| HbA1c at booking | 203 | 1.27 (1.17; 1.36) | <0.001 | | 1.25 (1.16; 1.35) | <0.001 |
| **Preeclampsia** | | | | | | |
| Late diagnosis | 210 | 1.02 (0.68; 1.53) | 0.918 | 201 | 1.17 (0.78; 1.74) | 0.446 |
| Center | 210 | 2.43 (1.50; 3.92) | <0.001 | | 2.41 (1.48; 3.91) | <0.001 |
| Chronic hypertension | 210 | 1.60 (1.04; 2.46) | 0.032 | | 1.51 (0.96; 2.37) | 0.076 |
| Obesity | 201 | 1.66 (0.97; 2.87) | 0.067 | | 1.65 (0.99; 2.76) | 0.054 |
| **Total gestational weight gain** | | | | | | |
| | | **Crude b (95% CI)** | **p** | **n** | **Adjusted b (95% CI)** | **p** |
| Late diagnosis | 198 | 4.34 (1.85; 6.84) | 0.001 | 184 | -0.39 (-1.81; 1.04) | 0.591 |
| Previous macrosomia | 198 | 3.11 (0.21; 6.02) | 0.036 | | 2.18 (0.64; 3.72) | 0.006 |
| Obesity | 196 | -6.77 (-9.24; -4.11) | <0.001 | | -1.98 (-3.53; -0.43) | 0.012 |
| Weight gain at booking | 197 | 0.96 (0.88; 1.04) | <0.001 | | 0.91 (0.82; 1.00) | <0.001 |
| Maternal inpatient care | 187 | 2.65 (0.08; 5.22) | 0.043 | | -0.40 (-1.75; 0.95) | 0.563 |
| **3rd-trimester Hba1c** | | | | | | |
| Late diagnosis | 170 | 0.44 (0.15; 0.73) | 0.003 | 169 | 0.29 (0.04; 0.55) | 0.026 |
| Previous macrosomia | 170 | 0.49 (0.17; 0.80) | 0.003 | | 0.24 (-0.04; 0.52) | 0.086 |
| Weight gain at booking | 169 | 0.03 (0.01; 0.05) | <0.001 | | 0.02 (0.01; 0.04) | 0.007 |
| HbA1c at booking | 170 | 0.36 (0.26; 0.46) | <0.001 | | 0.33 (0.23; 0.42) | <0.001 |
| **Neonatal outcomes** | | | | | | |
| **Hypoglycemia** | | | | | | |
| | **n** | **Crude RR (95% CI)** | **p** | **n** | **Adjusted RR (95% CI)** | **p** |
| Late diagnosis | 187 | 0.74 (0.41; 1.32) | 0.302 | 186 | 0.93 (0.54; 1.61) | 0.788 |
| Smoking | 187 | 2.46 (1.42; 4.27) | 0.001 | | 1.86 (1.08; 3.18) | 0.025 |
| Preeclampsia | 186 | 1.89 (1.09; 3.27) | 0.023 | | 1.59 (0.94; 2.69) | 0.086 |
| Gestational age at delivery | 187 | 0.85 (0.75; 0.96) | 0.011 | | 0.86 (0.75; 0.99) | 0.032 |
| Male gender | 187 | 2.31 (1.17; 4.56) | 0.016 | | 1.88 (0.95; 3.73) | 0.071 |
| Macrosomia | 187 | 1.99 (1.12; 3.52) | 0.019 | | 1.99 (1.16; 3.42) | 0.013 |
| **NICU admission** | | | | | | |
| Late diagnosis | 187 | 0.91 (0.62; 1.33) | 0.632 | 152 | 0.86 (0.54; 1.35) | 0.497 |
| Smoking | 187 | 2.08 (1.47; 2.94) | <0.001 | | 2.22 (1.47; 3.36) | <0.001 |
| Preeclampsia | 186 | 1.70 (1.18; 2.44) | 0.004 | | 1.56 (1.04; 2.35) | 0.033 |
| 3rd-trimester HbA1c | 152 | 1.29 (1.06; 1.56) | 0.010 | | 1.39 (1.14; 1.68) | 0.001 |
| Gestational age at delivery | 187 | 0.86 (0.81; 0.92) | <0.001 | | 0.85 (0.77; 0.93) | <0.001 |

RR: relative risk; CI: confidence interval; HbA1c: glycated hemoglobin.
Data are presented as crude and adjusted relative risk (95% CI) or b coefficient (95% CI).
Statistics tests: Poisson's regression with robust variances, linear regression.

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after referral to a specialized unit; another 27% arrived less than 30 days before, and almost 20% never sought the service (25). A multifactorial issue, one of the reasons for such delays includes the public health system's prioritized attention on more prevalent infectious diseases. Paucity of specialized teams and the socioeconomic, educational, and cultural characteristics of patients also contribute (24). Although women diagnosed in the first trimester arrived at specialists around mid-pregnancy, they had received nutritional counseling and medications, including insulin, in the primary setting, gaining less weight (~3.0 kg) compared with those arriving later.

Most outcomes evaluated showed no relation with early diagnosis of overt diabetes, but did correlate with other clinically relevant variables. Although chronic hypertension, a well-known risk factor for preeclampsia (19), was more prevalent (~ three times) among women diagnosed with overt diabetes during the first trimester, it showed no significant association with preeclampsia after adjustments. This finding suggests that the presence of chronic hypertension in early pregnancy prompted clinicians and obstetricians to screen for diabetes earlier. Chronic hypertension has been inadequately reported in studies on overt diabetes (≥ 100 participants) — ~4.0% in one study (26), ~9.0% in another (27), compared to ~17.0% in our cohort — limiting our evaluation of chronic hypertension as a risk factor for overt diabetes.

As expected, neonatal hypoglycemia showed a protective association with a higher gestational age at delivery, whereas neonatal macrosomia was associated with increased risk of this outcome (28).

NICU admission was independently related to higher gestational age at delivery (a protective role) and to maternal third-trimester glycemia, highlighting the role of late-pregnancy glycemic control in reducing neonatal morbidity, as found in other types of diabetes in pregnancy (29). Preeclampsia and smoking were other independent risk factors, possibly related to placental function.

Overt diabetes remains poorly characterized in the literature. Many studies of diabetes in pregnancy excluded this group (30,31). The reported prevalence of overt diabetes varies widely, ranging from 0.23% among US women with "gestational diabetes progressing to type 2 diabetes" (32) to 0.4% in a French national cohort (10) and up to 21.7% (7) in a high-risk population. Whether earlier diagnosis and intervention translate into improved outcomes is unclear. No randomized controlled trials have addressed this question so far. One observational study reported comparable outcomes in women with well-controlled overt diabetes and those with gestational diabetes or normoglycemic pregnancies (33).

Lack of postpartum follow-up data hindered evaluating persistent diabetes in our cohort. Previous studies reported postpartum diabetes rates between 19.5% (7) and 70.7% (4). In a meta-analysis, the risk of diabetes in women presenting overt diabetes was 6-fold compared with women with previous GDM and 25-fold compared with women without hyperglycemia (3). Many women in our sample probably had undiagnosed type 2 diabetes, as most of them presented severe hyperglycemia already at diagnosis: more than 75% of those diagnosed in the first trimester had a fasting plasma glucose ≥ 126 mg/dL, the current diagnostic cut-off point for diabetes in nonpregnant adults. Moreover, the traditional cut point for diabetes control, a HbA1c of 7.0%, was found for half of the sample. Only two participants presented diabetes complications, but their presence ratifies the existence of an unknown hyperglycemia before pregnancy. The small sample size did not allow further sensitivity analyses according to hyperglycemia severity.

As strengths of our work, this is one of the first studies with over 100 participants to assess the impact of an early diagnosis of overt diabetes on pregnancy outcomes. Early diagnosis was correlated with some improved maternal parameters during pregnancy, such as less total gestational weight gain in women with obesity and a lower 3rd-trimester HbA1c, but not with better pregnancy outcomes. This may be due to the homogeneous sample enrolled, as age and BMI were quite similar between the groups.

A strong limitation of our study was the relatively small sample size which may have reduced the power to detect differences in rarer outcomes, such as perinatal death and congenital anomalies. Study power for the main outcomes was high for neonatal

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hypoglycemia and NICU admission (90-100%) and moderate for preeclampsia (~76%). Some results with a null association (when it was expected to occur) may reflect type II error, related to the study power. Another limitation is the influence of the study center on the outcomes, which reflects, first, the retrospective and secondary collection of information, and second, the confidence in the diagnostic definitions made by the medical team caring for the participants, likely due to different patterns of assistance. The generalization of the results is limited due to the exclusive inclusion of women who used the public health system, mostly having a lower income profile associated with a lower educational level (only 4.1% had over 11 years of schooling). Data on high-risk prenatal care in the private setting in Brazil (as accessed in PubMed and Embase), which could yield different results associated with higher educational and socioeconomic backgrounds, is lacking. Nevertheless, the cohort is likely representative of Latina women with overt diabetes: they presented higher rates for family history of diabetes (~60% compared with <23% in one Chinese cohort (13) and ~57% in a New Zealand cohort (7)), and obesity was common (73% compared with < 42% in a Chinese cohort (13)). Finally, lack of postpartum data limits firm conclusions on diabetes persistence, a key factor for a definitive classification of hyperglycemia.

In conclusion, in the present cohort of women with overt diabetes cared for in the public health system, early diagnosis was associated with better maternal control, but this did not translate into improved clinically relevant pregnancy outcomes. Our findings underscore the need for prospective trials to determine a timely diagnosis and intervention, helping to improve pregnancy outcomes in these women.

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**Data availability:** the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Inquiries can be directed to the corresponding author (areichelt@hcpa.edu.br) or to Vania Hirakata (vhirakata@hcpa.edu.br).

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# SUPPLEMENTARY MATERIAL

**Supplementary Table 1.** Overt diabetes in pregnancy: univariable analyses by outcome

| Maternal | | | | | | |
|---|---|---|---|---|---|---|
| Characteristic/outcome | Inpatient glycemic control | | p | Preeclampsia | | p |
| | no | yes | | no | yes | |
| | n = 87 | n = 117 | | n = 144 | n = 66 | |
| Late diagnosis | 26 (29.9) | 56 (47.9) | 0.014 | 60 (41.7) | 28 (42.4) | >0.999 |
| Center | | | 0.006 | | | <0.001 |
| Center 1 | 48 (55.2) | 41 (35.0) | | 79 (54.9) | 17 (25.8) | |
| Center 2 | 39 (44.8) | 76 (65.0) | | 65 (45.1) | 49 (74.2) | |
| Maternal age | 32.1 ± 5.8 | 31.8 ± 6.5 | 0.760 | 32.1 ± 6.0 | 31.3 ± 6.1 | 0.380 |
| White skin color | 61 (70.1) | 80 (68.4) | 0.910 | 103 (71.5) | 44 (66.7) | 0.581 |
| ≤ 11 years in school | 81 (93.1) | 115 (98.3) | 0.128 | 138 (95.8) | 65 (98.5) | 0.562 |
| Smoking | 11 (12.6) | 12 (10.3) | 0.757 | 17 (11.8) | 8 (12.1) | >0.999 |
| Family history of diabetes | 52 (59.8) | 78 (66.7) | 0.386 | 87 (60.4) | 43 (65.2) | 0.615 |
| Previous gestational diabetes | 29 (33.3) | 38 (32.5) | >0.999 | 50 (34.7) | 18 (27.3) | 0.362 |
| Family history of hypertension | 44 (50.6) | 60 (51.3) | >0.999 | 65 (45.1) | 38 (57.6) | 0.127 |
| Chronic hypertension | 16 (18.4) | 19 (16.2) | 0.829 | 19 (13.2) | 16 (24.2) | 0.073 |
| Diabetes complications | 1 (1.1) | 1 (0.9) | >0.999 | 1 (0.7) | 1 (1.5) | >0.999 |
| FPG ≥ 126 mg/dL | 61 (70.9) | 86 (78.9) | 0.265 | 98 (70.0) | 49 (80.3) | 0.178 |
| | 86 | 109 | | 140 | 61 | |
| Gestational age at booking | 22.1 ± 7.7 | 25.4 ± 8.4 | 0.005 | 23.7 ± 8.0 | 25.1 ± 8.4 | 0.262 |
| Obesity | 59 (72.0) | 81 (71.7) | >0.999 | 93 (68.4) | 53 (81.5) | 0.074 |
| | 82 | 113 | | 136 | 65 | |
| Weight gain at booking (kg) | 3.5 ± 6.7 | 6.5 ± 8.5 | 0.010 | 5.0 ± 7.1 | 5.8 ± 9.3 | 0.489 |
| | 83 | 113 | | 138 | 64 | |
| HbA1c at booking | 6.5 ± 1.1 | 7.5 ± 1.2 | <0.001 | 7.0 ± 1.4 | 7.1 ± 1.1 | 0.515 |
| | 86 | 117 | | 143 | 66 | |
| Weight gain adequacy | | | 0.061 | | | 0.082 |
| Insufficient | 41 (51.9) | 38 (35.2) | | 65 (47.1) | 18 (31.0) | |
| Adequate | 15 (19.0) | 32 (29.6) | | 33 (23.9) | 15 (25.9) | |
| Excessive | 23 (29.1) | 38 (35.2) | | 40 (29.0) | 25 (43.1) | |
| | 79 | 108 | | 138 | 58 | |
| 3rd-trimester HbA1c | 6.0 ± 0.8 | 6.5 ± 1.0 | 0.002 | 6.3 ± 1.0 | 6.4 ± 0.8 | 0.466 |
| | 67 | 95 | | 119 | 49 | |

| Characteristic/outcome | Total gestational weight gain | | p | 3rd-trimester HbA1c | | p |
|---|---|---|---|---|---|---|
| | n | b (95% CI) | | n | b (95% CI) | |
| Late diagnosis | 198 | 4.34 (1.85; 6.84) | 0.001 | 170 | 0.44 (0.15; 0.73) | 0.003 |
| Center | 198 | 0.04 (-2.49; 2.57) | 0.975 | 170 | 0.11 (-0.18; 0.40) | 0.462 |
| Maternal age | 198 | -0.08 (-0.28; 0.13) | 0.471 | 170 | -0.02 (-0.04; 0.01) | 0.208 |
| White skin color | 198 | -1.33 (-4.08; 1.41) | 0.340 | 170 | -0.03 (-0.35; 0.29) | 0.857 |
| ≤ 11 years in school | 198 | -0.03 (-0.99; 0.94) | 0.951 | 170 | -0.08 (-0.19; 0.04) | 0.195 |
| Smoking | 198 | -0.91 (-4.69; 2.88) | 0.638 | 170 | -0.19 (-0.66; 0.28) | 0.431 |
| Family history of diabetes | 198 | -0.01(-2.61; 2.59) | 0.993 | 170 | 0.20 (-0.10; 0.49) | 0.195 |
| Previous gestational diabetes | 198 | -1.08 (-3.74; 1.57) | 0.422 | 170 | 0.16 (-0.14; 0.46) | 0.300 |
| Previous macrosomia | 198 | 3.11 (0.21; 6.02) | 0.036 | 170 | 0.49 (0.17; 0.80) | 0.003 |

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| Characteristic/outcome | Total gestational weight gain | | p | 3rd-trimester HbA1c | | p |
|---|---|---|---|---|---|---|
| | n | b (95% CI) | | n | b (95% CI) | |
| Chronic hypertension | 198 | -0.25 (-3.63; 3.12) | 0.884 | 170 | -0.13 (-0.52; 0.26) | 0.515 |
| Diabetes complications | 198 | -1.01 (-18.75; 16.74) | 0.911 | 170 | -0.29 (-1.63; 1.04) | 0.666 |
| Number of pregnancies | 198 | 0.40 (-0.39; 1.18) | 0.321 | 170 | 0.01 (-0.08; 0.11) | 0.782 |
| Pregestational BMI | 197 | -0.45 (-0.59; -0.31) | <0.001 | 168 | 0.00 (-0.02; 0.01) | 0.626 |
| Obesity | 196 | -6.77 (-9.42; -4.11) | <0.001 | 167 | -0.05 (-0.37; 0.27) | 0.766 |
| Gestational age at booking | 198 | 0.30 (0.15; 0.45) | <0.001 | 170 | 0.03 (0.01; 0.05) | 0.001 |
| Weight gain at booking | 197 | 0.96 (0.88; 1.04) | <0.001 | 169 | 0.03 (0.01; 0.05) | <0.001 |
| HbA1c at booking | 197 | 0.94 (-0.04-1.92) | 0.060 | 170 | 0.36 (0.26; 0.46) | <0.001 |
| Maternal inpatient care | 187 | 2.65 (0.08; 5.22) | 0.043 | 162 | 0.46 (0.17; 0.76) | 0.002 |
| 3rd-trimester HbA1c | 162 | 3.01 (1.59; 4.43) | <0.001 | --- | | |
| Total gestational weight gain | --- | | | 162 | 0.03 (0.02; 0.05) | <0.001 |
| Gestational age at delivery | 189 | 0.00 (-0.44; 0.45) | 0.986 | 162 | 0.03 (-0.04; 0.09) | 0.411 |

**Neonatal**

| Characteristic/outcome | Hypoglycemia | | p | NICU admission | | p |
|---|---|---|---|---|---|---|
| | no | yes | | no | yes | |
| | n = 147 | n = 40 | | n = 117 | n = 70 | |
| Late diagnosis | 65 (44.2) | 14 (35.0) | 0.387 | 51 (43.6) | 28 (40.0) | 0.743 |
| Center | | | >0.999 | | | 0.509 |
| Center 1 | 70 (47.6) | 19 (47.5) | | 53 (45.3) | 36 (51.4) | |
| Center 2 | 77 (52.4) | 21 (52.5) | | 64 (54.7) | 34 (48.6) | |
| Maternal age | 32.1 ± 6.4 | 30.6 ± 5.1 | 0.162 | 32.5 ± 6.4 | 30.7 ± 5.6 | 0.063 |
| White skin color | 103 (70.1) | 26 (65.0) | 0.673 | 81 (69.2) | 48 (68.6) | >0.999 |
| ≤ 11 years in school | 142 (96.6) | 39 (97.5) | >0.999 | 113 (96.6) | 68 (97.1) | >0.999 |
| Maternal smoking | 14 (9.5) | 11 (27.5) | 0.007 | 8 (6.8) | 17 (24.3) | 0.002 |
| Obesity | 107 (76.4) | 24 (63.2) | 0.150 | 84 (75.0) | 47 (71.2) | 0.706 |
| Preeclampsia | 40 (27.2) | 18 (46.2) | 0.038 | 28 (23.9) | 30 (43.5) | 0.009 |
| Total gestational weight gain | 7.5 ± 9.0 | 10.4 ± 7.2 | 0.062 | 7.3 ± 8.7 | 9.5 ± 8.5 | 0.099 |
| | 139 | 38 | | 112 | 65 | |
| 3rd-trimester HbA1c | 6.2 ± 1.0 | 6.5 ± 0.8 | 0.165 | 6.2 ± 0.9 | 6.6 ± 1.1 | 0.012 |
| | 122 | 30 | | 101 | 51 | |
| Gestational age at birth | 37.9 ± 1.6 | 37.2 ± 2.0 | 0.027 | 38.1 ± 1.2 | 37.2 ± 2.2 | 0.004 |
| Preterm birth | 20 (13.6) | 18 (45.0) | <0.001 | 11 (9.4) | 27 (38.6) | <0.001 |
| Male gender | 81 (55.1) | 31 (77.5) | 0.017 | 64 (54.7) | 48 (68.6) | 0.086 |
| Macrosomia (≥ 4,000 g) | 19 (12.9) | 11 (27.5) | 0.047 | 16 (13.7) | 14 (20.0) | 0.350 |
| Birth weight categories* | | | 0.085 | | | 0.126 |
| SGA | 9 (6.1) | 1 (2.5) | | 9 (7.7) | 1 (1.4) | |
| AGA | 85 (57.8) | 17 (42.5) | | 65 (55.6) | 37 (52.9) | |
| LGA | 53 (36.1) | 22 (55.0) | | 43 (36.8) | 32 (45.7) | |

*Birth weight categories classified according to the World Health Organization chart.

FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; BMI body mass index; NICU: neonatal intensive care unit SGA: small for gestational age; AGA: adequate for gestational age; LGA: large for gestational age.

Data are presented as n (%) or mean ± standard deviation unless otherwise highlighted.

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**Supplementary Table 2.** Sample size and power according to the main pregnancy outcomes

| Outcome | Sample size | RR | % (literature) | Power | RR | % (this study) |
|---|---|---|---|---|---|---|
| Preeclampsia | 120 | 2.0 | 28.2 | 75.8 | 2.0 | 30.1 |
| Hypoglycemia | 178 | 2.0 | 22.0 | 90.8 | 2.0 | 23.8 |
| NICU | 220 | 2.0 | 18.9 | 100.0 | 2.0 | 38.6 |
| Congenital anomaly | 880 | 2.0 | 5.9 | 23.3 | 2.0 | 13.6 |

RR: relative risk; %: frequency.

All parameters were calculated using the PSS Health online version (17), with a power of 80% and a level of significance of 5%, plus 10% for losses.

Since the frequency of outcomes varied in the literature, we estimated the mean prevalence of hypoglycemia and NICU. The prevalence of preeclampsia and congenital anomalies was based on the study of Jovanovič and cols. (32).

The relative risk of 2.0 or all calculations was arbitrarily set based on the study of Jayasinghe and cols. (12).

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original

# Development and validation of a clinical prediction model for postoperative weight regain after bariatric surgery using inflammatory, metabolic, and ferritin biomarkers

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## ABSTRACT

**Objective:** Postoperative weight regain remains a challenge after bariatric surgery and affects long-term outcomes. This study aimed to develop a clinical model to predict weight regain within 12 months, prior to surgery by using preoperative inflammatory, metabolic, and ferritin as biomarkers. **Subjects and methods:** This retrospective observational study included 394 patients with obesity who underwent bariatric surgery (2020–2023), including laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB). Patients were divided into a training set (70%, n = 276) and a validation set (30%, n = 118) using a random number table. Weight regain was defined as a ≥ 10% increase from the postoperative nadir (median time to regain: 8.2 months). Key variables included peripheral blood inflammatory markers [systemic immune-inflammation index (SII, calculated as platelet count × neutrophil count/lymphocyte count), neutrophil-to-lymphocyte ratio (NLR)], glycolipid metabolism indicators [low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)], and ferritin levels. Multivariate logistic regression was used to identify independent predictive variables, and the nomogram model was validated via calibration, area under the receiver operating characteristic curve (AUC), and decision curve analysis (DCA). **Results:** The weight regain rate was 19.9% (55/276) in the training set. Independent predictive variables included elevated SII (OR=1.004; 95% CI = 1.000–1.007), LDL-C (OR = 1.873; 95% CI = 1.054–3.329), ferritin (OR = 1.005; 95% CI = 1.003–1.008), and reduced HDL-C (OR = 0.103; 95% CI = 0.013–0.844) (all P < 0.05). The model showed strong discrimination (training AUC = 0.852, 95% CI = 0.795–0.910; validation AUC = 0.812, 95% CI = 0.709–0.915) and good calibration (Hosmer–Lemeshow P > 0.05). DCA confirmed the model's clinical utility across threshold probabilities. **Conclusion:** Preoperative SII, LDL-C, ferritin, and HDL-C levels effectively predict postoperative weight regain. Early monitoring of these biomarkers may guide personalized interventions to improve long-term outcomes.

**Keywords:** Bariatric surgery; postoperative weight regain; biomarkers; key predictors

## INTRODUCTION

With the development of the global economy and changes in people's lifestyles, the incidence of obesity has been increasing rapidly (1). The World Health Organization reported that in 2022, the number of people with obesity worldwide exceeded 1 billion (2,3). Obesity not only severely affects an individual's physical health but also significantly increases the risk of chronic diseases such as cardiovascular diseases, diabetes, hypertension, and fatty liver (4,5). Bariatric surgery has been widely recognized for its safety and effectiveness after years of development and practice (6). By changing the structure of the gastrointestinal tract and the digestion process, bariatric surgery can significantly reduce the weight of patients and improve glycolipid metabolism disorders (7). However, postoperative weight regain is a critical issue affecting the long-term efficacy of bariatric surgery, with reported

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rates ranging from 15% to 35% within 2–5 years postoperatively (8).

This study focused on weight regain within 12 months postoperatively (the early stage) with the goal of establishing a predictive tool that does not cover the long-term weight regain cycle (2–5 years) most commonly reported in clinical practice. Postoperative weight regain not only leads to weight rebound in people with obesity but also may trigger the recurrence and progression of metabolic diseases such as diabetes and hypertension, which may cause psychological problems such as depression and anxiety (9,10).

Currently, research on postoperative weight regain in people with obesity focuses mainly on anatomical factors, diet and exercise habits, gut hormones and psychological status (11). Some studies have also indicated that after bariatric surgery, the secretion levels of peptides that promote satiety, such as peptide YY, glucagon-like peptide-1, and gastric inhibitory polypeptide, increase, whereas the secretion level of ghrelin, which promotes appetite, decreases (12,13). In addition, a low exercise frequency and insufficient exercise duration and intensity after bariatric surgery are considered important reasons for postoperative weight regain (14).

Although many factors have been found to be closely related to postoperative weight regain after bariatric surgery, there is still a clinical need to identify specific biological markers that can predict the risk of postoperative weight regain earlier and with greater objectivity. Previous studies have proposed new ideas for understanding the risk of weight regain from the perspectives of inflammation and glycolipid metabolism disorders (15-18). Ferritin is the main form of stored iron, but its level is also affected by systemic inflammation. Thus, changes in ferritin levels may reflect both iron metabolism status and inflammatory activity, which together have implications for people with obesity (19).

Despite the existing research on postoperative weight regain and related indicators in people with obesity, providing clinicians with a precise prediction tool to guide personalized treatment and prevent weight regain is currently not possible. Therefore, this study aimed to construct a model to predict postoperative weight regain before surgery by using preoperative inflammatory markers, glycolipid metabolism indicators, and ferritin levels to enable early risk assessment and personalized interventions.

## SUBJECTS AND METHODS

### Study design and participants

This study was an analytical, observational, open-label retrospective study. The experimental designers, data collectors, and statistical analysts were independent of each other, and the data collectors and statistical analysts were blinded to the experimental design. People with obesity who underwent bariatric surgery in our hospital from January 2020 to January 2023 were selected as the research subjects.

**Inclusion criteria:** (1) aged 18–65 years; (2) body mass index (BMI) ≥ 30 kg/m² (WHO criteria); (3) underwent bariatric surgery [laparoscopic sleeve gastrectomy (LSG) or laparoscopic Roux-en-Y gastric bypass (LRYGB)] at our hospital; (4) complete preoperative laboratory data (inflammatory markers, glycolipid metabolism, iron metabolism) and postoperative follow-up records (≥ 12 months); and (5) voluntarily signed informed consent.

**The exclusion criteria were as follows:** (1) patients who had malignant tumors or hematological/autoimmune diseases affecting inflammation or iron metabolism; (2) patients who used drugs to modify inflammation, glycolipid metabolism, or iron metabolism within 3 months preoperatively; (3) patients affected by mental/cognitive impairment that made it impossible to cooperate with follow-up; (4) pregnant or lactating women; and (5) patients who had incomplete clinical or follow-up data.

**Sample size:** In accordance with previous studies on postoperative weight regain in people with obesity (20), the expected weight regain rate of enrolled patients after bariatric surgery was 20%. The confidence interval was set at 95%, and the allowable error was 5%. The following sample size estimation formula was used: $n=Z^2\times p\times(1-p)/E^2$, where $n$ is the sample size, $Z$ = 1.96, the expected pass rate ($p$) = 0.20, and the allowable error ($E$) = 0.05. Substituting into the above formula, $n\approx245.86$. Rounding up and considering that 10% of cases might be unable to be included in the

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analysis, at least 274 people with obesity who underwent bariatric surgery needed to be included. A total of 394 eligible people with obesity were ultimately included in this study.

**Division of sets and groups:** The enrolled patients were divided into a training set ($n$ = 276) and a validation set ($n$ = 118) at a 7:3 ratio using the random number table method. A patient enrollment flowchart is shown in **Figure 1**. All enrolled patients were evaluated for postoperative weight regain according to the following criteria: weight gain ≥ 10% compared with the lowest postoperative weight within 12 months after bariatric surgery was defined as weight regain. Patients with weight regain were defined as the weight regain group, and patients who did not meet the above criteria were defined as the nonweight regain group. In this study, only a retrospective statistical analysis of the previous inpatient database was conducted, and the patient names and contact information were kept confidential. Accordingly, the ethics committee waived any specific ethical requirements for this study.

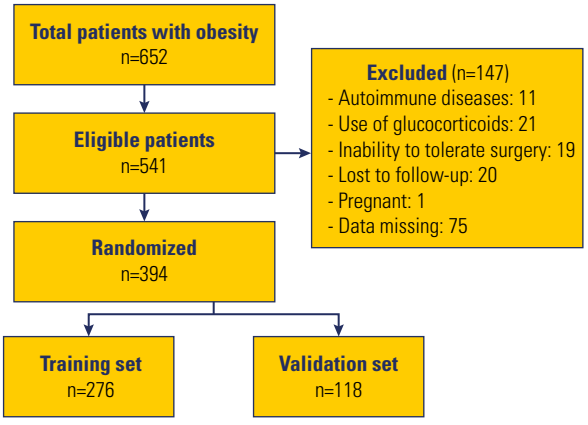



**Figure 1.** Flowchart of the enrollment of research subjects.

## Data collection and outcome measures

### *General clinical data*

The general clinical data of the enrolled patients, including age, sex, preoperative BMI, waist–hip ratio, length of hospital stay, operation time, surgical approach, obesity type, and comorbidity status, were collected. The obesity types were divided into abdominal obesity and generalized obesity. The surgical approaches included mainly LSG and laparoscopic Roux-en-Y gastric bypass. The comorbidities included mainly type 2 diabetes mellitus (T2DM), fatty liver, obstructive sleep apnea hypopnea syndrome (OSAHS), hypertension, hyperlipidemia, hyperuricemia, and thyroid nodules.

### *Peripheral blood composite inflammatory markers*

Preoperative fasting peripheral venous blood was collected. The neutrophil count (N), lymphocyte count (L), platelet count (PLT), and monocyte count (MONO) were measured. The derived indices included the neutrophil-to-lymphocyte ratio (NLR) = N/L, the platelet-to-lymphocyte ratio (PLR) = PLT/L, the monocyte-to-lymphocyte ratio (MLR) = MONO/L, the systemic immune-inflammation index (SII) = PLT × N/L, and the systemic inflammatory response index (SIRI) = (MONO × N)/L.

### *Detection of glycolipid metabolism indicators*

Preoperative fasting blood was used to measure the levels of glycated hemoglobin (HbA1c), insulin, C-peptide, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

### *Detection of iron metabolism indicators*

Preoperative fasting blood was used to measure serum iron, ferritin, total iron-binding capacity (TIBC), and unsaturated iron-binding capacity (UIBC, calculated as TIBC minus serum iron). Transferrin saturation (TSAT), a key indicator of iron utilization, was further calculated as (serum iron/TIBC) × 100% to complement the iron status assessment.

## Statistical analysis

SPSS 26.0 software and R 4.2.1 software were used for statistical analysis. For measurement data, a normality test was first performed. Data conforming to a normal distribution are expressed as the mean ± standard deviation ($\bar{x}\pm s$), and comparisons between two groups were performed using an independent-samples t test. Measurement data that did not conform to a normal distribution are expressed as medians (interquartile ranges) [M (Q1, Q3)], and comparisons between two groups were performed using the Mann–Whitney U test.

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Count data are expressed as the number of cases (percentage) [n (%)], and comparisons between groups were performed using the $\chi^2$ test. When the theoretical frequency was less than 5, Fisher's exact probability method was used. Factors with P < 0.05 in the univariate analysis were included in the multivariate logistic regression analysis, and the forward stepwise regression method was used to determine the variables independently associated with postoperative weight regain. On the basis of the results of the multivariate logistic regression analysis, the rms package of R software was used to construct a nomogram prediction model. In the training set, the bootstrap resampling method (1000 repeated samples) was used for internal validation of the model, and in the validation set, external validation of the model was performed. Calibration curves were plotted to observe the consistency between the predicted probability and the actual probability; decision analysis curves (DCAs) were plotted to analyze the net benefit of the model at different threshold probabilities; and receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was calculated to evaluate the predictive efficacy of the model. The larger the AUC is, the stronger the predictive ability of the model. P < 0.05 was considered to indicate statistical significance.

## RESULTS

### Comparison of clinical data between the training set and the validation set

There were no significant differences in the general clinical data, such as age, sex, preoperative BMI, disease duration, obesity type, surgical approach, or comorbidity status, between the training set and the validation set (P > 0.05; **Table 1**), indicating that the clinical data of the patients in the two datasets were comparable and could be used for subsequent research and validation.

**Table 1.** Comparison of general clinical data between the training set and the validation set [($\bar{x}\pm s$), $M$ ($Q_1$, $Q_3$), n (%)]

| General clinical data | Training set (n = 276) | Validation set (n = 118) | t/Z/$\chi^2$ | P |
|---|---|---|---|---|
| Age (years) | 31.87 ± 7.45 | 31.59 ± 7.69 | 0.334 | 0.739 |
| Sex | | | 0.648 | 0.421 |
| Male | 80 (29.0) | 39 (33.1) | | |
| Female | 196 (71.0) | 79 (66.9) | | |
| Preoperative BMI (kg/m$^2$) | 40.32 ± 6.24 | 39.88 ± 5.50 | 0.655 | 0.513 |
| Waist–hip ratio | 0.95 (0.90, 1.00) | 0.94 (0.89, 1.00) | -0.626 | 0.532 |
| Number of hospitalization days (d) | 6 (5, 6) | 6 (5, 6) | -0.811 | 0.417 |
| Operation time (min) | 90 (75, 105) | 90 (70, 100) | -1.070 | 0.285 |
| Type of obesity | | | 0.329 | 0.566 |
| Abdominal obesity | 141 (51.1) | 64 (54.2) | | |
| Generalized obesity | 135 (48.9) | 54 (45.8) | | |
| Surgical approach | | | 0.937 | 0.333 |
| LSG | 135 (48.9) | 64 (54.2) | | |
| LRYGB | 141 (51.1) | 54 (45.8) | | |
| Comorbidities | | | | |
| T2DM | 108 (39.1) | 40 (33.9) | 0.965 | 0.326 |
| Fatty liver | 266 (96.4) | 115 (97.5) | 0.059 | 0.809 |
| OSAHS | 209 (75.7) | 90 (76.3) | 0.013 | 0.908 |
| Hypertension | 93 (33.7) | 33 (28.0) | 1.247 | 0.264 |
| Hyperlipidemia | 162 (58.7) | 79 (66.9) | 2.371 | 0.124 |
| Hyperuricemia | 126 (45.7) | 45 (38.1) | 1.901 | 0.168 |
| Thyroid nodule | 121 (43.8) | 60 (50.8) | 1.634 | 0.201 |

Note: BMI: body mass index; T2DM: type 2 diabetes mellitus; OSAHS: obstructive sleep apnea hypopnea syndrome.

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### Comparison of observed indicators between the weight regain group and the nonweight regain group in the training set

In the training set, 55 patients experienced weight regain within 12 months of surgery, with a weight regain rate of 19.9%. These patients were all included in the weight regain group, and the other 221 patients were included in the nonweight regain group. The prevalences of T2DM, hypertension, and hyperlipidemia and the levels of NLR, PLR, SII, SIRI, HbA1c, C-peptide, LDL-C, and ferritin were greater in the weight regain group than in the nonweight regain group, while the level of HDL-C was lower ($P < 0.05$). There were no significant differences in age, sex, preoperative BMI, waist–hip ratio, length of hospital stay, operation time, obesity type, surgical type, prevalence of fatty liver, OSAHS, hyperuricemia, thyroid nodules, MLR, insulin, TC, TG, serum iron, TIBC, or UIBC between the two groups ($P > 0.05$; **Table 2**).

### Risk factors for postoperative weight regain

The postoperative weight regain status of people with obesity was assigned as the dependent variable (no = 0, yes = 1), and the intergroup differential indicators, including T2DM, hypertension, hyperlipidemia, NLR, PLR, SII, SIRI, HbA1c, C-peptide, HDL-C, LDL-C and ferritin (all original values), were assigned as independent variables. Multivariate logistic regression analysis revealed that higher SII values [OR (95% CI) = 1.004 (1.000–1.007)], LDL–C levels [OR (95% CI) = 1.873 (1.054–3.329)], and ferritin levels [OR (95% CI) = 1.005 (1.003–1.008)] were risk factors for postoperative weight regain, and a higher level of HDL-C [OR (95% CI) = 0.103 (0.013–0.844)] was a protective factor ($P < 0.05$; **Table 3**).

### Nomogram model for the risk of postoperative weight regain

On the basis of the results of the multivariate logistic regression analysis, a nomogram model for the risk of postoperative weight regain was constructed using the SII, LDL-C level, ferritin level, and HDL-C level, as shown in **Figure 2**. It can be intuitively seen from the nomogram that the higher the preoperative levels of SII, LDL-C, and ferritin and the lower the level of HDL-C are, the higher the score and the probability of postoperative weight regain.

### Goodness of fit of the nomogram model for the risk of postoperative weight regain

The goodness-of-fit of the nomogram model for the risk of postoperative weight regain was evaluated by calibration curves in the training set and the validation set. The results showed that in the training set, the Hosmer–Lemeshow $\chi^2$ of the model was 7.7129 ($P = 0.462$), while in the validation set, the Hosmer–Lemeshow $\chi^2$ of the model was 12.577 ($P = 0.127$), indicating that the model had a high goodness-of-fit in both the training set and the validation set (see **Figure 3**).

### Predictive efficacy of the nomogram model for the risk of postoperative weight regain

The predictive efficacy of the nomogram model for the risk of postoperative weight regain was analyzed by ROC curves in the training set and the validation set. The results showed that in the training set, the AUC (95% CI) of the model was 0.852 (0.795–0.910); in the validation set, the AUC (95% CI) of the model was 0.812 (0.709–0.915), indicating that the model had high predictive efficacy in both the training set and the validation set (see **Figure 4**).

### Clinical value of the nomogram model for the risk of postoperative weight regain

The clinical net benefit of the nomogram model for the risk of postoperative weight regain was analyzed through DCA in the training set and the validation set. The results revealed that in the training set, the clinical net benefit of the model was greater than 0 within a threshold probability range of 0–0.95. In the validation set, the clinical net benefit of the model was greater than 0 within a threshold probability range of 0–0.82. These results suggested that the model had high clinical application value in both the training set and the validation set (as shown in **Figure 5**).

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**Table 2.** Comparison of the observed indicators between the weight regain group and the nonweight regain group in the training set [($\bar{x}\pm s$), *M* ($Q_1$, $Q_3$), n (%)]

| Index | Weight regain group (n = 55) | Nonweight regain group (n = 221) | t/Z/$\chi^2$ | P |
|---|---|---|---|---|
| General clinical data | | | | |
| Age (years) | 32.53 ± 6.66 | 31.71 ± 7.64 | 0.731 | 0.465 |
| Sex | | | 0.955 | 0.328 |
| Male | 13 (23.6) | 67 (30.3) | | |
| Female | 42 (76.4) | 154 (69.7) | | |
| Preoperative BMI (kg/m$^2$) | 41.05 ± 6.17 | 40.14 ± 6.26 | 0.970 | 0.333 |
| Waist–hip ratio | 0.96 (0.91, 1.02) | 0.95 (0.90, 1.00) | -0.856 | 0.392 |
| Number of hospitalization days (d) | 6 (6,6) | 6 (5, 6) | -0.720 | 0.471 |
| Operation time (min) | 90 (75, 110) | 90 (70, 105) | -0.144 | 0.886 |
| Type of obesity | | | 2.362 | 0.124 |
| Abdominal obesity | 23 (41.8) | 118 (53.4) | | |
| Generalized obesity | 32 (58.2) | 103 (46.6) | | |
| Surgical approach | | | 0.765 | 0.382 |
| LSG | 24 (43.6) | 111 (50.2) | | |
| LRYGB | 31 (56.4) | 110 (49.9) | | |
| Comorbidities | | | | |
| T2DM | 29 (52.7) | 79 (35.7) | 5.331 | 0.021 |
| Fatty liver | 54 (98.2) | 212 (95.9) | 0.641 | 0.423 |
| OSAHS | 42 (76.4) | 167 (75.6) | 0.015 | 0.902 |
| Hypertension | 27 (49.1) | 66 (29.9) | 7.287 | 0.007 |
| Hyperlipidemia | 40 (72.7) | 122 (55.2) | 5.578 | 0.018 |
| Hyperuricemia | 23 (41.8) | 103 (46.6) | 0.407 | 0.524 |
| Thyroid nodule | 18 (32.7) | 103 (46.6) | 3.446 | 0.063 |
| NLR | 2.38 ± 0.71 | 1.74 ± 0.66 | 6.316 | <0.001 |
| PLR | 142.32 ± 36.95 | 115.49 ± 34.08 | 5.138 | <0.001 |
| MLR | 0.24 ± 0.07 | 0.21 ± 0.11 | 1.639 | 0.102 |
| SII | 764.71 ± 252.04 | 498.89 ± 232.07 | 7.470 | <0.001 |
| SIRI | 1.24 (0.96,1.54) | 0.79 (0.59, 1.09) | 5.955 | <0.001 |
| HbA1c (%) | 7.03 ± 2.05 | 6.49 ± 1.24 | 2.509 | 0.013 |
| Insulin (mU/L) | 20.80 (13.60, 26.40) | 21.70 (16.35, 30.45) | -1.255 | 0.209 |
| C-peptide (ng/mL) | 4.51 ± 1.95 | 3.99 ± 1.48 | 2.176 | 0.030 |
| TC (mmol/L) | 4.93 ± 0.87 | 4.98 ± 0.92 | -0.402 | 0.688 |
| TG (mmol/L) | 1.72 (1.29, 2.68) | 1.84 (1.43, 2.61) | -1.037 | 0.300 |
| HDL-C (mmol/L) | 1.01 ± 0.21 | 1.09 ± 0.19 | -2.549 | 0.011 |
| LDL-C (mmol/L) | 3.54 ± 0.83 | 3.18 ± 0.65 | 3.390 | 0.001 |
| Serum iron (μmol/L) | 14.05 ± 5.46 | 14.61 ± 5.34 | -0.700 | 0.484 |
| TIBC (μmol/L) | 66.76 ± 10.7 | 68.14 ± 12.92 | -0.728 | 0.467 |
| UIBC (μmol/L) | 54.40 (42.80, 61.40) | 52.20 (42.00, 61.85) | 0.167 | 0.867 |
| Ferritin (ng/mL) | 146.05 (51.44, 446.11) | 94.67 (46.80, 188.95) | 2.831 | 0.005 |

Note: BMI: body mass index; T2DM: type 2 diabetes mellitus; OSAHS: obstructive sleep apnea hypopnea syndrome; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio; SII: systemic immune-inflammation index; SIRI: systemic immune response index; HbA1c: glycated hemoglobin; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TIBC: total iron binding capacity; UIBC: unsaturated iron binding capacity.

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**Table 3.** Multivariate logistic regression analysis of the risk of postoperative weight regain in people with obesity

| Index | β | S.E. | Wald $\chi^2$ | P | OR | Lower limit of 95% CI | Upper limit of 95% CI |
|---|---|---|---|---|---|---|---|
| T2DM | 0.201 | 0.455 | 0.194 | 0.660 | 1.222 | 0.501 | 2.983 |
| Hypertension | 0.414 | 0.422 | 0.962 | 0.327 | 1.512 | 0.662 | 3.455 |
| Hyperlipidemia | 0.099 | 0.413 | 0.058 | 0.810 | 1.104 | 0.491 | 2.482 |
| NLR | 0.378 | 0.589 | 0.412 | 0.521 | 1.460 | 0.460 | 4.633 |
| PLR | 0.008 | 0.008 | 1.046 | 0.306 | 1.008 | 0.992 | 1.024 |
| SII | 0.004 | 0.002 | 4.503 | 0.034 | 1.004 | 1.000 | 1.007 |
| SIRI | -0.539 | 0.672 | 0.645 | 0.422 | 0.583 | 0.156 | 2.175 |
| HbA1c | 0.148 | 0.148 | 1.013 | 0.314 | 1.160 | 0.869 | 1.549 |
| C-peptide | 0.101 | 0.125 | 0.660 | 0.417 | 1.107 | 0.866 | 1.414 |
| HDL-C | -2.273 | 1.073 | 4.487 | 0.034 | 0.103 | 0.013 | 0.844 |
| LDL-C | 0.628 | 0.293 | 4.572 | 0.033 | 1.873 | 1.054 | 3.329 |
| Ferritin | 0.005 | 0.001 | 15.068 | 0.000 | 1.005 | 1.003 | 1.008 |
| Constant | -7.192 | 1.967 | 13.366 | 0.000 | | | |

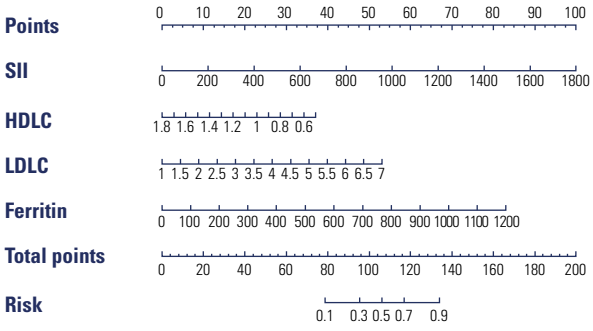



**Figure 2.** Nomogram model for the risk of postoperative weight regain.

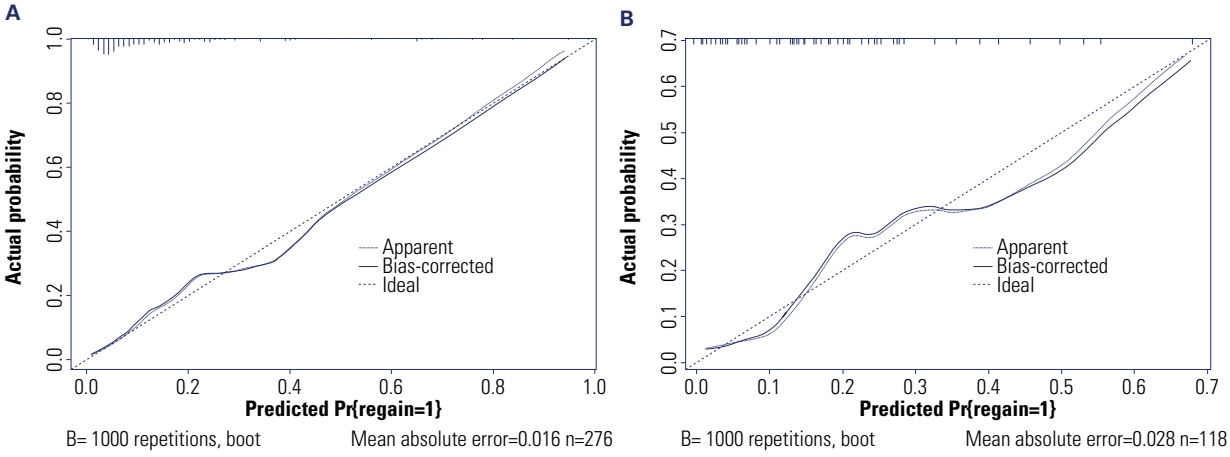



**Figure 3.** (**A**) Calibration curve analysis of the nomogram model for the risk of postoperative weight regain in the training set; (**B**) Calibration curve analysis of the nomogram model for the risk of postoperative weight regain in the validation set.

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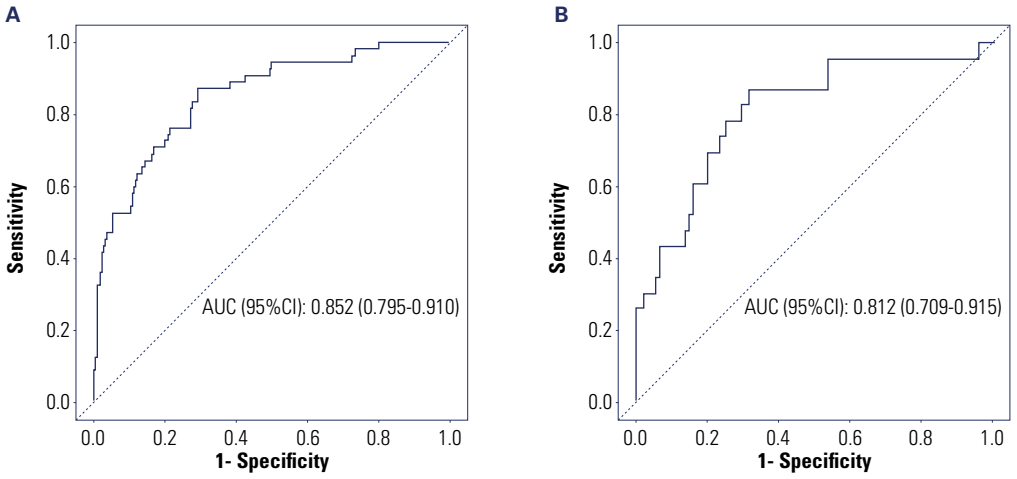



**Figure 4.** (**A**) ROC curve analysis of the nomogram model for the risk of postoperative weight regain in the training set; (**B**) ROC curve analysis of the nomogram model for the risk of postoperative weight regain in the validation set.

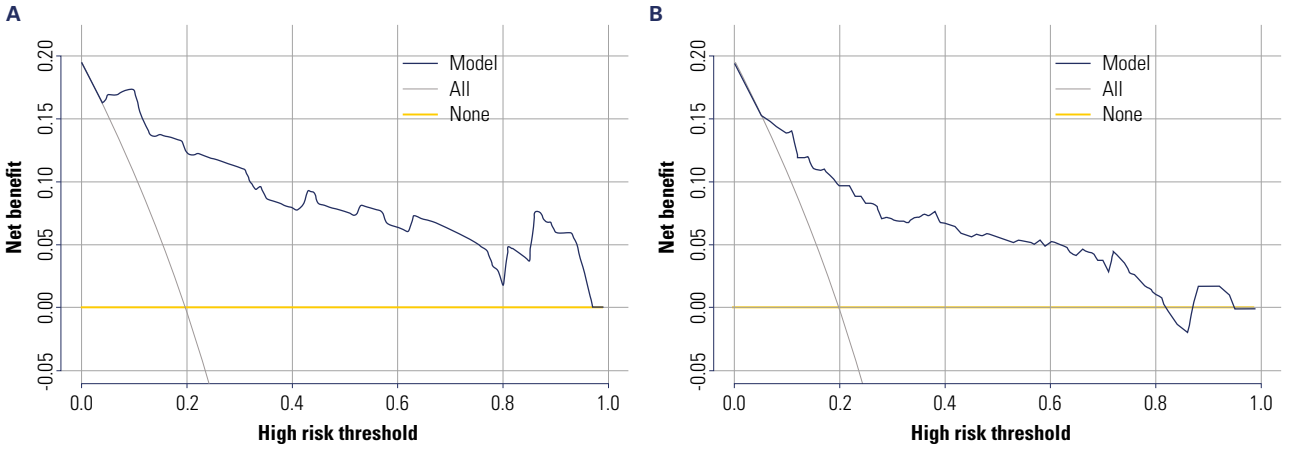



**Figure 5.** (**A**) DCA curve analysis of the nomogram model for the risk of postoperative weight regain in the training set; (**B**) DCA curve analysis of the nomogram model for the risk of postoperative weight regain in the validation set.

## DISCUSSION

This retrospective analysis of 394 people with obesity who underwent bariatric surgery revealed that prior to surgery, higher levels of LDL-C and ferritin, a greater SII value, and a lower level of HDL-C, were significantly associated with postoperative weight regain. The nomogram prediction model constructed on the basis of the results of multivariate logistic regression analysis demonstrated excellent goodness-of-fit, good predictive efficacy, and high clinical application value through internal and external validation. These findings suggest that the establishment of this model can provide a powerful tool for clinicians to accurately assess the risk of weight regain within 12 months postoperatively at an early stage, facilitating timely adjustment of short-term intervention strategies. Notably, the model cannot support claims about durable weight control, as the 12-month follow-up period is insufficient to reflect long-term outcomes.

The results of this study indicated that the SII value in the weight regain group was significantly greater than that in the nonweight regain group, and multivariate logistic regression analysis revealed that a higher SII value was associated with an increased risk of postoperative weight regain. Previous studies have explored the role of the SII in predicting the outcomes of cancer patients and found that a high SII value is related to a poor prognosis (21). A chronic inflammatory state may disrupt fat metabolism and energy balance, thereby increasing the risk of postoperative weight

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regain (22). Although differences in the NLR and PLR were observed between the weight regain group and the nonweight regain group in this study, they did not show independent predictive value in the multivariate analysis. Some studies have suggested that the NLR can reflect the body's inflammatory response and immune status and that an elevated NLR in people with obesity may be associated with adipose tissue inflammation and metabolic disorders (23). The inflammatory response affects fat metabolism and weight regulation through multiple pathways, leading to postoperative weight regain (24). Inflammatory factors can interfere with the insulin signaling pathway, resulting in insulin resistance, which promotes fat synthesis and storage while inhibiting fat breakdown, ultimately leading to weight gain (25,26).

This study also revealed that a higher LDL-C level was a risk factor for postoperative weight regain, whereas a higher HDL-C level was a protective factor, which is consistent with the general understanding of lipid metabolism abnormalities in people with obesity. An elevated LDL-C level reflects abnormal lipid metabolism in the body and may be related to postoperative weight regain (27). In contrast, HDL-C has an anti-atherosclerotic effect. It can transport cholesterol from peripheral tissues back to the liver for metabolism, reducing cholesterol deposition in the vascular wall (28). A decrease in the HDL-C level may indicate a decrease in the body's ability to clear lipids, thereby increasing the risk of weight regain (29). In this study, although differences in the HbA1c level, C-peptide level, and LDL-C level were observed between the weight regain group and the nonweight regain group, only the LDL-C level showed independent predictive value in the multivariate analysis. Some studies have suggested that HbA1c can reflect long-term blood glucose control and that abnormal blood glucose metabolism is closely related to the development of obesity (30,31). The differences in the research results may be related to factors such as the baseline characteristics of the study subjects, surgical methods, postoperative follow-up duration, and lifestyle interventions. In people with obesity, a decrease in the HDL-C level may be related to reduced HDL synthesis, increased HDL degradation, and impaired liver function (32,33). Previous studies have also suggested that inflammatory factors can modify HDL, causing it to lose its normal antioxidant and anti-inflammatory functions and rendering it unable to effectively perform reverse cholesterol transport (34,35).

This study is the first to investigate the ferritin level as a factor influencing postoperative weight regain in people with obesity and found that an elevated ferritin level is a risk factor for postoperative weight regain. Ferritin is the main form of stored iron, but it is also an acute-phase reactant — it is closely regulated by chronic low-grade inflammation, which is a core feature of obesity. In people with obesity, inflammatory cytokines can upregulate hepatic ferritin synthesis, whereas elevated ferritin further exacerbates inflammation by promoting oxidative stress in adipocytes (36-38). This bidirectional interaction jointly disrupts energy metabolism: iron overload impairs fat decomposition via lipid peroxidation (39), and inflammation disrupts insulin signaling and energy balance (25), ultimately increasing the risk of postoperative weight regain. Ferritin levels in children with obesity are positively correlated with indicators such as the BMI and body fat percentage (40). Changes in the level of ferritin, which is the main form of stored iron, are closely related to energy metabolism and fat accumulation (41,42). Iron overload can increase lipid peroxidation in adipocytes, disrupting cell membrane structure and function and thus affecting fat synthesis and metabolism (38,39). Iron overload may also interfere with the signal transduction pathway in adipocytes, affecting the expression of genes related to fat metabolism by upregulating the gene expression of fatty acid synthase (FAS) in adipocytes and promoting fatty acid synthesis while inhibiting the activity of enzymes related to fat breakdown, resulting in fat accumulation (43,44). In people with obesity, iron overload may lead to energy metabolism imbalance, decreased fat oxidation, and increased fat synthesis, thus promoting weight gain. Changes in the expression of genes related to energy metabolism in the adipose tissue of people with obesity may be related to changes in ferritin levels (45,46).

The nomogram prediction model constructed in this study is highly important for clinical prediction

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and assessment. This model incorporates preoperative measures of SII, LDL-C, ferritin, and HDL–C, providing clinicians with an intuitive, convenient, and accurate tool for assessing the risk of postoperative weight regain in people with obesity considering bariatric surgery. For patients with higher SII, LDL-C, and ferritin levels and lower HDL-C levels, doctors can ascertain that they have a greater risk of postoperative weight regain. For such patients, doctors can strengthen preoperative health education, enabling patients to fully understand the risk of postoperative weight regain and the importance of a healthy lifestyle. After surgery, doctors can more closely monitor patients at risk in regard to weight changes, metabolic indicators, and lifestyles; promptly detect signs of weight regain; and take corresponding intervention measures. This may include increasing the frequency of follow-up and regularly measuring patients' blood glucose, blood lipids, and inflammatory markers to adjust the treatment plan in a timely manner. Compared with the previous lack of effective prediction tools, where doctors could rely only on experience and some simple clinical indicators to predict the patient's risk of weight regain, the prediction model in this study has greater accuracy and reliability.

Some limitations of this study should be considered. First the sample size remains insufficient compared with that of large-scale multicenter studies. In research on risk factors for postoperative weight regain in patients with obesity, an inadequate sample size may result in the failure to detect some weak yet real associations, thereby affecting the accuracy and reliability of the results. Second, this study defines weight regain as an increase of ≥ 10% from the lowest weight within 12 months after surgery. While this enables the early identification of potential risks, clinically significant weight regain mostly occurs 2–5 years postoperatively. Therefore, the predictive efficacy of this model for weight regain beyond 1 year after surgery still needs to be verified. Third, ferritin is an acute-phase reactant and cannot be used alone as a marker of iron stores in people with obesity, as its level is confounded by both iron metabolism and systemic inflammation. Although we calculated TSAT to improve the iron utilization assessment, this study lacked data on C-reactive protein and hepcidin levels. This limitation prevents us from distinguishing whether elevated ferritin reflects iron overload or inflammatory activation, weakening the mechanistic interpretation of ferritin's role. Fourth, considering that obesity is a complex multifactorial disease, postoperative weight regain may be influenced by many other factors, such as the gut microbiota, genetic polymorphisms, and psychological factors.

To address these limitations, multicenter, large-scale studies should be conducted in the future, encompassing patients with obesity from different regions and age groups, to increase the diversity and representativeness of the sample. Moreover, the follow-up duration should be extended to 2–5 years to more accurately distinguish between early fluctuations and persistent regain, further improving the external validity of the model. In addition to focusing on peripheral blood composite inflammatory markers, glycolipid metabolism disorders, and ferritin characteristics, indicators such as the gut microbiota, gene polymorphisms, and psychological factors should be incorporated into the research. Through a comprehensive analysis of the relationships between these indicators and postoperative weight regain, the mechanism underlying postoperative weight regain can be elucidated more deeply, and a more robust prediction model can be established.

In conclusion, the findings of this study suggest that higher preoperative levels of SII, LDL-C, and ferritin and a lower level of HDL-C in people with obesity are important factors for predicting the risk of postoperative weight regain. Timely monitoring of these markers can help predict the risk of postoperative weight regain at an early stage, providing ideas for improving patients' surgical prognosis, adjusting postoperative treatment strategies in a timely manner, and enhancing the clinical treatment effect.

**Ethics approval and consent to participate:** this study were approved by the Medical Ethics Committee of Tianjin Medical University General Hospital. Written Informed consent were obtained from all the participates.All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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**Authors contribution:** Yaxin Liu is the guarantor of the entire study. Yaxin Liu and Longhao Sun designed this study. Yaxin Liu, Chenxi Fu and Longhao Sun acquired the data. Chenxi Fu analyzed the data and did the clinical investigation. Yaxin Liu did the literature research and drafted the paper. Longhao Sun reviewed and revised the paper. All authors have read and approved the final paper.

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**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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# Death cause and age at death among individuals with diabetes: a 40-year retrospective Brazilian study

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## ABSTRACT

**Objective:** To evaluate the association between specific/all-cause mortality and the individual's age at death. **Materials and methods:** This was a retrospective study that employed STROBE guidelines to its development. Medical records of 1,367 individuals diagnosed with diabetes mellitus (DM) who died between 1981 and 2021, in Bauru, São Paulo state, Brazil, were evaluated. Demographic, clinical, and obituary data were collected from medical records and death certificates, serving as adjustment variables in the analysis. Statistics were developed by using Cox-regression models. **Results:** The final model, adjusted by male sex and T2DM, revealed Hazard Ratios of 0.74 (95% CI: 0.59–0.92; $p < 0.01$) for infectious causes and 1.92 (95% CI: 1.12–3.37; $p < 0.01$) for gastrointestinal conditions. These findings suggest that infectious causes were associated with later ages at death, while gastrointestinal conditions were related to younger ages at death. The other evaluated variables and groups of death causes did not show statistical significance. **Conclusion:** Our study demonstrates that sex and DM type significantly influenced age at death. These findings emphasize the importance of considering demographic and clinical factors when assessing mortality patterns in DM, contributing to improved risk stratification and clinical management strategies.

**Keywords:** Diabetes; mortality; death causes; sex; diabetes type

## INTRODUCTION

Diabetes mellitus (DM) encompasses a group of metabolic disorders whose hallmark is hyperglycemia (1). The most common types of DM are type 1 DM (T1DM), which derives, predominantly, from an autoimmune condition; and type 2 DM (T2DM), whose most meaningful characteristic is insulin resistance (1).

Globally, it is estimated that more than 500 million individuals are living with DM in 2025 (2). These estimates are expected to rise to over 1.31 billion by 2050, mostly due to the increasing prevalence of T2DM (2). In Brazil, DM affects almost 10% of the population, with significant regional disparities and high underreporting rates (2). The rising prevalence of DM in Brazil mirrors global trends, underscoring the urgent need for robust public health interventions to address its growing burden (2).

Regarding mortality, DM is a leading contributor to death, due to its systemic nature its systemic nature (3). Indeed, a long-standing hyperglycemia, through complex pathophysiological mechanisms, causes protein glycation and cellular dysfunction, disrupting the individual's homeostasis (4). This process generates endothelial dysfunction that contributes to significant occurrence of cardiovascular (CVD) events and functional impairment of the kidneys in individuals with DM (4). Thereto, individuals with DM tend to present higher mortality rates when compared with the whole population (3).

Under this perspective, several studies have been conducted comparing mortality patterns between patients with and without DM, particularly concerning

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specific and all-cause mortality (3-5). In parallel, other studies have focused exclusively on mortality risk due to distinct causes among patients with DM, using age as the crucial adjustment factor in their analyses (3-5). However, a significant gap exists in understanding the interplay between age at death and its association with specific and all-cause mortality in patients with DM (5). Therefore, the present study aimed to evaluate the association between specific/all-cause mortality and the age at death.

## MATERIALS AND METHODS

### Study design

This was a retrospective study conducted following the STROBE guidelines.

The main objective of this study was to evaluate the association between specific/all-cause mortality and the age at death. Since other variables such as sex, ethnicity, marital status, decade of death, DM type, DM duration, treatment modality and body mass index (BMI) may influence this relationship, they were also considered in the final analysis as adjustment variables. Consequently, the secondary aim of this study was to evaluate the interaction between these variables on patients specific and all-cause mortality on the age of death. **Figure 1** shows a graphic representation of the study design and aims.

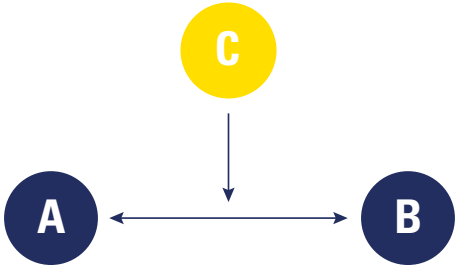



**Figure 1.** Study design and aims graphic representation. (**A**) Patients' age at death; (**B**) Patients' death cause; (**C**) Other considered variables such as sex, ethnicity, marital status, death's decade, DM type, DM duration, proposed treatment, BMI.

### Setting

The study was carried out in Bauru city, located in the central-western region of São Paulo state, Brazil, with data collection being carried out between 1981 and 2021.

Previous studies conducted in Bauru with specific populations pointed to an increasing trend in DM cases, both for T1DM and for T2DM. However, precise epidemiological data on these conditions remain scarce and limited (6).

All patients were treated and followed at the same endocrinology clinic, which simultaneously acts as a private practice and as a referral center for the public health system in Bauru and surrounding areas. This dual function ensures a balanced representation of the local population, as approximately half of the patients were privately insured or self-paying, and the other half were referred by the public sector – specifically, the *Associação dos Diabéticos de Bauru* (ADB).

ADB is a public, non-profit organization affiliated with the *Faculdade de Medicina de Bauru*, *Universidade de São Paulo* (FMBRU-USP). It provides free care to individuals with DM and works in close partnership with the mentioned endocrinology clinic.

While care in both settings was coordinated by the same endocrinologist throughout the 40-year study period, ADB also includes a multidisciplinary team with nurses and other health professionals involved in patients' education and support, as well as an important work driven by medical students.

Importantly, all provided care was guided by a personalized and humanized approach. There was no rigid schedule for follow-up visits; instead, the interval between consultations was individually determined based on the patients' clinical needs, personal circumstances, and availability. This approach aligns with the concept of a therapeutic care plan, aiming to respect the uniqueness of each patient's health journey.

### Participants

This study included 1,367 individuals with a confirmed diagnosis of DM who died between 1981 and 2021. Patients with a documented diagnosis of DM and complete data in both medical records and death certificates were included. Patients who did not meet these criteria were excluded from the analysis.

### Variables

The primary outcome was individuals' age at death. Explanatory variables included: Demographic: sex,

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ethnicity (self-reported: White, Black, Brown, Yellow, Indigenous), marital status (single, married, widowed, divorced), and decade of death; Clinical: DM type (T1DM or T2DM), DM duration (in years), treatment modality (diet, oral antidiabetic agents, insulin, or combinations), and BMI; Mortality: cause of death.

Causes of death were grouped into six main categories: CVD, external causes (E), acute complications of chronic diseases (AC), infectious causes (I), neoplasms (N), gastrointestinal diseases (GD), and Ill-defined causes (ID). In the present study, causes of death were reclassified into broader etiological categories based on the underlying cause of death, rather than the immediate or terminal mechanisms reported in death certificates. Conditions such as cardiorespiratory arrest, multiple organ failure, shock, and respiratory failure were considered final common pathways of death and were therefore grouped according to their most plausible underlying etiological cause, following epidemiological, WHO, and Brazilian Health Minister recommendations for mortality analysis. This reclassification aimed to improve analytical coherence, reduce misclassification bias, and allow meaningful comparisons across cause-specific mortality groups.

The study also examined interactions between the independent variables and their influence on the age at death.

## Data sources and measurement

Demographic and clinical data were extracted from patients' medical records, maintained by the same endocrinologist to ensure consistency. Obituary data were manually collected from two official Registry Offices in Bauru, with oversight by authorized professionals. Death certificates followed Brazil's standardized format, prioritizing the underlying cause of death in epidemiological analyses.

BMI was calculated dividing weight (kg) by the square of the height ($m^2$) and categorized into: underweight (<18.5), normal weight (18.6–24.9), overweight (25–29.9), obesity grade I (30–34.9), grade II (35–39.9), and grade III (>40) (6). Diagnosis and treatment of DM were done according to Brazilian Diabetes Society and American Diabetes Association guidelines (7,8).

## Bias

To minimize bias, all clinical data were collected by a single physician using uniform documentation practices. However, retrospective data collection from death certificates may be limited due to inconsistencies in how causes of death were recorded, especially in Ill-defined cases.

## Study size

The initial dataset included 1,367 individuals. The final analytical sample resulted from the availability of valid and complete data records. No sample size calculation was performed due to the retrospective design of the study and full inclusion of eligible records.

## Quantitative variables

Quantitative variables such as DM duration, decade of death, and BMI were analyzed both as continuous and categorical variables. Ethnicity was recategorized as White vs. non-White to enhance statistical power.

## Statistical methods

Normality of continuous variables was assessed using the Shapiro-Wilk test. Descriptive statistics were followed by bivariate analyses using Chi-square, Student's t-test, and ANOVA to examine associations between demographic/clinical variables and age at death and cause of death.

Variables with statistically significant associations ($p < 0.05$) were included in survival analysis using Kaplan–Meier curves and Cox proportional hazards models, which death was the event of interest. The likelihood ratio test was used to compare nested models. Hazard ratios (HR) > 1 indicated higher risk of earlier death; HR < 1 indicated longer survival. In this context, Cox proportional hazards models were applied to assess the temporal ordering of age at death across cause-of-death groups rather than its classical approach.

The average age at death across the cohort served as the reference for comparison. All statistical analyses were performed using R software version 4.2.0, and graphs were generated using R® and Canva®.

## Ethics approval

This study was approved by the Research Ethics Committee of the School of Dentistry of the University

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of São Paulo (Protocol No. 37022220.0.0000.5417). Judicial authorization was obtained for accessing and analyzing death certificates.

## RESULTS

A total of 555 individuals were excluded from the analysis due to inconsistencies identified during the data selection process. Among them, 208 were confirmed not to have been residents of Bauru at the time of death, which made it impossible to retrieve their obituary data from other local registry offices. Furthermore, 247 patients lacked reliable information regarding the year of death or had no identifiable death certificate available in the municipal records. These issues were primarily attributed to duplicated names that prevented proper identification, absence of key identifiers such as maternal names, or misspellings and inconsistencies on how names were recorded in official documents. While the exact reasons for each missing record could not be fully verified, these hypotheses were suggested by registry office staff as the most likely explanations. Therefore, the final sample was composed of 812 individuals.

The mean age at death among patients was 72.35 ± 14.06 years. **Supplementary table 1** provides an overview of the mean ages at death and their corresponding confidence intervals across all evaluated subgroups.

Statistical analysis revealed a significant association between patients' age at death and cause of death with sex ($p < 0.01$) and DM type ($p < 0.01$). Other variables did not show statistical significance (**Supplementary table 1**).

An initial survival model, excluding interactions between sex and DM type, was created. Subsequently, separate models were developed for each subgroup: men, women, T1DM, and T2DM. Statistically significant findings were observed only in the models for men ($p = 0.007$) and T2DM ($p = 0.006$). Based on these findings, a final model was developed, including both men and T2DM concurrently as shown in **Table 1**.

Patients who died from infectious causes had a notably reduced likelihood of the event of interest occurring (death at a specific age) when compared to the reference group in the models that demonstrated statistical significance. Specifically, the likelihood of the interest event occur was reduced by approximately: 20% (HR: 0.80; 95% CI: 0.69–0.93; $p < 0.01$) in the model including only causes of death; 26% (HR: 0.74; 95% CI: 0.60–0.91; $p < 0.01$) in the model focusing men; and 19% (HR: 0.81; 95% CI: 0.69–0.93; $p < 0.01$) in the model focusing T2DM. This suggests that patients who died from infectious causes tended to present higher age at death **Table 1**.

Interestingly, patients who died from gastrointestinal causes showed a significantly higher likelihood of the interest event occurring than the reference group in the model that considered men as an adjustment variable (HR: 1.97; 95% CI: 1.15–3.35; $p < 0.01$). This suggests that patients who died from gastrointestinal causes tended to present lower ages at death. Other

**Table 1.** Cox regression results

| Models | CVD | AC | E | I | N | ID | GD | Model's p-value |
|---|---|---|---|---|---|---|---|---|
| Without subgroups | 1.09; 0.97 - 1.22; 0.14 | 0,91; 0.71 - 1.16; 0.45 | 0.94; 0.55 - 1.60; 0.84 | 0.80; 0.69 - 0.93; < 0.01 | 1.18; 0.93 - 1.49; 0.15 | 1.09; 0.90 - 1.30; 0.38 | 1.50; 0.98 - 2.29; 0.06 | 0.003 |
| Men | 1.10; 0.92 - 1.32; 0.25 | 0.82; 0.53 - 1.28; 0.38 | 0.88; 0.48 - 1.62; 0.70 | 0.74; 0.60 - 0.91; < 0.01 | 1.14; 0.83 - 1.56; 0.42 | 1.25; 0.96 - 1.63; 0.09 | 1.97; 1.15 - 3.35; 0.01 | 0.002 |
| Women | 1.08; 0.92 - 1.27; 0.30 | 1.01; 0.75 - 1.36; 0.93 | 0.94; 0.30 - 2.95; 0.91 | 0.85; 0.70 - 1.03; 0.10 | 1.16; 0.82 - 1.64; 0.89 | 0.98; 0.77 - 1.26; 0.38 | 1.03; 0.51 - 2.01; 0.91 | 0.60 |
| T1DM | 1.03; 0.57 - 1.89; 0.92 | 1.03; 0.31 - 3.36; 0.96 | 0.52; 0.16 - 1.70; 0.27 | 0.74; 0.35 - 1.51; 0.40 | * | 1.72; 0.91 - 3.23; 0.09 | 1.07; 0.14 - 7.90; 0.94 | 0.45 |
| T2DM | 1.09; 0.98 - 1.28; 0.13 | 0.92; 0.71 - 1.17; 0.47 | 1.19; 0.66 - 2.15; 0.57 | 0.81; 0.69 - 0.93; < 0.01 | 1.22; 0.97 - 1.55; 0.08 | 1.22; 0.97 - 1.55; 0.88 | 0.49; 0.97 - 2.30; 0.06 | 0.004 |
| Men and T2DM | 1.12; 0.93 - 1.34; 0.21 | 0.79; 0.51 - 1.25; 0.32 | 1.10; 0.55 - 2.22; 0.78 | 0.74; 0.59 - 0.92; < 0.01 | 1.17; 0.85 - 1.60; 0.34 | 1.17; 0.88 - 1.54; 0.26 | 1.93; 1.12 - 3.37; 0.01 | 0.004 |

Presentation standard: T1DM, type 1 diabetes; T2DM, type 2 diabetes; Hazard Ratio; 95% Confidence Interval, p-value. *, an insufficient sample to allow statistical analysis.
CVD: cardiovascular diseases; E: external causes; AC: acute complications of chronic diseases; I: infectious causes; N: neoplasms; GD: gastrointestinal diseases; ID: III-defined causes.

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variables did not exhibit statistical significance in any model (**Supplementary table 1**).

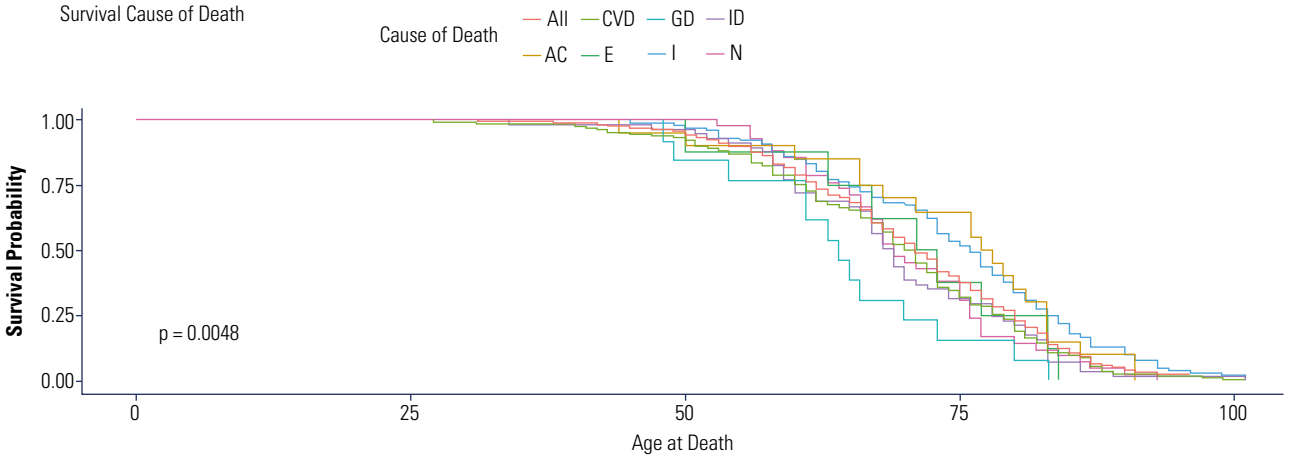
In the final model, patients who died from infectious causes had a likelihood of the interest event occurring of approximately 26% lower than the reference group (HR: 0.74; 95% CI: 0.59–0.92; p < 0.01).; and patients who died from gastrointestinal causes exhibited a 93% higher likelihood of the interest event occurring compared to the reference group (HR: 1.92; 95% CI: 1.12–3.37; p < 0.01) – **Figure 2**. These results align with the trends displayed by the other models. **Supplementary figures 1 to 5** bring the other models' graphical presentations.

## DISCUSSION

It was found that patients who died from infectious causes tended to be older at death, while those who died due to gastrointestinal tract-related causes tended to die at younger ages. The other causes of death did not present statistical significance. Some conditions such as male sex and T2DM, seem to modulate the association between age at death and its causes. The other conditions did not show statistical significance.

In the present study, it was found that patients who died from infectious causes tended to be older at the time of death. Studies from the Netherlands have shown that older adults with DM tend to be more frequently hospitalized and decease from infectious conditions compared with younger individuals with DM (9,10). Possibly, these findings are explained by an interplay between immunosenescence and the chronic immune dysfunction associated with it, which makes older individuals with DM more susceptible not only to acquiring infections, but also to dying from them (9,10). However a Taiwanese study suggested that the association between DM, infectious diseases and mortality may be mediated by glycemic control levels and the presence of individuals' comorbidities, proposing a more complex relationship between these variables (11). Under this perspective, differences in healthcare systems, early detection, and effective treatment protocols may explain these conflicting observations (11).

Interestingly, our study observed a significant mortality burden attributed to gastrointestinal tract-related diseases. These findings are consistent with the results of an Italian study, which found that young patients with DM tended to die more from gastrointestinal causes compared to other conditions (12). However, a British study observed that the burden of gastrointestinal causes of death was higher in older individuals with DM (13). These discrepancies may be attributed to regional differences in cause-of-death certification, access to early interventions, or the possibility that undiagnosed or misclassified complications (e.g., acute pancreatitis, severe hepatic steatosis) were more prevalent in specific study cohorts (12,13).




p: p-value; CVD: cardiovascular diseases; E: external causes; AC: acute complications of chronic diseases; I: infectious causes; N: neoplasms; GD: gastrointestinal diseases; ID: Ill-defined causes.

**Figure 2.** Kaplan-Meier curve for the final model.

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Our study identified a high proportion of deaths attributed to gastrointestinal causes among individuals with DM. This finding aligns with a North American cohort study that also reported a substantial burden of gastrointestinal mortality in this population (14). This may be explained by the high prevalence of non-alcoholic fatty liver disease (NAFLD), steatohepatitis, and other chronic gastrointestinal complications commonly associated with T2DM, especially when coexisting with obesity and metabolic syndrome (14). These conditions often progress silently and may only be recognized in advanced stages, contributing to increased mortality (14). Conversely, a British cohort study reported a low proportion of deaths attributed to gastrointestinal causes among individuals with DM (15). This discrepancy may reflect underdiagnosis and underreporting of these conditions, especially when death certificates prioritize more apparent causes such as cardiovascular events (15). Moreover, the predominance of macrovascular complications as the leading cause of death in individuals with DM — particularly in high-income countries — may overshadow the contribution of gastrointestinal diseases to overall mortality (14,15).

In our analysis, cardiovascular causes of death did not show a statistically significant association with age at death. This is consistent with findings from a North American population-based study that also failed to observe a strong link between specific causes of death such as Alzheimer and the age at which individuals died (16). One possible explanation is that people with DM frequently live with multiple comorbidities (16). As a result, the impact of any single condition — such as CVD — on age at death may be diluted within a broader pattern of complex, overlapping health issues. On the other hand, multicenter cohort studies have demonstrated important age-related patterns in the causes of death among patients with DM. For instance, a large international meta-analysis showed that cardiovascular risk is higher among those with early-onset or long-standing DM, contributing to younger age at death due to cardiac and cerebrovascular events (17). In parallel, neoplastic causes tend to be more commonly found among older individuals with DM, particularly liver, pancreatic, and colorectal cancers (18). These findings are likely driven by the cumulative effects of chronic hyperglycemia, insulin resistance, and systemic inflammation, which increase cancer risk over time (18). Thus, while DM may accelerate the development of certain neoplasms, their clinical impact tend to become more pronounced in later life (17,18).

We also observed that male sex significantly modified the association between age and cause of death. This was also supported by a Canadian study that found a significant interaction between sex and cause-specific mortality patterns, with men showing stronger age-related associations than women (19). The authors attributed these differences to poorer glycemic control and higher rates of macrovascular complications and infections among men, all of which contribute to earlier mortality (19). Interestingly, the same study highlighted that women with T2DM had proportionally higher cardiovascular mortality compared to men, especially when compared to women without DM (20). This may reflect the loss of endogenous hormonal protection in women with DM, as well as later recognition and suboptimal management of cardiovascular conditions in this group, contributing to premature death (20).

Furthermore, T2DM emerged as a significant modifier in the relationship between age and cause of death. This is in line with findings from the UK Biobank cohort, which demonstrated that individuals with T2DM exhibited signs of accelerated biological aging and had higher all-cause mortality rates (21). Chronic hyperglycemia, persistent low-grade inflammation, and the accumulation of advanced glycation end products may drive this process and influence the observed mortality patterns (21). However, not all studies have confirmed such associations. For example, a population-based study conducted in southern Wisconsin, found no significant relationship between T2DM, age at death, and cause-specific mortality after adjusting for confounders such as hypertension, smoking, and dyslipidemia (22). These results suggest that in some settings, the risk profile of individuals with DM is heavily influenced by coexisting conditions, making it difficult to isolate the direct impact of DM on mortality (22).

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The lack of statistical significance found for other adjustment variables suggests a context-dependent or minimal influence on age-specific mortality patterns in this cohort. Another study observed that socioeconomic status, for instance, impacted DM outcomes through its effect on healthcare access, education, and lifestyle behaviors (23). Ethnic disparities may also have a role on age-specific mortality patterns; in the United States, African-Americans and Hispanic individuals experience higher rates of DM complications but may differ in their mortality profiles due to both genetic and structural determinants (24). Furthermore, epigenetic changes induced by chronic stress, poor diet quality, and environmental exposures may modify individuals' vulnerability to DM complications across different causes of death (25). The non-significant findings may also reflect sample size limitations or residual confounders, reinforcing the need for larger, stratified studies to unravel the complex interaction between DM, age, and mortality.

One of the key strengths of this study lies in its robust epidemiological design, which integrates a comprehensive population-based approach with an extensive 40-year follow-up dataset. The inclusion of patients from both public and private healthcare settings enhances the representativeness of the sample, ensuring a more accurate reflection of real-world DM-related mortality trends. Furthermore, the meticulous data collection process, rigorously standardized, reinforces the reliability of clinical and demographic records.

Despite its strengths, this study is subject to some limitations. For instance, the lack of clinical confounders (e.g., individuals' comorbidities) may have impacted the explored relationships. Furthermore, the potential misclassification or reporting bias from death certificates should be mentioned by the nature of data collection. As previously demonstrated in another study conducted by our group, data underreporting DM as the cause of death may lead to biases and interpose confounding factors in this analysis (26,27).

It should be mentioned that, as 40.6% of eligible individuals were excluded due to missing or inconsistent death records, there is a potential for selection bias that warrants careful consideration. Although exclusions were derived from administrative and logistical issues, the excluded individuals may differ systematically from those included in key sociodemographic or clinical characteristics. Unfortunately, due to the absence of complete identifiers and death records, a direct comparison between included and excluded subjects was not feasible. Nonetheless, this limitation may impact the generalizability of the findings, particularly if the missing cases had distinct mortality patterns or disease severity. Differences in the distribution of causes of death compared with our previous publication reflect a deliberate reclassification based on underlying etiological mechanisms rather than terminal events listed on death certificates. This approach allowed a more coherent and robust analysis of age-related mortality patterns.

In conclusion, this study provides insights into the impact of cause-specific and all-cause mortality on the age at death among patients with DM. Our findings highlight that sex and DM type significantly influenced the age at death, with men and individuals with T2DM showing the most pronounced associations. Patients who died from infectious diseases tended to have a higher age at death, whereas those who died from gastrointestinal diseases tended to present a lower age at death.

These results underscore the necessity of considering both demographic and clinical factors when evaluating mortality patterns in patients with DM. Understanding these associations may help refine risk stratification strategies and improve clinical management, particularly in populations with a high burden of DM-related complications. Future research should explore potential underlying mechanisms and the role of additional factors, such as healthcare access and socioeconomic disparities, in shaping these mortality trends.

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**Data availability:** the original data underlying the analyses presented in this study are confidential and are securely maintained by the research team. Due to ethical and privacy considerations, the data are not publicly available. Data collection

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was approved by an Ethics Committee, as previously described in the manuscript.

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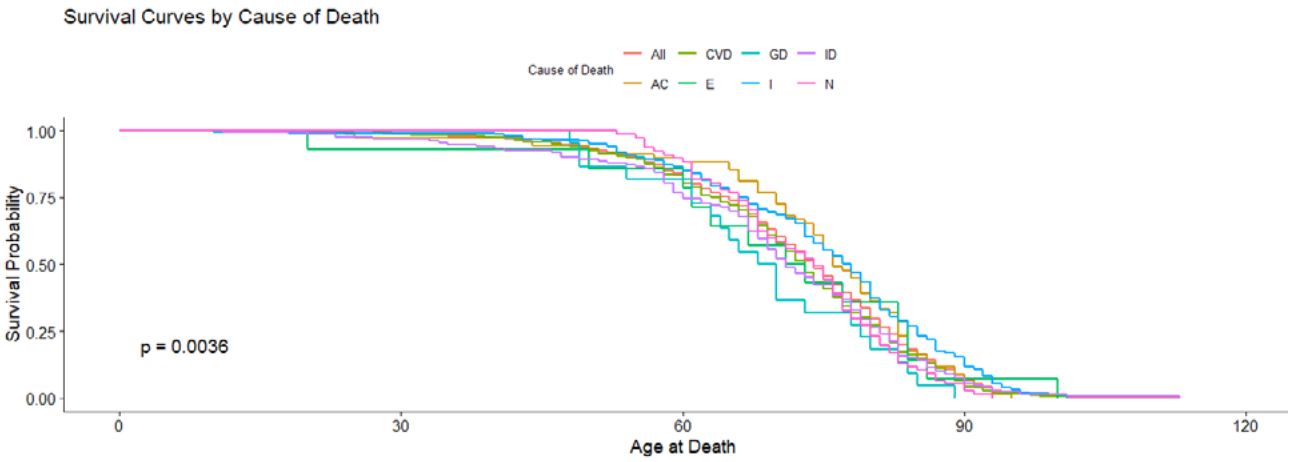

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**Supplementary table 1.** Patient's average death by evaluated categories

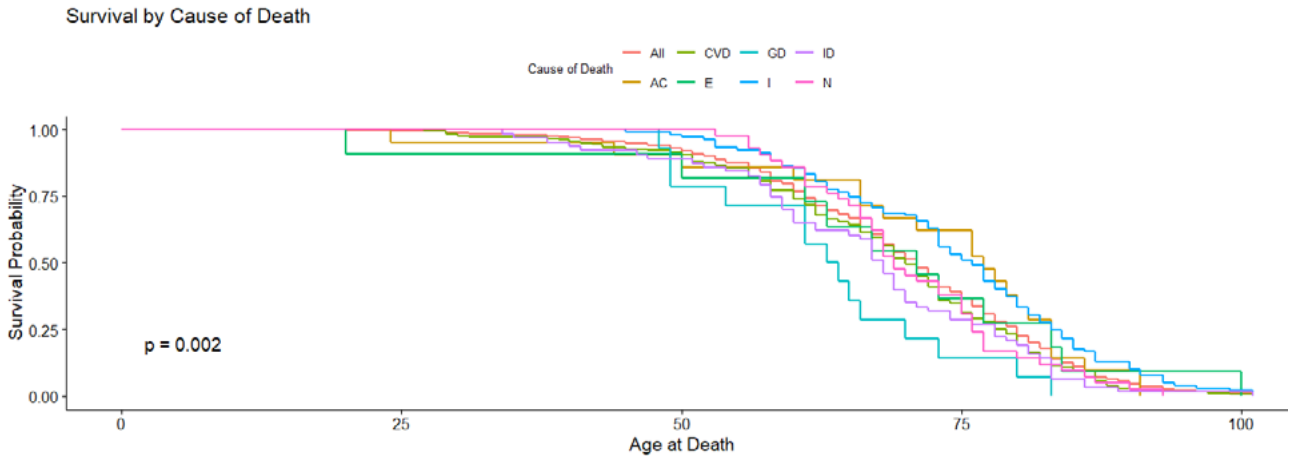
| Variable | Number of observations/ Average and Confidence interval | Average patient's death age | p-value for variable's association with patient's death age | p-value for variable's association with patient's death cause |
|---|---|---|---|---|
| Total | 812 (100%) | 72.35 ± 14.06 | - | |
| Sex | | | < 0.01 | < 0.01 |
| Men | 370 (45.56%) | 69.42 ± 18.93 | - | |
| Women | 442 (54.44%) | 75.44 ± 17.15 | - | |
| Self-reported ethnicity | | | 0.61 | 0.15 |
| Whites | 717 (88.30%) | 73.18 ± 13.88 | - | |
| Non-whites | 95 (11.70%) | 71.25 ± 15.764 | - | |
| Marital status | | | 0.90 | 0.57 |
| Single | 74 (9.11%) | 71.92 ± 17.55 | - | |
| Married | 585 (72.05%) | 73.06 ± 14.19 | - | |
| Widowed | 126 (15.52%) | 72.14 ± 8.24 | - | |
| Divorced | 27 (3.32%) | 73.93 ± 11.24 | - | |
| Diabetes type | | | < 0.01 | < 0.01 |
| T1DM | 40 (4.93%) | 70.22 ± 15.70 | - | |
| T2DM | 772 (95.07%) | 72.98 ± 10.87 | - | |
| Diabetes duration | | | 0.19 | 0.10 |
| < 10 years | 125 (15.39%) | 73.40 ± 12.19 | - | |
| 10-19 years | 294 (36.21%) | 72.16 ± 18.81 | - | |
| 20-29 years | 278 (34.23%) | 72.44 ± 15.29 | - | |
| 30-39 years | 91 (11.21%) | 71.19 ± 14.27 | - | |
| ≥ 40 years | 24 (2.96%) | 71.98 ± 13.18 | - | |
| Diabetes treatment | | | 0.52 | 0.17 |
| Only diet | 40 (4.92%) | 74.00 ± 18.27 | - | |
| Only Insulin | 412 (50.74%) | 73.06 ± 13.17 | - | |
| Only Antidiabetic oral drugs | 206 (25.38%) | 72.17 ± 15.18 | - | |
| Insulin and Antidiabetic oral drugs | 154 (18.96%) | 72.49 ± 9.76 | - | |
| BMI | | | 0.13 | 0.21 |
| Underweight | 15 ( 1.84%) | 75.92 ± 11.28 | - | |
| Normal | 210 (25.88%) | 72.17 ± 14.29 | - | |
| Overweight | 297 (36.58%) | 72.88 ± 15.14 | - | |
| Obesity I | 193 (23.77%) | 71.88 ± 13.27 | - | |
| Obesity II | 59 (7.26%) | 71.85 ± 15.19 | - | |
| Obesity III | 38 (4.67%) | 70.49 ± 15.67 | - | |
| Decade of death | | | 0.43 | 0.27 |
| 1981-1989 | 34 (4.19%) | 71.19 ± 15.29 | - | |
| 1990-1999 | 169 (20.83%) | 72.29 ± 14.06 | - | |
| 2000-2010 | 340 (41.84%) | 72.54 ± 13.98 | - | |
| 2011-2021 | 269 (33.14%) | 71.10 ± 14.87 | - | |
| Death causes | | | - | - |
| CVD | 167 (20.56%) | 72.52 ± 15.24 | - | |
| AC | 138 (16.99%) | 72.49 ± 18.25 | - | |
| E | 45 (5.54%) | 69.20 ± 18.13 | - | |
| I | 89 (10.96%) | 74.22 ± 12.60 | - | |
| N | 93 (11.45%) | 73.44 ± 15.73 | - | |
| ID | 173 (21.30%) | 72.27 ± 14.82 | - | |
| GD | 107 (13.17%) | 70.98 ± 12.24 | - | |
| Numeric variables | | | | |
| DM duration | 14.29 ± 20.78 | - | 0.09 | 0.16 |
| BMI | 23.98 ± 15.74 | - | 0.25 | 0.12 |

Presentation standard: Hazard Ration; 95% Confidence Interval; p-value. Cardiovascular diseases (CVD), external causes (E), acute complications of chronic diseases (AC), infectious causes (I), neoplasms (N), gastrointestinal diseases (GD), and III-defined causes (ID).

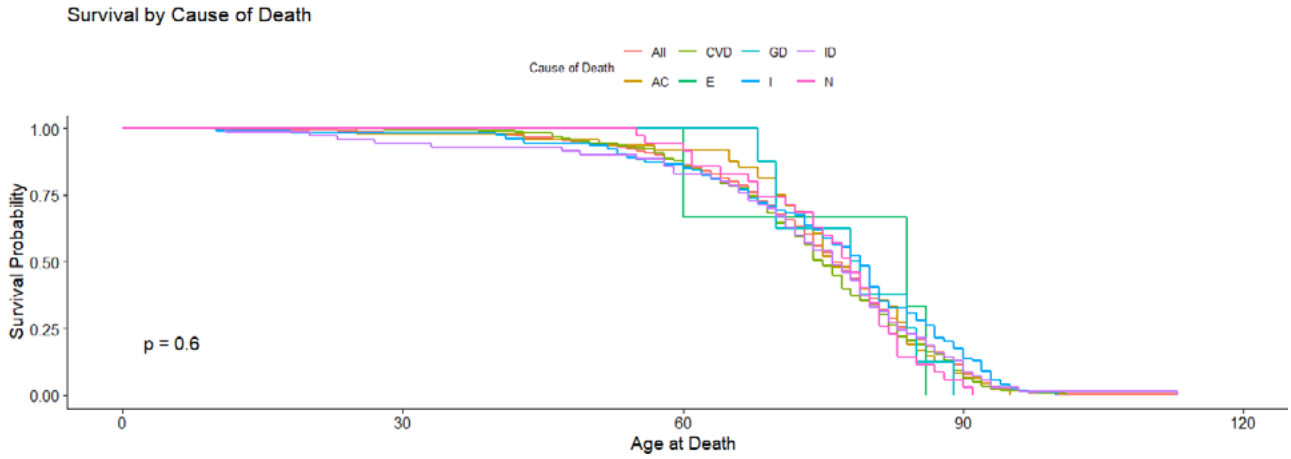
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**Supplementary figure 1.** Kaplan-Mayer curves without adjustment variables.

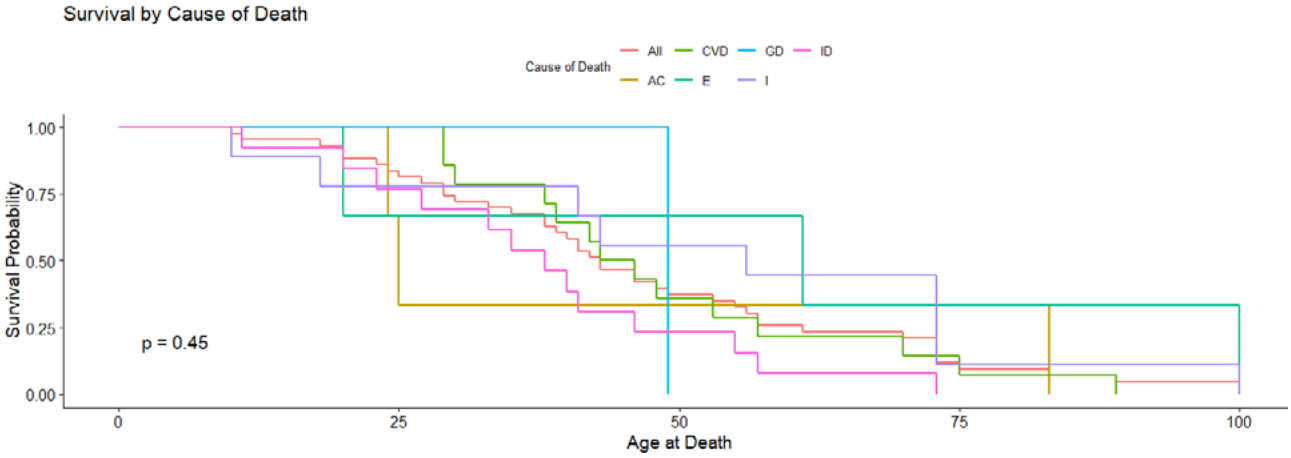



**Supplementary figure 2.** Kaplan-Mayer curves adjusted by men's model.

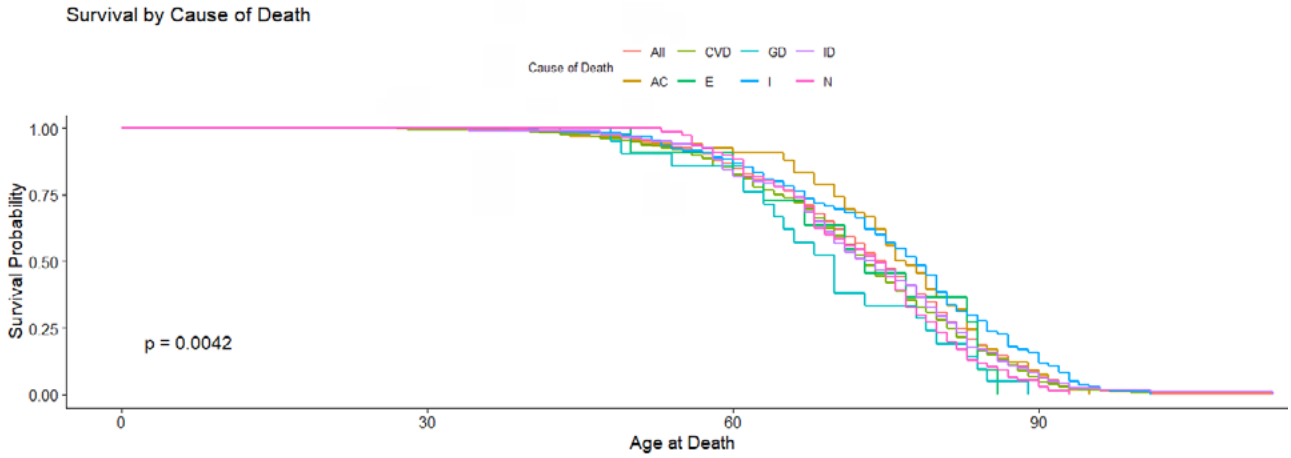



**Supplementary figure 3.** Kaplan-Mayer curves adjusted by women's model.

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**Supplementary figure 4.** Kaplan-Mayer curves adjusted by T1DM's model.




**Supplementary figure 5.** Kaplan-Mayer curves adjusted by T2DM's model.

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original

# Diabetes Distress Scale (DDS-17): European Portuguese Validation

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## ABSTRACT

**Objective:** Diabetes mellitus (DM) is currently one of the main public health challenges, not only because of its physical complications, but also because of the emotional impact associated with its chronic management. Diabetes distress (DD) is defined as the emotional response that results from living with DM and the consequent self-care required to manage it. The Diabetes Distress Scale (DDS-17) is an instrument used to assess DD. The aim of this study was to translate and culturally adapt the DDS-17 into European Portuguese, assessing its psychometric properties. **Subjects and methods:** Cross-sectional study divided into three phases: translation and back-translation; cultural adaptation with pre-testing in 30 patients; and psychometric validation (descriptive analysis of the items, exploratory factor analysis and reliability analysis) with 170 patients with type 2 DM from three Primary Care Units of the Braga Local Health Unit. **Results:** The European Portuguese version of the DDS-17 replicated the four original factors. Internal consistency was high (total Cronbach's alpha = 0.89), with all subscales scoring above 0.70. The overall DD was considered low (M = 1.68), but the "emotional burden related to DM" subscale showed values suggestive of moderate DD (M = 2.07). **Conclusion:** The European Portuguese version of the DDS-17 showed adequate validity and reliability and could be used in clinical and research contexts to better understand and manage DD in patients with type 2 DM in Portugal.

**Keywords:** Diabetes mellitus, type 2; psychological distress; primary health care

## INTRODUCTION

Diabetes mellitus (DM) has reached significant proportions worldwide and represents one of the major public health challenges in adulthood. According to the International Diabetes Federation, in 2021, 10.5% of individuals aged 20 to 79 were estimated to have DM, of whom approximately half remained unaware of their condition. Projections indicate that by 2045, one in eight adults is expected to have DM, representing a 46% increase (1). In Portugal, the estimated prevalence of DM within the same age group was 14.1% in 2021, corresponding to approximately 1.1 million individuals (2).

DM is associated with numerous physical complications, such as neuropathy, nephropathy and retinopathy, amongst others (3). However, the emotional and psychological burden linked to its chronic management has gained increasing recognition (4).

Since 2023, the American Diabetes Association has recommended a holistic approach to the management

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of DM, incorporating not only glycemic control and pharmacological treatment but also focus on emotional and behavioral factors. A key recommendation is the early identification of psychological conditions such as Diabetes Distress (DD), given their significant impact on patients' quality of life and clinical outcomes (5).

DD refers to a negative emotional response associated with living with DM, including feelings of frustration, fear, overwhelm, and exhaustion related to the self-care required for disease management — such as strict glucose monitoring, treatment adherence, and concern about future complications (6). When unrecognized or inadequately addressed, DD can substantially affect treatment adherence, metabolic control, and disease progression, increasing the risk of long-term complications (7,8). Some studies have shown that DD may affect glycemic control more profoundly than major depression, representing a distinct clinical entity requiring specific assessment (9).

To address this need, psychometric tools have been developed to identify and quantify DD. The Diabetes Distress Scale (DDS-17) is one such instrument, originally created and validated in the United States by Polonsky and cols. (2005). It comprises 17 items distributed across four subscales: emotional burden (5 items); physician-related distress (4 items); regimen-related distress (5 items); and interpersonal distress (3 items). The DDS-17 demonstrated excellent internal consistency, with an overall Cronbach's alpha of 0.93 and subscale values ranging from 0.80 to 0.95. Its use enables the accurate identification of individuals with elevated DD levels, facilitating tailored interventions and improving overall health and well-being (10).

Given the rising prevalence of DM and DD in Portugal, and the absence of validated psychometric instruments in European Portuguese, there is a clear need for a culturally adapted and validated tool to assess DD (2,5).

Currently, no specific data are available on the prevalence of diabetes distress (DD) among individuals with type 2 diabetes mellitus (T2DM) followed in primary healthcare settings in Portugal. While some studies have assessed quality of life, self-care behaviors, and disease knowledge in this population, the lack of concrete data on DD reveals a major gap in national literature and underscores the need for further research (2).

Although the DDS-17 has been widely applied in other international contexts — such as Brazilian Portuguese, Mandarin, Indonesian, and Turkish — it has not yet been translated or validated for the cultural and linguistic context of European Portuguese (11-14). The absence of an adapted version represents a significant limitation for both clinical practice and research in Portugal, as it hinders the early identification of patients experiencing meaningful emotional distress.

Therefore, the present study aims to translate, culturally adapt, and validate the DDS-17 for European Portuguese, with the goal of providing a reliable, sensitive, and clinically relevant tool. This instrument will allow for a more accurate assessment of the emotional impact of DM in Portugal; enhance clinical practice by enabling early identification of patients requiring intervention for DD; and support scientific research by providing robust data on DD in the Portuguese population.

## SUBJECTS AND METHODS

This was a cross-sectional analytical study conducted in three distinct phases: translation, cultural adaptation, and psychometric validation of the DDS-17 (**Appendix 1**) for European Portuguese.

The study population consisted of patients registered in the Family Health Units (FHUs) of Minho, Gualtar and Sá de Miranda, all part of the Local Health Unit (LHU) of Braga. A convenience sample of 170 patients was obtained, selected from those seen in DM consultations at these units between March and May 2025, who met the predefined inclusion and exclusion criteria.

The sample size was determined based on methodological recommendations for validation studies of psychometric instruments, which suggest at least 100 participants and 5 to 10 participants per item (15). As the DDS-17 includes 17 items, a sample of 170 participants was chosen to ensure statistical robustness.

The inclusion criteria for participants in this study were as follows: (1) Age ≥ 18 years; (2) native European Portuguese speaker; (3) diagnosis of type 2 Diabetes Mellitus (T2DM) for more than 1 year; (4) follow-up in DM consultation at FHU of Minho, Gualtar or Sá de

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Miranda; and (5) under treatment with oral antidiabetic drugs and/or injectable therapies (insulin or non-insulin). Participants with (1) diagnosis of type 1 DM; (2) diagnosis of dementia or cognitive impairment; (3) severe psychiatric disorders such as bipolar disorder, schizophrenia, or psychotic disorders; and (4) conditions preventing effective verbal communication or comprehension were excluded.

## Study phases

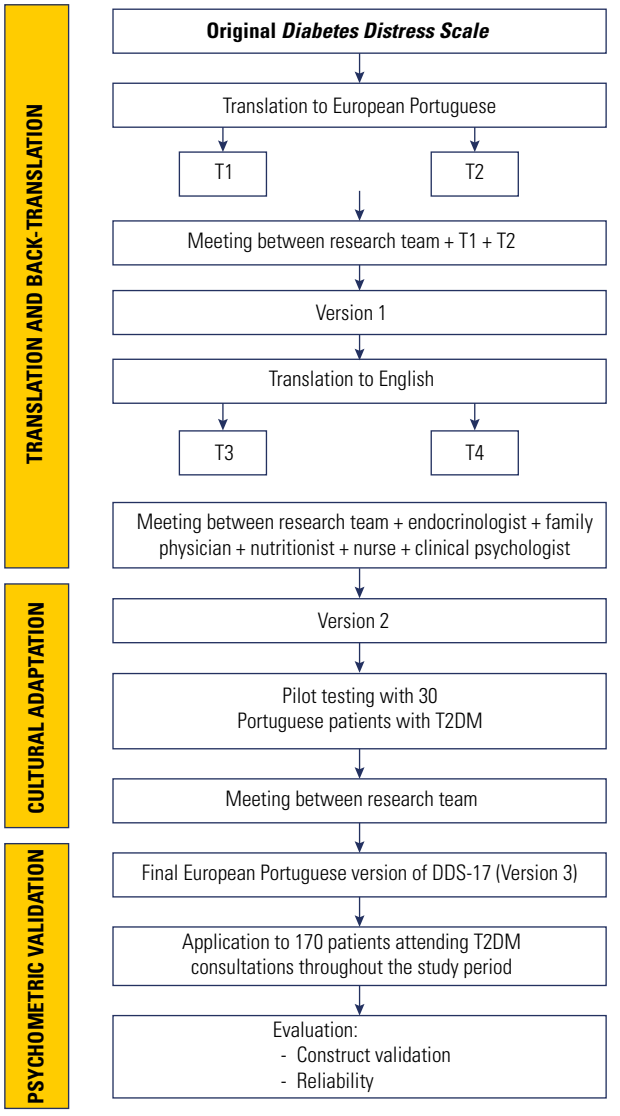
### *Phase 1: Translation and back-translation*

The translation phase was carried out in three stages, following specific international recommendations (16). The flowchart in **Figure 1** outlines the different phases of the study.

- **Initial translation:** The original DDS-17 (in English) was translated into European Portuguese (PT) by two independent translators (T1 and T2), both native PT speakers fluent in English.
- **Synthesis of translations:** Each translator produced a report, which was reviewed jointly by the research team and both translators to create a consensus version of the questionnaire (Version 1).
- **Back-translation:** Version 1 was then back-translated into English by two native English speakers (T3 and T4), both fluent in PT and blind to the original version. The resulting reports were compared with the original DDS-17 and analyzed for conceptual equivalence.
- **Expert panel review:** An expert panel was assembled, including an endocrinologist, a family physician with expertise in DM, a DM specialized nurse, and a clinical psychologist. Each expert received the original DDS-17, the two PT translations, Version 1, and both back-translations. A consensus meeting between the researchers and the expert panel resulted in Version 2 (pre-test version) of the scale.

### *Phase 2: Cultural adaptation*

To ensure cultural adaptation, Version 2 was applied to 30 patients with T2DM followed at FHU Sá de Miranda, after confirming the inclusion and exclusion criteria. These participants did not take part in Phase 3. This phase aimed to assess the comprehension, clarity, and cultural relevance of the items. Participant feedback was compiled and reviewed by the research team, leading to the final version (Version 3), used in the next phase. All participants in Phases 2 and 3 provided written informed consent, in accordance with the principles of the Declaration of Helsinki and the Oviedo Convention.




DDS-17: Diabetes Distress Scale; T1: Translator 1; T2: Translator 2; T3: Translator 3; T4: Translator 4; T2DM: Diabetes Mellitus type 2.

**Figure 1.** Representative flowchart of the study phases.

### *Phase 3: Psychometric validation*

Version 3 of the DDS-17 (**Appendix 2**) was applied to the 170 selected participants.

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### Study variables

In Phases 2 and 3, sociodemographic data were collected using a structured form developed for the study. This was followed by the application of Version 3 of the DDS-17.

Authorization for the translation and validation of the DDS-17 for European Portuguese was obtained from Polonsky and cols.

### Structure of the original DDS-17

The 17 items of the DDS-17 assess DD across four subscales: emotional burden (5 items), physician distress (4 items), regimen distress (5 items) and interpersonal distress (3 items). Each item is scored using a 6-point Likert scale reflecting the frequency of emotional burden in the past month. The final score is calculated as the mean of all items and scores are interpreted as < 2.0 means little or no distress, 2.0 to 2.9 moderate distress and ≥ 3.0 high distress. Results can be analyzed as a total mean score or separately by subscale. Mean scores suggesting moderate to high distress indicate the need for clinical intervention (17).

### Statistical analysis

Data were entered and analyzed using IBM SPSS® Statistics, version 29.0.2.0, 2023. Sociodemographic characteristics of the sample were described using absolute frequencies, relative frequencies, means (M), and standard deviations (SD).

Next, the psychometric properties of the Portuguese version of the DDS-17 were assessed through item-level descriptive analysis, exploratory factor analysis, and reliability analysis.

For the descriptive analysis of the 17 DDS-17 items, results were presented as mean (M), standard deviation (SD), median (Mdn), interquartile range ($25^{th}$–$75^{th}$ percentile), and observed range (minimum–maximum), allowing for a comprehensive assessment of DD levels within the study sample.

The factorial structure of the instrument was assessed using exploratory factor analysis with Varimax rotation, after verifying the underlying assumptions for its use — namely, a Kaiser-Meyer-Olkin (KMO) value greater than 0.50 and a statistically significant Bartlett's test of sphericity (p-value < 0.05) (18). Given the sample size in this study, factor loadings equal or greater than 0.45 were considered acceptable (15).

Finally, scale reliability was assessed based on internal consistency. Cronbach's alpha coefficient ($\alpha$) was used, with values equal or greater than 0.70 interpreted as indicating good internal consistency (18). Additionally, corrected item-total correlation was considered as a complementary reliability criterion.

## RESULTS

A pre-test was conducted with 30 patients with T2DM using Version 3 of the DDS-17. Participants reported no difficulties in understanding the items or completing the scale, and no modifications to the translated version were deemed necessary.

According to **Table 1**, a total of 170 participants were included, recruited from three FHUs of the LHU of Braga, with a relatively balanced distribution. The sample comprised 53.5% male, with a mean age of 69.55 years (SD = 12.55), reflecting a predominantly older adult sample. Age distribution was assessed using the Kolmogorov–Smirnov (with Lilliefors correction) and Shapiro–Wilk tests, both of which indicated normality (p-value = 0.09 and p-value = 0.08, respectively) (15).

**Table 1.** Total sample characterization (n = 170)

| Variable | n (%) |
|---|---|
| FHU of origin | |
| Minho | 60 (35.3) |
| Gualtar | 51 (30.0) |
| Sá de Miranda | 59 (34.7) |
| Sex | |
| Male | 91 (53.5) |
| Female | 79 (46.5) |
| Age (years) | |
| Mean ± standard deviation | 69.55 ± 12.55 |
| Age range | 39-96 |
| Education (years) | |
| < 4 | 27 (15.9) |
| 4 | 63 (37.1) |
| 6-9 | 42 (24.7) |
| 12 | 17 (10.0) |
| Higher education | 21 (12.4) |

FHU: Family Health Unit.

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Educational attainment was generally low, with 53.0% of participants having completed four or fewer years of formal education, and only 12.4% having attended higher education. There were no missing data in the sociodemographic variables, and all participants completed the questionnaire in its entirety.

**Table 2** summarizes the descriptive statistics for the 17 items of the DDS-17. Overall, participants provided very low scores across all items, with a median of 1.00 ("Not a problem"), indicating low levels of DD. Items 1, 7, and 12 stood out, with a median of 2.00 ("A slight problem"). Items 2, 5, 9, 11, 15, and 17 showed the least variability, with at least 75.0% of participants selecting the lowest score option on the scale (P75 = 1.00).

An exploratory factor analysis with Varimax rotation was conducted to examine the factorial structure of the instrument. Sampling adequacy was confirmed with a KMO > 0.50 and a significant Bartlett's test (18). Given the sample size, factor loadings equal or greater than 0.45 were considered acceptable (15).

An initial exploratory factor analysis extracted three factors, accounting for 62.81% of the total variance. However, since four factors were expected according to the original scale, a new exploratory factor analysis was conducted, forcing a four-factor solution, which explained 68.05% of the total variance (**Table 3**) (10). The first factor correspondend to the original subscale "Physician Distress", the second to "Emotional Burden", the third to "Regimen Distress", and the fourth to "Interpersonal Distress". Notably, item 3 showed a cross-loading with a relatively high loading on the first factor (0.539). However, its exclusion would result in a decrease in Cronbach's alpha ($\alpha = 0.67$) and, given its strong item-total correlation with factor 3 (0.66), it was retained in its original subscale. All items demonstrated factor loadings above 0.50.

**Table 4** presents the descriptive statistics and internal consistency results for the four DDS-17 subscales and the total score. Overall, mean scores across the subscales were low, reflecting a generally low level of DD in this sample. The "Emotional Burden related to DM" subscale showed the highest mean score (M = 2.07), exceeding the threshold of 2.0, which indicate moderate to high levels of DD that could merit clinical attention (17).

**Table 2.** Descriptive of DDS-17 scores about diabetes distress (n = 170)

| DDS-17 question | M (SD) | Mdn (P25-75) | Min-Max |
|---|---|---|---|
| 1. Feeling that diabetes is taking up too much of my mental and physical energy every day. | 2.25 (1.56) | 2.00 (1.00-3.00) | 1-6 |
| 2. Feeling that my doctor doesn't know enough about diabetes and diabetes care. | 1.18 (0.74) | 1.00 (1.00-1.00) | 1-6 |
| 3. Not feeling confident in my day-to-day ability to manage diabetes. | 1.56 (1.13) | 1.00 (1.00-2.00) | 1-6 |
| 4. Feeling angry, scared and/or depressed when I think about living with diabetes. | 1.99 (1.41) | 1.00 (1.00-3.00) | 1-6 |
| 5. Feeling that my doctor doesn't give me clear enough directions on how to manage my diabetes. | 1.22 (0.78) | 1.00 (1.00-1.00) | 1-6 |
| 6. Feeling that I am not testing my blood sugars frequently enough. | 1.62 (1.12) | 1.00 (1.00-2.00) | 1-6 |
| 7. Feeling that I will end up with serious long-term complications, no matter what I do. | 2.24 (1.56) | 2.00 (1.00-3.00) | 1-6 |
| 8. Feeling that I am often failing with my diabetes routine. | 1.82 (1.24) | 1.00 (1.00-2.00) | 1-6 |
| 9. Feeling that friends or family are not supportive enough of self-care efforts (e.g. planning activities that conflict with my schedule, encouraging me to eat the "wrong" foods). | 1.45 (1.01) | 1.00 (1.00-1.00) | 1-6 |
| 10. Feeling that diabetes controls my life. | 2.05 (1.46) | 1.00 (1.00-3.00) | 1-6 |
| 11. Feeling that my doctor doesn't take my concerns seriously enough. | 1.21 (0.81) | 1.00 (1.00-1.00) | 1-6 |
| 12. Feeling that I am not sticking closely enough to a good meal plan. | 2.28 (1.43) | 2.00 (1.00-3.00) | 1-6 |
| 13. Feeling that friends or family don't appreciate how difficult living with diabetes can be. | 1.60 (1.15) | 1.00 (1.00-2.00) | 1-6 |
| 14. Feeling overwhelmed by the demands of living with diabetes. | 1.82 (1.32) | 1.00 (1.00-2.00) | 1-6 |
| 15. Feeling that I don't have a doctor who I can see regularly enough about my diabetes. | 1.24 (0.84) | 1.00 (1.00-1.00) | 1-6 |
| 16. Not feeling motivated to keep up my diabetes self management. | 1.60 (1.13) | 1.00 (1.00-2.00) | 1-6 |
| 17. Feeling that friends or family don't give me the emotional support that I would like. | 1.45 (1.01) | 1.00 (1.00-1.00) | 1-6 |

M: mean; Max: maximum; Mdn: median; Min: minimum; P25: 25th percentile; P75: 75th percentile; SD: standard deviation.

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**Table 3.** Exploratory factor analysis results

| Items | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2. Feeling that my doctor doesn't know enough about diabetes and diabetes care. | 0.899 | | | |
| 11. Feeling that my doctor doesn't take my concerns seriously enough. | 0.892 | | | |
| 15. Feeling that I don't have a doctor who I can see regularly enough about my diabetes. | 0.866 | | | |
| 5. Feeling that my doctor doesn't give me clear enough directions on how to manage my diabetes. | 0.846 | | | |
| 3. Not feeling confident in my day-to-day ability to manage diabetes. | | | 0.523 | |
| 4. Feeling angry, scared and/or depressed when I think about living with diabetes. | | 0.809 | | |
| 10. Feeling that diabetes controls my life. | | 0.779 | | |
| 14. Feeling overwhelmed by the demands of living with diabetes. | | 0.761 | | |
| 1. Feeling that diabetes is taking up too much of my mental and physical energy every day. | | 0.672 | | |
| 7. Feeling that I will end up with serious long-term complications, no matter what I do. | | 0.652 | | |
| 6. Feeling that I am not testing my blood sugars frequently enough. | | | 0.776 | |
| 12. Feeling that I am not sticking closely enough to a good meal plan. | | | 0.599 | |
| 16. Not feeling motivated to keep up my diabetes self management. | | | 0.572 | |
| 8. Feeling that I am often failing with my diabetes routine. | | | 0.566 | |
| 9. Feeling that friends or family are not supportive enough of self-care efforts (e.g. planning activities that conflict with my schedule, encouraging me to eat the "wrong" foods). | | | | 0.766 |
| 13. Feeling that friends or family don't appreciate how difficult living with diabetescan be. | | | | 0.705 |
| 17. Feeling that friends or family don't give me the emotional support that I would like. | | | | 0.690 |
| % of variance explained | 23.60 | 18.87 | 13.22 | 12.36 |

**Table 4.** Descriptive statistics and internal consistency of the DDS-17 subscales

| Subscales | M (SD) | Min-Max | α |
|---|---|---|---|
| Emotional Burden related to DM | 2.07 (1.13) | 1.00-5.40 | 0.83 |
| Physician-Related Distress | 1.21 (0.74) | 1.00-6.00 | 0.96 |
| Interpersonal Distress | 1.50 (0.89) | 1.00-5.67 | 0.79 |
| Regimen-Related Distress | 1.78 (0.87) | 1.00-4.80 | 0.76 |
| Total | 1.68 (0.72) | 1.00-5.06 | 0.89 |

DM: diabetes mellitus; M: mean; Max: maximum; Min: minimum; SD: standard deviation; α: Cronbach's alfa.

All subscales and the total scale demonstrated good internal consistency, with Cronbach's alpha values above the recommended threshold of 0.70. Additionally, all items showed corrected item-total correlations above 0.30, ranging from 0.380 (item 6) to 0.924 (item 2) (18).

## DISCUSSION

The present study aimed to translate, culturally adapt, and psychometrically validate the European Portuguese version of the DDS-17, an instrument widely used to assess the emotional response associated with DM management (11-14), in a sample of 170 patients with T2DM recruited from three FHUs within the LHU of Braga.

Overall, the mean DDS-17 score was 1.68, suggesting a generally well-controlled level of DD. This may suggest effective DM management and support within the FHUs, where patients are regularly monitored and receive continuous care. Nonetheless, higher distress scores on items related to concerns about long-term complications and adherence to meal plans highlight areas that may require targeted clinical attention. These specific distress domains could affect patient self-management behaviors and ultimately glycemic control, emphasizing the need for healthcare providers to address emotional and regimen-related challenges during consultations.

The exploratory factor analysis confirmed the original four-factor structure of the DDS-17, explaining 68.05% of the total variance. The four factors — "*Physician-Related Distress*", "*Emotional Burden*", "*Regimen-Related Distress*", and "*Interpersonal Distress*" — showed strong factor loadings and internal consistency, with Cronbach's alpha ranging from 0.76 to 0.96, exceeding the recommended minimum threshold (≥ 0.70) across all subscales and for the total scale, demonstrating good reliability.

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Notably, the "*Physician-related distress*" subscale presented a particularly high value ($\alpha$ = 0.96), exceeding that reported by Polonsky and cols. (2005), who found $\alpha$ = 0.88 (10). This same subscale revealed low DD levels (M = 1.21), suggesting that patients in this sample feel supported by healthcare professionals, in contrast with international studies that identify the doctor–patient relationship as a significant source of DD (19,20). This finding may be explained by the organization of Portuguese primary care services, which promote continuity and proximity between patients and physicians.

The "*Emotional burden*" subscale yielded the highest mean score (M = 2.07), reaching the threshold for moderate distress, underscoring the emotional challenges faced by individuals with T2DM, even when overall distress levels are low. This finding is consistent with previous studies that identify the emotional impact of the disease as one of the main challenges in DM self-management, particularly regarding frustration, constant worry, and feelings of overload associated with daily self-care (7,8). This result is especially relevant given the age profile of the sample, as older adults may experience additional emotional strain related to comorbidities, social isolation, or declining autonomy. Besides, highlights the importance of approaches that specifically recognize and address the psychological burden of T2DM as a chronic illness (21).

The "*Regimen-related distress*" subscale had the second-highest mean (M = 1.78), which, while not indicating clinically significant DD, may reflect a sense of monotony or fatigue associated with the daily repetition of self-care demands, even when adherence is good. These findings reinforce the need for educational approaches that foster intrinsic motivation, empowerment, and autonomy in individuals with T2DM (22).

Regarding the "*Interpersonal distress*" subscale (M = 1.50), the results suggest an overall positive perception of emotional support from family and friends. However, the low variability in responses may also reflect barriers to emotional expression, particularly among older individuals, which should be addressed in clinical practice (23).

The results indicate that the European Portuguese version of the scale demonstrates robust psychometric indicators of validity and reliability, making it suitable for clinical and academic use in Portugal. In line with the original structure of the scale, the present findings confirmed that the distribution of items across the four proposed subscales is appropriate for the Portuguese context. Studies conducted in Brazil, China, Turkey, and Indonesia have also yielded similar structures, reinforcing the relevance of the subscale domains and confirming the psychometric stability of the DDS-17 across diverse settings, regardless of cultural differences or healthcare system characteristics (11-14).

Despite promising results, this study presents limitations that should be considered. The sample was one of convenience and geographically restricted to the Minho region, which may limit the generalizability of findings to other populations. The study sample was characterized predominantly by older adults (mean age 69.55 ± 12.55 years) with relatively low educational attainment, characteristics that may influence how DD is experienced and reported. Although this age group represents a substantial proportion of the Portuguese population with T2DM, it limits broader applicability (2). Reduced health literacy may have affected item comprehension or subjective interpretation, potentially leading to underreporting of emotional distress. Another key limitation is the absence of temporal stability testing (test-retest reliability) and sensitivity to change over time which are critical aspects for using the DDS-17 in longitudinal clinical monitoring or intervention evaluation.

Nonetheless, this study makes a relevant contribution to clinical practice in Portugal by providing a psychometrically validated tool to assess DD. Its regular use may support early detection of clinically significant emotional distress, enabling targeted and personalized interventions, in alignment with American Diabetes Association guidelines for comprehensive DM care. Future studies should extend the application of the DDS-17 to more diverse populations and examine its association with other relevant variables such as treatment adherence, disease duration, presence of complications, and mental health indicators. Evaluating concurrent validity with instruments such as the PHQ-9 or HADS could further strengthen the

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diagnostic utility of the European Portuguese version of the DDS-17. Additionally, assessment of its temporal stability would help confirm its applicability in longitudinal research and clinical follow-up contexts.

In conclusion, the validation of the DDS-17 for European Portuguese represents a valuable contribution to clinical practice in Portugal, addressing the lack of culturally adapted instruments for assessing DD. Based on its strong internal consistency, robust factorial structure, and ease of use, the DDS-17 proves to be suitable for application in the Portuguese context, particularly within Primary Health Care, allowing for the early detection of emotional dimensions that are often overlooked in the management of T2DM. The systematic use of the DDS-17 may support a biopsychosocial, person-centered approach to T2DM care, facilitating the early identification of DD and guiding personalized interventions that complement metabolic control. Future studies are needed to explore associations with other variables and to assess the temporal stability of the DDS-17.

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**Appendix 1.** Diabetes Distress Scale

| | Not a Problem | A Slight Problem | A Moderate Problem | Somewhat Serious Problem | A Serious Problem | A Very Serious Problem |
|---|---|---|---|---|---|---|
| 1. Feeling the diabetes is taking up too much of my mental and physical energy every day. | 1 | 2 | 3 | 4 | 5 | 6 |
| 2. Feeling that my doctor doesn't know enough about diabetes and diabetes care. | 1 | 2 | 3 | 4 | 5 | 6 |
| 3. Not feeling confident in my day-to-day ability to manage diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 4. Feeling angry, scared and/or depressed when I think about living with diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 5. Feeling that my doctor doesn't give me clear enough directions on how to manage my diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 6. Feeling that I am not testing my blood sugars frequently enough. | 1 | 2 | 3 | 4 | 5 | 6 |
| 7. Feeling that I will end up with serious long-term complications, no matter what I do. | 1 | 2 | 3 | 4 | 5 | 6 |
| 8. Feeling that I am often failing with my diabetes routine. | 1 | 2 | 3 | 4 | 5 | 6 |
| 9. Feeling that friends or family are not supportive enough of self-care efforts (e.g. planning activities that conflict with my schedule, encouraging me to eat the "wrong" foods). | 1 | 2 | 3 | 4 | 5 | 6 |
| 10. Feeling that diabetes controls my life. | 1 | 2 | 3 | 4 | 5 | 6 |
| 11. Feeling that my doctor doesn't take my concerns seriously enough. | 1 | 2 | 3 | 4 | 5 | 6 |
| 12. Feeling that I am not sticking closely enough to a good meal plan. | 1 | 2 | 3 | 4 | 5 | 6 |
| 13. Feeling that friends or family don't appreciate how difficult living with diabetes can be. | 1 | 2 | 3 | 4 | 5 | 6 |
| 14. Feeling overwhelmed by the demands of living with diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 15. Feeling that I don't have a doctor, who I can see regularly enough about my diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 16. Not feeling motivated to keep up my diabetes self-management. | 1 | 2 | 3 | 4 | 5 | 6 |
| 17. Feeling that friends or family don't give me the emotional support that I would like. | 1 | 2 | 3 | 4 | 5 | 6 |

Polonsky WH, Fisher L, Earles J, Dudl RJ, Lees J, Mullan J, et al. Assessing Psychosocial Distress in Diabetes Development of the Diabetes Distress Scale. 2005.

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**Appendix 2.** Diabetes Distress Scale

| | Não é um problema | É um problema ligeiro | É um problema moderado | É um problema algo sério | É um problema sério | É um problema muito sério |
|---|---|---|---|---|---|---|
| 1. Sinto que a diabetes está a tirar-me muito da minha energia mental e física todos os dias. | 1 | 2 | 3 | 4 | 5 | 6 |
| 2. Sinto que o meu médico não sabe o suficiente sobre a diabetes e os cuidados de saúde relativos à diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 3. Não me sinto confiante com a minha capacidade de gerir a diabetes no meu dia-a-dia. | 1 | 2 | 3 | 4 | 5 | 6 |
| 4. Sinto-me zangado(a), assutado(a) e/ou deprimido(a) quando penso em viver com diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 5. Sinto que o meu médico não me dá orientações claras o suficiente sobre como gerir a minha diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 6. Sinto que não estou a testar os meus níveis de açúcar no sangue com a regularidade suficiente. | 1 | 2 | 3 | 4 | 5 | 6 |
| 7. Sinto que vou acabar por ter complicações sérias a longo-prazo, independentemente do que faça. | 1 | 2 | 3 | 4 | 5 | 6 |
| 8. Sinto que falho frequentemente com a minha rotina da diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 9. Sinto que os meus amigos ou família não me apoiam o suficiente nos meus esforços de autocuidado (por exemplo: planeiam atividades que entram em conflito com o meu horário, incentivam-me a comer os alimentos "errados"). | 1 | 2 | 3 | 4 | 5 | 6 |
| 10. Sinto que a diabetes controla a minha vida. | 1 | 2 | 3 | 4 | 5 | 6 |
| 11. Sinto que o meu médico não leva em conta as minhas preocupações de forma séria o suficiente. | 1 | 2 | 3 | 4 | 5 | 6 |
| 12. Sinto que não estou a cumprir adequadamente um bom plano alimentar. | 1 | 2 | 3 | 4 | 5 | 6 |
| 13. Sinto que amigos ou família não valorizam o quão difícil pode ser viver com diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 14. Sinto-me sobrecarregado(a) com as exigências de viver com diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 15. Sinto que não tenho um médico a quem possa recorrer com a regularidade suficiente acerca da minha diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 16. Não me sinto motivado(a) para manter a autogestão da minha diabetes. | 1 | 2 | 3 | 4 | 5 | 6 |
| 17. Sinto que amigos ou família não me dão o apoio emocional que gostaria. | 1 | 2 | 3 | 4 | 5 | 6 |

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# Assessment of body composition, quality of life, and depression in women with polycystic ovary syndrome

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## ABSTRACT

**Objective:** This study aimed to evaluate body composition, quality of life, and depression risk in women with polycystic ovary syndrome (PCOS) compared to women without PCOS. **Subjects and methods:** This prospective, cross-sectional study assessed quality of life (QoL) and depressive symptoms in women with polycystic ovary syndrome (PCOS) compared with controls with and without overweight or obesity. Assessments included body composition, QoL, health-related quality of life (HRQoL), and depressive symptoms. Participants without PCOS were divided into two groups according to BMI: < 25 kg/m² [median age 30 years (27–33)] and ≥ 25 kg/m² [median age 32 years (29–35)]. **Results:** The study included 47 women with polycystic ovary syndrome (PCOS) phenotypes A and B. The median age was 29 years (25–35), and the median body mass index (BMI) was 32.08 kg/m² (28.48–36.40). Women with PCOS showed a higher risk of depression (24% with moderate to severe risk) compared with women without PCOS, both with and without overweight or obesity (5% and 3%, respectively). Additionally, women with PCOS consistently reported lower QoL and HRQoL scores, particularly in the physical, environmental, and overall QoL domains, as well as in functional capacity, pain, general health status, vitality, emotional well-being, and mental health domains of HRQoL. **Conclusion:** Based on these findings, we concluded that body composition does not appear to be a determining factor for increased risk of depressive symptoms or poorer perceptions of QoL and HRQoL in women with PCOS.

**Keywords:** Polycystic ovary syndrome; body composition; depression; quality of life; obesity.

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, affecting approximately 10%–13% of this population. The syndrome exhibits a complex etiology involving reproductive, metabolic, and psychological factors, and often remains undiagnosed until later stages (1). Although its exact cause is not fully understood (2), multiple factors are believed to contribute to the development of PCOS, including genetic predisposition (2), metabolic disturbances (3), behavioral factors (4), hormonal alterations (5), and overweight or obesity (6). According to the 2003 Rotterdam Consensus (7), PCOS is diagnosed when at least two of the following three criteria are present: clinical and/or biochemical hyperandrogenism (HA), oligo/anovulation (AO), and polycystic ovary morphology (POM). Patients may be categorized into one of four phenotypes: A) HA + AO + POM, B) HA + AO, C) HA + POM, and D) AO + POM (8).

PCOS is associated with an elevated risk of various metabolic conditions, including insulin resistance, type 2 diabetes mellitus, hypertension, and dyslipidemia. Psychological disorders are also among the comorbidities linked to the syndrome (9). In a 2023 narrative review, the authors highlighted the bidirectional

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relationship between metabolic syndrome and major depressive disorders, demonstrating that heightened activity of pro-inflammatory enzymes, combined with a diminished response of anti-inflammatory enzymes — both common in metabolic diseases — can result in dysfunctional metabolism and neurodegeneration (10). These effects, whether occurring individually or collectively, can significantly impair quality of life (QoL).

In its position statement, the European Society of Endocrinology (Gerard and cols., 2014) recommends that psychological assessment for patients with PCOS should be conducted not only at initial evaluation, but as an integral part of ongoing care (11). The 2023 International Guide to the Evaluation and Management of PCOS further emphasizes the importance of assessing the increased risks of anxiety and depression in women diagnosed with PCOS, advocating for continuous psychological monitoring in this population (12).

Nevertheless, it remains unclear which factors most strongly contribute to the lower perceived QoL and higher prevalence of depressive symptoms observed in women with PCOS. Overweight and obesity, which are common in PCOS, are significantly linked to these emotional outcomes (13). Additionally, metabolic and reproductive complications, as well as physical manifestations such as hirsutism, hair loss, and acne, may also play a role (14).

In this context, we aimed to assess signs and symptoms of depression, perceived QoL, and health-related QoL (HRQoL) in women with PCOS classified as phenotypes A and B, and to compare them with two control groups of women without PCOS, both with and without overweight or obesity. The hypothesis was that PCOS is associated with poorer perceptions of QoL and HRQoL, regardless of overweight or obesity status. Therefore, for practical application during the diagnostic process, it is important to evaluate emotional health, refer patients for psychological assessment, and promote comprehensive treatment to achieve better outcomes.

## SUBJECTS AND METHODS

### Ethical considerations

This study was approved by the Institutional Ethics Committee (no. 04057312.6.0000.525). All participants received clear and objective information about the study and signed an informed consent form.

### Study design and sample

This prospective cross-sectional study included 47 women diagnosed with PCOS phenotypes "A" and "B" (median age: 29 years [interquartile range – IQR: 25–35]; median body mass index [BMI]: 32.08 kg/m$^2$ [IQR: 28.48–36.40]). Two control groups without PCOS were also assessed: the first comprised 47 women with BMI < 25 kg/m$^2$ (median age: 30 years [IQR: 27–33]; median BMI: 22 kg/m$^2$ [IQR: 20.55–23.15]) (control BMI < 25 kg/m$^2$), and the second comprised 32 women with BMI ≥ 25 kg/m$^2$ (median age: 32 years [IQR: 29–35]; median BMI: 28.6 kg/m$^2$ [IQR: 26.6–31.00]) (control BMI ≥ 25 kg/m$^2$). Participants with PCOS were recruited from the Endocrinology outpatient clinics at Clementino Fraga Filho University Hospital (Federal University of Rio de Janeiro). Control participants were recruited among female students and staff at the Federal University of Rio de Janeiro, with no history of PCOS or other chronic diseases, and were divided into two groups based on BMI.

Sample size calculations were performed with Epi Info 6.0 software (StartCalc – Sample Size and Power command), assuming an 80% statistical power, a significance level of 5% ($p < 0.05$), and a population prevalence of PCOS between 8%–10%. For continuous variables, the minimum detectable difference between groups was estimated according to prior studies, with the expected standard deviation reflective of the study population. For categorical variables, the expected prevalence and an acceptable margin of error were considered. Assuming a study power of 99% and a confidence level of 95% ($p = 0.05$), an estimated sample size of 60 participants was determined, with 20 in the PCOS group and 20 controls in each subgroup. The final sample size was increased to account for potential dropouts, ensuring statistical robustness.

Inclusion criteria for women with PCOS were: age 18–45 years, medical records at the university hospital, and fulfillment of at least two of the three Rotterdam Consensus criteria for PCOS diagnosis (7), with exclusion of other causes of hyperandrogenism. Eligible women were evaluated during clinical

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consultations and invited to participate in the study. Women with chronic diseases, such as diebtes mellitus, dyslipidemia, hypertension, or hypothyroidism, were excluded. Clementino Fraga Filho University Hospital is a tertiary-level university hospital providing specialized, high-complexity care. As a result, patients treated at this institution often present with more severe cases of PCOS, likely explaining why only phenotypes A and B were observed in the sample.

## Procedures

Women with PCOS registered at the outpatient clinic were contacted by the study coordinator, informed about the study, and scheduled for a physician consultation. After verification of diagnosis and eligibility, they received detailed information about the study and, upon agreeing to participate, completed and signed the informed consent form. Women with PCOS then underwent anamnesis during a clinical consultation with an endocrinologist participating in the study. Demographic data were collected, and patients were asked about amenorrhea, hirsutism, acne, anovulation, baldness, and weight gain. In addition to these signs and symptoms, patients were questioned regarding diabetes, systemic arterial hypertension, family history of PCOS or metabolic diseases, and cardiovascular diseases. Following anamnesis, a comprehensive physical examination was performed to assess hirsutism, acne, and acanthosis nigricans.

At the end of the consultation, patients underwent body composition assessment and completed three questionnaires: the WHOQOL-Bref for perceived QoL (15), the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) for HRQoL (16), and the Beck Depression Inventory for assessment of depressive symptoms (17). All questionnaires had been translated and validated in Portuguese. Data collected from women with PCOS were analyzed and compared with those from two non-PCOS control groups defined according to BMI: < 25 kg/m² and ≥ 25 kg/m². The control group with BMI ≥ 25 kg/m² was not matched to the PCOS group because the PCOS group consisted predominantly of individuals with obesity, whereas the control group was mainly composed of women with overweight.

## Body composition

Body composition was estimated using standard anthropometric methods. Total body mass (kg) was measured with an electronic scale (Filizola; precision: 0.1 kg), and height (cm) was measured with a stadiometer (Filizola; precision: 0.1 cm). Seven skinfold thicknesses were measured in millimeters using a skinfold caliper (CESCORF; precision: 1 mm) at the abdominal, suprailiac, subscapular, pectoral, mid-axillary, triceps, and thigh sites. Waist, abdominal, and hip circumferences (cm) were measured with a flexible metal tape (Cardiomed; precision: 1 mm). Bone diameters (femur and humerus bi-epicondylar, and bi-styloid) were measured in centimeters with a pachymeter (Cardiomed; precision: 1 mm). From these measurements, the following variables were calculated: body mass index (BMI; kg/m$^2$) (18), body density (19), fat percentage (%F) (20), fat mass, lean mass, and muscle mass (21).

The Jackson and Pollock 7-site skinfold protocol for women, widely validated in the literature, was utilized. As DEXA, the gold standard for body composition assessment, was unavailable during the study period, a validated method appropriate for the studied age group was chosen, thus ensuring reliable and comparable results. All measurements were performed in accordance with International Society for the Advancement of Kinanthropometry standards (22), and always by the same assessor, who had prior experience in anthropometric assessment. The assessor demonstrated excellent reliability, with intraclass correlation coefficients ranging from 0.978 to 0.998 for the seven skinfold sites (i.e., abdominal, suprailiac, subscapular, pectoral, mid-axillary, triceps, and thigh).

## Signals and symptoms of depression

To assess depressive symptoms, the Beck Depression Inventory (BDI), validated in Portuguese, was administered. The BDI covers items related to sadness, pessimism, feelings of failure, guilt and punishment, dissatisfaction, self-deprecation, self-accusation, suicidal ideation, crying episodes, irritability, social withdrawal, decision-making difficulties, distorted self-image, work inhibition, sleep disturbances, fatigue, appetite and weight loss, worry, and decreased libido (17). BDI scores are classified as follows for individuals with

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no prior history of psychological disorders: up to 10 points, absent or minimal symptoms; 10–18 points, mild depression; 19–29 points, moderate to severe depression; and 30–63 points, severe depression (23).

### Quality of life and health-related quality of life

The self-administered WHOQOL-Bref questionnaire, translated and validated in Portuguese, was used to assess QoL. It contains 26 questions divided into four domains: physical health, psychological, social relationships, and environment, two general QoL questions. Patients were instructed to answer based on their experiences over the prior fifteen days. Responses were provided on Likert-type scales, with higher scores indicating more positive perceptions, except for questions 3, 4, and 26, which are reverse-scored (15). The self-administered SF-36 questionnaire, also translated and validated in Portuguese, was applied to assess HRQoL (16). This instrument comprises 36 items on a Likert-type scale, covering eight domains: functional capacity, physical functioning, pain, general health, vitality, social functioning, emotional aspects, and mental health. Each domain yields an independent score (0–100), with higher scores reflecting better perceived HRQoL.

### Statistical analysis

The Kolmogorov-Smirnov test was used to assess the distribution of variables. Parametric data were analyzed using one-way analysis of variance with Bonferroni post hoc test. Non-parametric data were analyzed with the Kruskal-Wallis test followed by Dunn's post hoc test. For assessment of depression risk, a frequency graph was constructed to quantify the proportion of scores in the categories: below 10, 10–18, and 19–29, across the three groups. Subsequently, Spearman's correlation test was applied to explore relationships between body composition and depression risk. A $p$-value less than 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 8.0 software.

## RESULTS

After clinical and laboratory evaluation, all patients exhibited hyperandrogenism. The primary complaints were oligo/anovulation (35.3%), assessed based on menstrual irregularities rather than second-phase progesterone levels, as it was not feasible to assess all patients; hirsutism, determined using the Ferriman-Gallwey scale with a cutoff point of 8 (the threshold used at the onset of data collection) (35.3%), acne (25.5%), infertility (23.5%), and baldness (7.9%). These characteristics confirm that our cohort corresponds to phenotypes A and B, classified as classic PCOS. Body composition was assessed by a physical education professional, who found that all women with PCOS were overweight or obese (**Figure 1**).

Of the 47 women evaluated, 32 adhered to the treatment protocol. During follow-up, we investigated risk indicators for metabolic syndrome, with the most prevalent being fasting glucose > 126 mg/dL, HDL cholesterol < 50 mg/dL, and abdominal circumference > 88 cm. **Table 1** presents the number and percentage of patients displaying one, two, or three metabolic syndrome risk indicators.

Frequency analysis (**Figure 2**) demonstrated that 24% of women with PCOS exhibited a moderate to severe risk of depression, compared to only 5% in the BMI > 25 control group and 3% in the BMI < 25 control group. Additionally, 38% of women with PCOS were classified as having a moderate risk of depression, whereas this proportion was 27% and 23% in the BMI > 25 and BMI < 25 control groups, respectively. Conversely, 38% of women with PCOS were classified as having no depression, in comparison to 72% and 70% in the BMI < 25 and BMI > 25 control groups, respectively. To assess the statistical difference in the incidence of depressive symptoms between groups, a Chi-square test was performed ($\chi^2$ = 15.54; $p$ = 0.0037), indicating a statistically significant difference in depression symptom incidence among groups.

Spearmans correlation analysis revealed no significant associations between depression risk and body composition parameters in women with PCOS: total body mass ($r$ = 0.09; $p$ = 0.56), BMI ($r$ = 0.08; $p$ = 0.59), fat percentage (%G) ($r$ = 0.08; $p$ = 0.61), and waist-to-hip ratio (WHR) ($r$ = 0.01; $p$ = 0.93) (**Figure 3**).

Regarding QoL, as measured by WHOQOL-Bref, statistically significant differences were observed in the physical ($p$ < 0.0011), environmental ($p$ < 0.0001),

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and overall QoL ($p$ < 0.0014) domains. Specifically, in the physical domain, the control group with BMI ≥ 25 kg/m² reported significantly better perceptions than both women with PCOS and controls with BMI < 25 kg/m². In the environmental domain, controls with BMI < 25 kg/m² exhibited better perceptions, with statistically significant differences compared to women with PCOS and controls with BMI ≥ 25 kg/m². In the overall QoL domain, women with PCOS had worse perceptions than the control group with BMI < 25 kg/m², with statistically significant differences (Table 2).

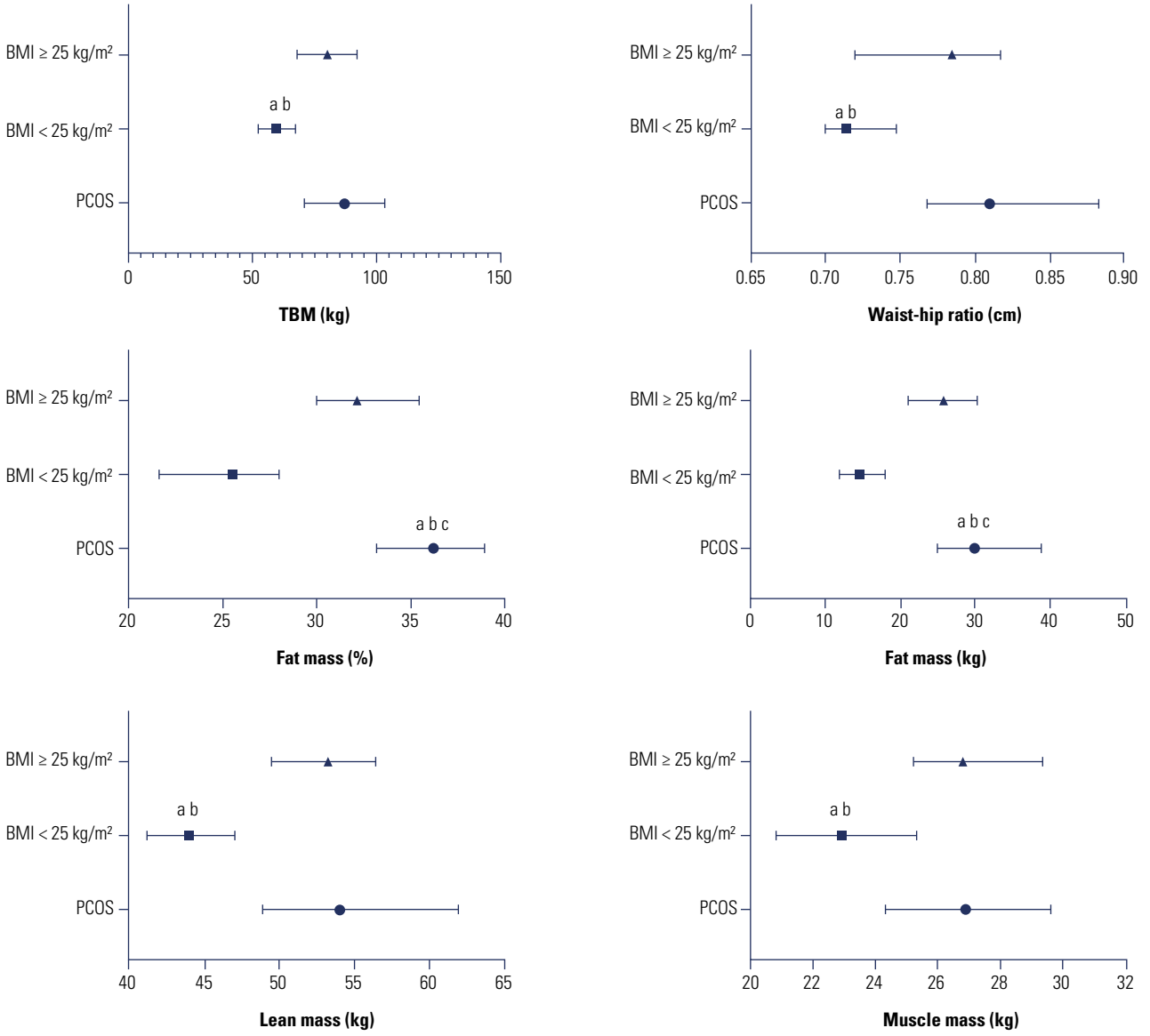



**Figure 1.** Body composition. Data are presented as median [first quartile – third quartile]; TBM = total body mass; statistical significance $p$ < 0.05; [a]difference between PCOS and control BMI < 25 kg/m²; [b]difference between control BMI < 25 kg/m² and control BMI ≥ 25 kg/m²; [c]difference between PCOS and control BMI ≥ 25 kg/m².

**Table 1.** Prevalence of metabolic syndrome risk factors in the study population

| | 3 or more MS risk factors | 2 MS risk factors | 1 MS risk factors |
|---|---|---|---|
| Number of patients | 11 patients | 12 patients | 9 patients |
| Percentage of patients | 34% | 38% | 28% |

MS: metabolic syndrome.

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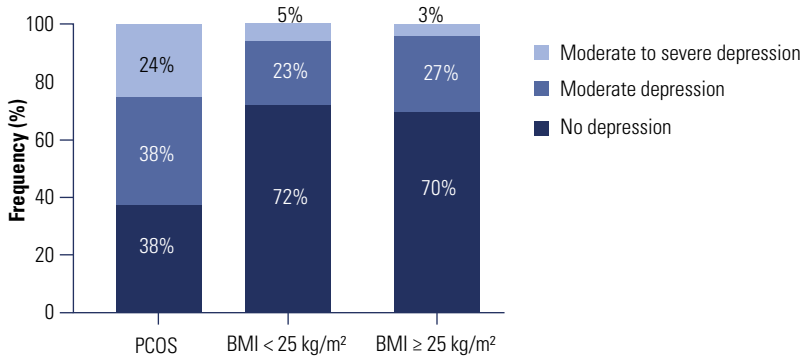
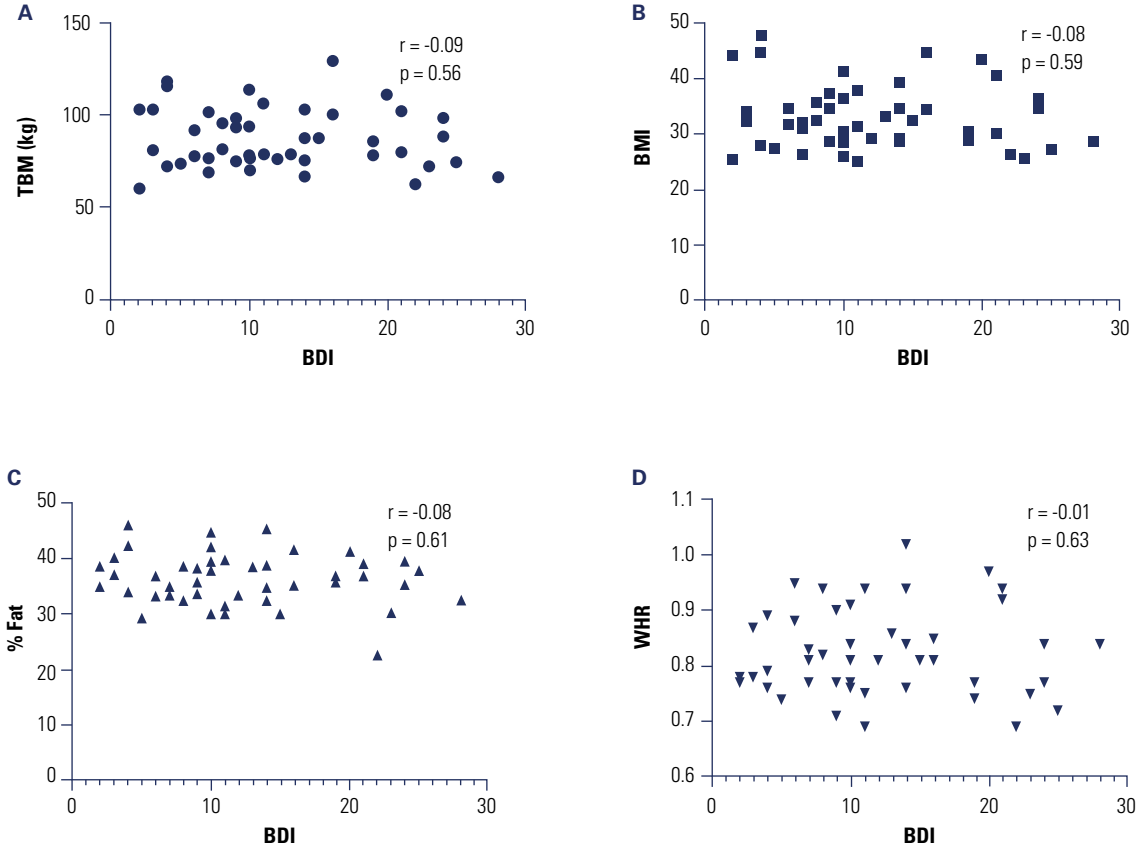



**Figure 2.** Beck Depression Inventory – percentage of women in the different groups based on risk of depression.




BDI: beck depression inventory; TBM: total body mass; BMI: body mass index; % Fat: body fat percentage; WHR: waist-to-hip ratio.

**Figure 3.** Body composition vs. symptoms of depression – correlation analysis between body composition data and risk of depression.

Patients with PCOS reported systematically lower scores in all SF-36 domains (**Table 2**), with statistically significant differences observed in "functional capacity" (PCOS ≠ control BMI < 25 kg/m²; $p < 0.041$), "pain" (PCOS ≠ control BMI < 25 kg/m²; PCOS ≠ control BMI ≥ 25 kg/m²; $p < 0.0070$), "general state of health" (PCOS ≠ control BMI < 25 kg/m²; PCOS ≠ control BMI ≥ 25 kg/m²; $p < 0.0001$) domains, as well as "vitality" (PCOS ≠ control BMI < 25 kg/m²; $p < 0.0219$), "emotional aspects" (PCOS ≠ control BMI < 25 kg/m²; $p < 0.0139$), "mental health" (PCOS ≠ control BMI < 25 kg/m²; $p < 0.0141$) (**Table 2**). There were no differences between the groups in the areas of "physical aspects" ($p = 0.2192$) and "social aspects" ($p = 0.1471$) (**Table 3**).

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**Table 2.** WHOQOL-Bref domain scores according to study subgroups

| | PCOS (n=47) | Control BMI < 25 kg/m² (n=47) | Control BM I ≥ 25 kg/m² (n=30) | *P-value |
|---|---|---|---|---|
| Physical | 13.10[bc] [10.30-17.70] | 13.10 [8.0-18.30] | 15.15 [9.70-19.40] | <0.0011 |
| Emotional | 13.30 [9.30-17.30] | 14.0 [9.30-18.0] | 14.70 [9.30-17.30] | <0.0504 |
| Social relations | 14.70 [6.70-20.0] | 14.00 [9.30-18.00] | 16.00 [8.00-20.00] | <0.0513 |
| Environmental | 13.00[ab] [5.0-18.0] | 16.00 [9.30-20.0] | 13.50 [9.50-16.50] | <0.0001 |
| General QoL issues | 12.00[a] [4.0-18.0] | 14.00 [8.00-20.00] | 14.00 [8.00-18.00] | <0.0014 |

Values described as median [minimum value – maximum value]; QoL = quality of life; *Kruskal-Wallis; statistical significance = 0.016%; [a]difference between PCOS and control BMI < 25 kg/m² by Mann-Whitney; [b]difference between control BMI < 25 kg/m² and control BMI ≥ 25 kg/m² by Mann-Whitney; [c]difference between PCOS and Control BMI ≥ 25 kg/m² by Mann-Whitney.

**Table 3.** Health-related quality of life – SF-36 according to the study subgroups

| | PCOS (n=47) | Control BMI < 25 kg/m² (n=47) | Control BM I ≥ 25 kg/m² (n=32) | *P-value |
|---|---|---|---|---|
| Functional capacity | 80.0[a] [35.0-100.0] | 90.0 [25.0-100.0] | 90.0 [45.0-100.0] | 0.0041 |
| Physical aspects | 75.0 [0.0-100.0] | 100.0 [0.0-100.0] | 100.0 [0.0-100.0] | 0.2192 |
| Pain | 51.5[a.c] [10.0-100.0] | 74.0 [40.0-100.0] | 72.0 [20.0-100.0] | 0.0070 |
| Genral satate of health | 44.50[a.c] [15.0-66.0] | 77.0 [30.0-100.0] | 69.5 [32.0-97.0] | <0.0001 |
| Vitality | 50.0[a] [10.0-85.0] | 60.0 [10.0-90.0] | 47.5 [10.0-85.0] | 0.0219 |
| Social aspects | 68.8 [12.50-100.0] | 75.0 [25.0-100.0] | 68.8 [25.0-100.0] | 0.1471 |
| Emocional aspects | 68.8[a] [12.5-100.0] | 100.0 [0.0-100.0] | 66.7 [00.0-100.0] | 0.0139 |
| Mental health | 64.0[a] [28.0-80.0] | 76.0 [40.0-92.0] | 70.0 [32.0-92.0] | 0.0141 |

Values described as median (minimum value – maximum value); QoL = quality of life; *Kruskal-Wallis; statistical significance = 5%; [a]difference between PCOS and control BMI < 25 kg/m² by Mann-Whitney; [b]difference between control BMI < 25 kg/m² and control BMI ≥ 25 kg/m² by Mann-Whitney; [c]difference between PCOS and control BMI ≥ 25 kg/m² by Mann-Whitney.

## DISCUSSION

This study aimed to evaluate women diagnosed with PCOS, specifically phenotypes A and B, and to compare them with two groups of women without a PCOS diagnosis (one overweight/obese and one of normal BMI) with respect to QoL, HRQoL, and risk of depression. The main findings indicated that all women with PCOS were classified as overweight or obese, although only 47.06% associated their weight gain with PCOS in the initial questionnaire. Additionally, women with PCOS exhibited higher rates of depression risk and reported lower perceived QoL and HRQoL than both control groups.

Obesity is recognized as a major comorbidity associated with PCOS (12) and plays a crucial role in the disease's pathophysiology (24). In this study, patients were randomly recruited during appointments; all were overweight or obese, with no selective bias for obesity. These patients reported poorer perceptions of both QoL and HRQoL, as well as more pronounced

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signs and symptoms of depression than women in the two control groups without PCOS, regardless of body weight. These results suggest that overweight/obesity alone is likely not the primary driver of adverse outcomes in women with PCOS.

These findings differ from those of Karsten and cols. (25), who compared women with obesity and a history of infertility (with and without PCOS) and found that in terms of symptoms of anxiety, depression, and QoL perception, poorer outcomes in women with PCOS were more strongly related to obesity than to other PCOS symptoms. Similarly, Himeleinand Thatcher (26) demonstrated that obesity, along with hirsutism, contributed significantly to reduced QoL, emotional disturbances, and reduced libido. Nevertheless, a 2019 review provides a complementary perspective on the interplay between PCOS and obesity, highlighting that elevated anxiety and depression in women with PCOS hinder the adoption of healthier lifestyles (e.g., regular physical activity, balanced diet), making weight loss — and consequently disease management — more difficult (6). This bidirectional relationship underscores the complexity of PCOS pathogenesis: obesity increases insulin resistance and hyperinsulinemia, which, in turn, aggravate the reproductive and metabolic dysfunctions typical of PCOS as well as depression (10).

With respect to depression risk, the current study demonstrated that women with PCOS have a higher risk of depression than women without the disease. This is evidenced by a much greater proportion of women with PCOS at risk of depression (23.5%) compared to the control groups, especially when compared to the BMI-matched group (3.1%) suggesting that depression risk is more closely associated with PCOS-related factors other than obesity. Another study using the Beck Inventory, conducted in Turkey — where the majority of the population is Muslim — concluded that depression was more closely related to infertility, while anxiety was more associated with overweight and obesity (27). These findings underscore the importance of considering cultural factors in the mental health assessment of women with PCOS.

Perceived QoL, anxiety, and depression risk are widely studied and well-known to be associated with PCOS (24,26,28–30). The 2023 guideline on PCOS diagnosis and management reiterates previous recommendations that screening for perceived QoL and depression should be conducted at the time of diagnosis using regionally validated instruments; screening should be repeated as needed by clinicians, particularly during specific life stages such as the puerperium. The guideline further recommends referring patients at increased risk for emotional disorders to appropriate treatment (12).

Regarding HRQoL, as measured by the SF-36 questionnaire, women with PCOS scored worse in domains including functional capacity, pain, general health status, vitality, emotional aspects, and mental health than controls without overweight or obesity (PCOS ≠ control BMI < 25 kg/m$^2$). When compared to overweight/obese controls, women with PCOS also reported significantly worse pain and general health status (PCOS ≠ control BMI ≥ 25 kg/m$^2$). These findings are consistent with prior research comparing women with obesity and infertility, with and without PCOS, which also reported lower emotional domain scores in women diagnosed with PCOS. In contrast, no significant differences were found between groups for physical aspects in that study (25).

A 2021 study assessed perceived QoL, HRQoL, and anxiety and depression levels in women with PCOS, comparing all four phenotypes. Results showed no significant differences among groups regarding infertility, body weight, and emotional domains; however, phenotypes A and B reported lower HRQoL scores with respect to hirsutism. The study thus recommends evaluating emotional issues in all women with PCOS, regardless of phenotype (30). These conclusions are supported by a 2013 Endocrine Society position statement, which stated that depression in women with PCOS does not necessarily require the presence of obesity or other disease sequelae, and also recommended assessing depression in all diagnosed patients (31). It is important to note that this study was conducted at a tertiary referral hospital that treats severe disease cases, including PCOS. Thus, all participants were classified as phenotypes A and B and exhibited all clinical manifestations, limiting comparisons across phenotypes. Moreover, a 2024 study found that

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infertile women, even without PCOS, require psychological support (32).

Delayed diagnosis and insufficient information about the disease are additional factors relevant to patient dissatisfaction, as reported in a 2017 study utilizing an online questionnaire with 1,385 women diagnosed with PCOS, aged 18–45, from 32 countries. Findings revealed high dissatisfaction with delayed diagnosis (over two years), consultations with multiple healthcare professionals who failed to identify the syndrome (three or more), and insufficient information on PCOS and its treatment. Importantly, most women from all regions reported not being evaluated for or referred for emotional support (1). Although we did not evaluate this issue and acknowledges it as a limitation, we also recognize its importance, as prolonged disease duration increases the risk of complications and, consequently, worsens QoL perceptions and depression risk.

Other limitations include the absence of a control group with another chronic disease, which would have allowed assessment of whether having a chronic illness per se is the main factor for low QoL, or whether this is specific to PCOS. Additionally, patients with phenotypes C and D were not included, as the outpatient clinic primarily treats complex disease cases. On the other hand, this study included a substantial sample of women with PCOS and two control groups stratified by body composition, facilitating identification of QoL impairments more closely linked to PCOS. These results reinforce prior evidence, emphasizing the importance of assessing QoL, anxiety, and depression risk in women with PCOS, particularly those with phenotypes A and B, who comprised the focus of this investigation.

In conclusion, the study confirmed previous data showing a higher prevalence of overweight and obesity among women with PCOS phenotypes A and B. However, this factor did not appear to be the primary cause of lower QoL, HRQoL, and increased risk of depression in this group. From a practical perspective, this study reinforces the importance of routine assessment of QoL, HRQoL, and depression risk in women with PCOS during clinical visits. Early identification of emotional and psychological issues can help prevent the onset of severe mental health disorders. The findings also support the need for a multidisciplinary treatment approach involving psychologists, physical educators, nutritionists, and psychiatrists when necessary, alongside endocrinologists and gynecologists, to optimize patient outcomes.

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**Data availability:** datasets related to this article will be available upon request to the corresponding author.

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original

# Sleep aspects and subclinical hypothyroidism: a four-year follow-up of the ELSA-Brasil study

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## ABSTRACT

**Objective:** Previous studies suggest a bidirectional relationship between thyroid dysfunction and sleep disorders. However, prospective evidence regarding the impact of sleep characteristics on subclinical hypothyroidism remains limited. This study aimed to evaluate the association between insomnia symptoms, sleep duration, and sleep debt and the incidence of subclinical hypothyroidism. **Subjects and methods:** We conducted a prospective cohort analysis of 7,983 euthyroid participants from the second wave (2012–2014) of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) who were not taking thyroid-related or psychiatric medications. Insomnia symptoms (initial, middle, and terminal), sleep duration, sleep debt, sociodemographic characteristics, and health behaviors were assessed via questionnaires. Subclinical hypothyroidism was defined as thyrotropin >4.0 µIU/mL and normal free thyroxine in the third wave (2016–2018). Crude and adjusted log-binomial regression models estimated relative risks (RR) and 95% confidence intervals (95% CI). **Results:** The incidence of subclinical hypothyroidism was 6.6% for both sexes. In women, middle insomnia was associated with a 35% reduced risk of subclinical hypothyroidism (RR: 0.65; 95% CI: 0.44–0.92). Among men, sleep debt was linked to a 30% increased incidence (RR: 1.30; 95% CI: 1.01–1.66), and in the continuous model, each additional hour of sleep debt raised the risk by 9% (RR: 1.09; 95% CI: 1.02–1.14). **Conclusion:** Of the sleep characteristics assessed, middle insomnia due to nocturnal awakenings appeared to be protective against subclinical hypothyroidism among women, while sleep debt increased the risk among men.

**Keywords:** Insomnia; sleep duration; sleep debt; subclinical hypothyroidism; thyroid disorders

## INTRODUCTION

Subclinical hypothyroidism, a prevalent form of thyroid dysfunction, is characterized by elevated thyrotropin (TSH) levels above the upper reference limit and normal free thyroxine (FT4) levels (1,2). This condition is commonly observed in clinical practice, with a significant proportion of cases being transient and reversible (3). Many patients are asymptomatic or exhibit nonspecific symptoms that can be easily mistaken for other conditions, such as obesity, menopause, or depression (4,5).

Brazilian studies report a prevalence of subclinical hypothyroidism ranging from 5.4% among adults and elderly to 6.5% among adults in the city of São Paulo (6,7). A 2007 survey in Rio de Janeiro found a prevalence of 12.3% for both clinical and subclinical hypothyroidism in adult women (8). The condition occurs more frequently in women than men, attributable to hormonal, autoimmune (loss of immunological tolerance), and genetic factors (3). Female sex hormones

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influence thyroid function, increasing women's susceptibility to thyroid diseases (9). Variations in prevalence are also affected by geographic and demographic factors (10-12). Advanced age, female sex, and white race/ethnicity are the main risk factors associated with elevated TSH (10,11). Subclinical hypothyroidism is thus a multifactorial disorder also associated with sleep disturbances (13).

High-quality sleep is vital for hormonal homeostasis and proper metabolic functioning (14,15). Endocrine disturbances may directly result from stressors, including poor sleep quality (16). Sleep disturbances can affect FT4 secretion depending on the severity and duration of the sleep problem, either by activating the hypothalamic-pituitary-adrenal axis or by modulating TSH levels — the primary marker of subclinical hypothyroidism — via negative feedback in the hypothalamic-pituitary-thyroid (HPT) axis (17).

Several studies over the past decade have shown that acute sleep restriction elevates serum TSH and reduces FT4 and free T3 (17-19). Conversely, chronic sleep restriction may induce adaptation, resulting in decreased TSH (18). An experimental study found that sleep deprivation may cause hypothyroxinemia (a reduction in T4), a state that can precede hyperthyrotropinemia (increased TSH) (19). Regarding insomnia, a cross-sectional study did not find differences in TSH between individuals with and without insomnia (20). Another population-based cross-sectional study observed that short and long sleep duration did not significantly affect TSH compared to normal sleep duration (7–8 hours); however, long sleep duration increased the odds of subclinical hypothyroidism by 97% (13).

Given the bidirectional relationship between sleep and the endocrine system (15,21), both clinical and subclinical thyroid dysfunction are associated with sleep abnormalities. Individuals with clinical hyperthyroidism often experience insomnia and short sleep, while those with clinical hypothyroidism exhibit excessive somnolence and prolonged sleep duration (22-24). Nonetheless, a cohort study of 682 older men found no differences in subjective or objective sleep measures when comparing individuals with subclinical thyroid diseases to euthyroid controls (25). Although sleep and thyroid hormones are interdependent under normal physiological conditions (15), health behaviors such as short sleep and sleep debt, which deregulate various hormones, may precede subclinical thyroid disease. Thus, the directionality of the association, in which sleep disturbances precede subclinical hypothyroidism, is also plausible, albeit understudied (25,26). It is not known thus far whether insomnia symptoms, analyzed prospectively, deregulate TSH levels.

Given the above, insomnia symptoms, sleep debt, and extremes of sleep duration may be risk factors for subclinical hypothyroidism, though few studies have addressed this direction of causality (10,13,14,17,20). The few existing studies differ in design and sleep-related exposures assessed. Therefore, we investigated the association between insomnia symptoms, sleep duration, and sleep debt, and the incidence of subclinical hypothyroidism in a cohort of Brazilian public employees, stratified by sex.

## SUBJECTS AND METHODS

### Study design

We conducted a prospective cohort analysis using data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a multicenter study conducted in five universities and one research center across six Brazilian states (Bahia, Espírito Santo, Minas Gerais, Rio Grande do Sul, São Paulo, and Rio de Janeiro). ELSA-Brasil's primary objective is to investigate the incidence and progression of chronic noncommunicable diseases, particularly diabetes and cardiovascular disease. Participants are active and retired public employees aged 35–74 at baseline (more information, see https://www.elsabrasil.org).

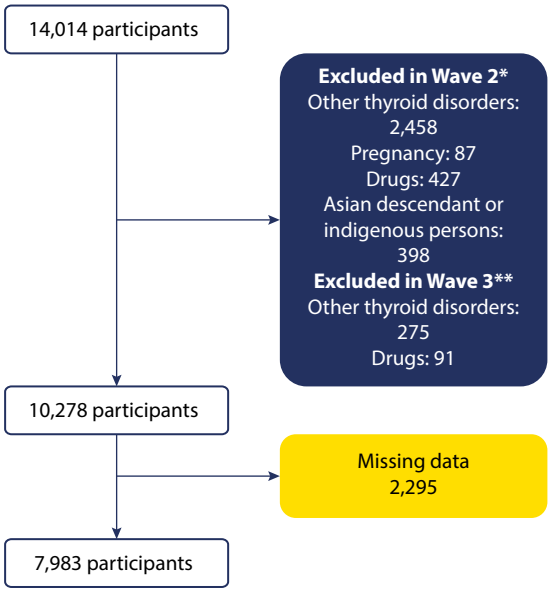
### Context and participants

The baseline assessment occurred between 2008 and 2010 and included 15,105 participants. The second wave (2012–2014) comprised 14,014 participants, and the third wave (2016–2018) included 12,636 participants of both sexes. This analysis utilized data from waves 2 and 3, as sleep data were unavailable at baseline. We only considered euthyroid individuals from wave 2 who were not using medications known to alter thyroid hormones (amiodarone, biotin, hydrocortisone, prednisolone, dexamethasone, phenobarbital,

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phenytoin, divalproex sodium, valproic acid, primidone, haloperidol, heparin, furosemide, carbidopa, levodopa, lithium, metoclopramide, carbamazepine, oxcarbazepine, or rifampicin) (27,28), antithyroid drugs (methimazole or propylthiouracil), or undergoing thyroid hormone replacement therapy (levothyroxine, L-thyroxine, or liothyronine) (28).

Participants using psychotropic medications, including antidepressants (tricyclics, selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, monoamine oxidase inhibitors, and atypical antidepressants), mood stabilizers, antipsychotics, tranquilizers and sedatives or hypnotics, as well as medications for attention-deficit/hyperactivity disorder (e.g., methylphenidate, lisdexamfetamine, and amphetamine salts) or autism spectrum disorder, were excluded. Individuals of Asian or indigenous descent were also excluded due to their small numbers (2.56% and 0.95%, respectively). Pregnant women and individuals with missing data for key variables were also excluded. Data from wave 3 included euthyroid individuals or those with subclinical hypothyroidism who were not using the aforementioned drugs and did not have missing data. After all exclusions, the final sample comprised 7,983 participants followed for four years (**Figure 1**).




*In Wave 2, individuals with clinical or subclinical hyperthyroidism, clinical or subclinical hypothyroidism, or central hyper-/hypothyroidism (hypopituitarism) were excluded. Individuals using medications known to affect thyroid function (e.g., amiodarone, biotin, hydrocortisone, prednisolone, dexamethasone, phenobarbital, phenytoin, divalproex sodium, valproic acid, primidone, haloperidol, heparin, furosemide, carbidopa, levodopa, lithium, metoclopramide, carbamazepine, oxcarbazepine, rifampicin, methimazole, propylthiouracil, liothyronine, or levothyroxine) were also excluded. Further exclusions were applied to individuals taking anxiolytics (e.g., bromazepam, clonazepam, diazepam, or lorazepam), antidepressants (e.g., SSRIs, SNRIs, or tricyclics), antipsychotics, or Z-drugs, as well as to pregnant individuals and those of Asian or Indigenous descent. **In Wave 3, individuals with subclinical hypothyroidism were not excluded; all other exclusion criteria remained unchanged.

**Figure 1.** Flowchart of selected participants.

## Subclinical hypothyroidism

Incident subclinical hypothyroidism was defined as normal thyroid function during the second wave of ELSA-Brasil (0.4–4.0 μIU/mL for TSH and 0.93–1.7 ng/dL for FT4), followed by TSH >4.0 μIU/mL and normal FT4 during the third wave. TSH and FT4 were measured in centrifuged serum samples collected after 12 hours of overnight fasting, using a third-generation immunoenzymatic assay (Roche Diagnostics, Germany) (27).

## Insomnia symptoms, sleep duration, and sleep debt

Sleep variables were measured using a questionnaire in the second wave of ELSA-Brasil. Insomnia was assessed with questions addressing initial (sleep-onset) insomnia ("*In the last 30 nights, have you had difficulty falling asleep?*"), middle insomnia ("*In the last 30 nights, have you woken up and had difficulty falling asleep again?*"), and terminal insomnia ("*In the last 30 nights, have you woken up before you wanted and been unable to fall asleep again?*") (29). Responses (never, rarely, sometimes, almost always, and always) were categorized as "yes" if "always" or "almost always", and "no" otherwise. Each question was analyzed separately, as well as a composite variable indicating any insomnia symptom.

Sleep duration was assessed with the question: "*How many hours on average do you sleep on a normal night?*" Responses were numeric and participants were classified in three groups short (≤6 hours), normal (>6 and ≤8 hours), and long (>8 hours) sleep duration (30). Sleep debt was measured as the difference between desired and actual hours of sleep, based on the question "*How many hours would you like to sleep to feel refreshed?*" (31). A difference was then categorized as "no" when it was ≤1 hour and "yes" when it was >1 hour. Sleep debt was also analyzed as a continuous

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variable, with negative values resulting from the difference between the questions set to zero.

### Confounders and effect modification

Adjustment variables were also measured with the questionnaire in the second wave of ELSA-Brasil and selected based on the literature for cross-sectional studies and included age, race/ethnicity (white, brown, or black), education (higher, secondary, or primary), smoking (non-smokers, former, or current), alcohol consumption (non-drinkers, former, or current), physical activity (intense, moderate, or light), coffee consumption (never, once a day, 2–3 times a day, and >3 times a day), and menopause (for females).

Alcohol consumption was assessed using the questions "*Do you currently consume alcohol?*" and "*Have you ever consumed alcohol?*". Participants who reported current consumption were classified as "current drinkers", those who reported past consumption were classified as "former drinkers", and those who answered "no" to both questions were classified as "non-drinkers".

Physical activity was measured using the International Physical Activity Questionnaire, to estimate the weekly time spent in light, moderate, and intense physical activities. Coffee consumption was using a question from the food consumption questionnaire, and answers were categorized as never/almost never, ≤1/day, 1–3/day, and >3/day.

Menopause was measured with closed questions on women's menstrual and defined as a cessation for >6 months or self-reported natural menopause. Given the higher prevalence of sleep problems and thyroid diseases in women, analyses were stratified by sex, which was considered an effect-modifying variable (32).

### Statistical analysis

Descriptive analysis used absolute and relative frequencies (incidence) for categorical variables and means and standard deviations for the quantitative variables, sleep deprivation and age. Incidence was calculated as the number of new cases among exposed individuals over the total exposed for each variable. Bivariate analysis employed Pearson's chi-squared test for categorical and Student's *t*-test for continuous variables.

Crude and adjusted log-binomial regression models were used to evaluate whether insomnia symptoms (overall and by type), sleep duration, and sleep debt increased the risk of subclinical hypothyroidism. Log-binomial regression was used to estimate the relative risks (RRs) and 95% confidence intervals (CIs). Model 1 adjusted for age, race/ethnicity, and education; Model 2 was adjusted for age, race/ethnicity, education, smoking, alcohol consumption, physical activity, and coffee consumption. The models for female sex were also adjusted for menopause. All analyses were conducted separately by sex using R software version 4.2.1 (R Foundation for Statistical Computing, Austria).

### Ethics

This study was approved by the Ethics Committee at all six research centers. All participants provided written informed consent.

## RESULTS

Over the four-year follow-up, the incidence of subclinical hypothyroidism was 6.6% in both men and women. Among women, the incidence was 5.4% among those with any insomnia symptoms, 5.6% for initial insomnia, 4.6% for middle insomnia, 6.1% for terminal insomnia, 6.6% for short sleep, 9.5% for long sleep duration, and 6.2% for sleep debt. Among men, the respective incidences were 6.1% (any insomnia),6.4% (initial insomnia), 6.0% (middle insomnia), 6.6% (terminal insomnia), 6.4% (short sleep), 9.3% (long sleep), and 7.1% (sleep debt) (**Table 1**).

As for sociodemographic and behavioral factors, higher mean age was observed in incident cases compared to euthyroid individuals in both women (mean = 55.8, SD = 8.5) and men (mean = 56.6, SD = 9.2). Incidence was 7.8% in white women, 5.3% in brown women, and 5.4% in black women; for men, 7.4% (white), 5.9% (brown), and 5.0% (black). No other variables showed statistically significant associations (**Table 1**).

Insomnia symptoms, initial insomnia, terminal insomnia, short and long sleep duration, and sleep debt were not significantly associated with incident subclinical hypothyroidism in women. However, women

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**Table 1.** Incidence of subclinical hypothyroidism according to subjective characteristics of sleep, sociodemographic conditions, and health behaviors, stratified by sex. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil, 2012–2018)

| Variables | | Women (n = 4112; 51.5%) | | Men (n = 3871; 48.5%) | |
|---|---|---|---|---|---|
| | | Euthyr. | Shypo. | Euthyr. | Shypo. |
| **n (%)** | | 3,839 (93.4) | 273 (6.6) | 3,615 (93.4) | 256 (6.6) |
| **Continuous variables** | | | | | |
| Age* | Mean (SD) | 54.6 (8.50) | 55.8 (8.55) | 54.3 (8.80) | 56.6 (9.23) |
| Sleep duration | Mean (SD) | 6.49 (1.39) | 6.55 (1.46) | 6.41 (1.25) | 6.54 (1.32) |
| Sleep debt | Mean (SD) | 1.65 (1.69) | 1.64 (2.24) | 1.26 (1.55) | 1.42 (1.87) |
| **Categorical variables** | | | | | |
| Insomnia symptoms | No | 2,813 (92.9) | 214 (7.1) | 2,950 (93.3) | 213 (6.7) |
| | Yes | 1,026 (94.6) | 59 (5.4) | 665 (93.9) | 43 (6.1) |
| Initial insomnia | No | 3,217 (93.2) | 236 (6.8) | 3278 (93.4) | 233 (6.6) |
| | Yes | 622 (94.4) | 37 (5.6) | 337 (93.6) | 23 (6.4) |
| Middle insomnia† | No | 3,192 (93.0) | 242 (7.0) | 3,194 (93.3) | 229 (6.7) |
| | Yes | 647 (95.4) | 31 (4.6) | 421 (94.0) | 27 (6.0) |
| Terminal insomnia | No | 3,249 (93.3) | 235 (6.7) | 3,247 (93.4) | 230 (6.6) |
| | Yes | 590 (93.9) | 38 (6.1) | 368 (93.4) | 26 (6.6) |
| Sleep duration (hours) | ≤ 6 | 1,926 (93.4) | 135 (6.6) | 1,933 (93.6) | 133 (6.4) |
| | > 6 and ≤ 8 | 1,770 (93.5) | 123 (6.5) | 1,594 (93.3) | 114 (6.7) |
| | > 8 | 143 (90.5) | 15 (9.5) | 88 (90.7) | 9 (9.3) |
| Sleep debt | No | 1,793 (92.9) | 137 (7.1) | 2,120 (93.7) | 142 (6.3) |
| | Yes | 2,046 (93.8) | 136 (6.2) | 1,495 (92.9) | 114 (7.1) |
| Race/Ethnicity* | White | 1,958 (92.2) | 166 (7.8) | 1,953 (92.6) | 157 (7.4) |
| | Brown | 1,065 (94.7) | 60 (5.3) | 1,128 (94.1) | 71 (5.9) |
| | Black | 816 (94.6) | 47 (5.4) | 534 (95.0) | 28 (5.0) |
| Level of education | Higher | 2,247 (93.6) | 153 (6.4) | 2,037 (93.7) | 137 (6.3) |
| | Secondary | 1,299 (93.3) | 94 (6.7) | 1,124 (92.7) | 88 (7.3) |
| | Primary | 293 (91.8) | 26 (8.2) | 454 (93.6) | 31 (6.4) |
| Alcohol consumption | Non-drinker | 746 (92.1) | 64 (7.9) | 267 (95.0) | 14 (5.0) |
| | Former drinker | 680 (93.5) | 47 (6.5) | 694 (93.9) | 45 (6.1) |
| | Current drinker | 2,413 (93.7) | 162 (6.3) | 2,654 (93.1) | 197 (6.9) |
| Smoking | Non-smoker | 2,480 (93.1) | 184 (6.9) | 1,945 (92.9) | 148 (7.1) |
| | Former smoker | 941 (93.6) | 64 (6.4) | 1235 (93.6) | 84 (6.4) |
| | Current smoker | 418 (94.4) | 25 (5.6) | 435 (94.8) | 24 (5.2) |
| Physical activity | Intense | 218 (94) | 14 (6) | 408 (93.8) | 27 (6.2) |
| | Moderate | 612 (93.6) | 42 (6.4) | 691 (93.1) | 51 (6.9) |
| | Light | 3,009 (93.3) | 217 (6.7) | 2,516 (93.4) | 178 (6.6) |
| Coffee consumption (daily) | Never | 301 (92.6) | 24 (7.4) | 286 (92.9) | 22 (7.1) |
| | ≤1 | 346 (91.8) | 31 (8.2) | 432 (94.1) | 27 (5.9) |
| | 2–3 | 2,144 (93.6) | 147 (6.4) | 1,649 (92.8) | 128 (7.2) |
| | >3 | 1,048 (93.7) | 71 (6.3) | 1,248 (94.0) | 79 (6.0) |
| Menopause | No | 1,300 (94.1) | 81 (5.9) | - | - |
| | Yes | 2,539 (93.0) | 192 (7.0) | - | - |

Euthyr.: Euthyroid; Shypo.: Subclinical hypothyroidism; SD: standard deviation; *$p$-value< 0.05 for women and men †$p$-value < 0.05 only in women; Chi-square test for categorical variables and Student t-test for continuous variables.

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with middle insomnia showed a 35% lower risk of subclinical hypothyroidism (RR: 0.65, 95% CI: 0.44–0.92) compared to those without middle insomnia (Table 2). Among men, none of the insomnia exposures or sleep duration categories were associated with subclinical hypothyroidism; however, sleep debt was positively associated. In the fully adjusted model (Model 2), sleep debt increased the risk of subclinical hypothyroidism by 30% (RR: 1.30, 95% CI: 1.01–1.66). In the continuous sleep debt model, each additional hour of sleep debt was associated with an 8% higher risk (RR: 1.08, 95% CI: 1.01–1.13) in Model 1, and a 9% higher risk (RR: 1.09, 95% CI: 1.02–1.14) in Model 2 (Table 2).

**Table 2.** Crude and adjusted associations between insomnia symptoms and subtypes, sleep duration, sleep debt, and subclinical hypothyroidism, stratified by sex. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil, 2012–2018)

| Variable | Women† (n = 4112; 51.5%) | Men (n = 3871; 48.5%) |
|---|---|---|
| **Insomnia symptoms** | **RR (95%CI)** | **RR (95%CI)** |
| Crude model | 0.77 (0.58–1.01) | 0.90 (0.65–1.22) |
| Model 1* | 0.76 (0.57–1.00) | 0.89 (0.64–1.20) |
| Model 2* | 0.77 (0.57–1.01) | 0.91 (0.65–1.24) |
| **Initial insomnia** | | |
| Crude model | 0.82 (0.58–1.13) | 0.96 (0.62–1.42) |
| Model 1* | 0.82 (0.57–1.13) | 0.98 (0.63–1.45) |
| Model 2* | 0.82 (0.58–1.14) | 1.01 (0.65–1.49) |
| **Middle insomnia** | | |
| Crude model | **0.65 (0.44–0.92)** | 0.90 (0.60–1.30) |
| Model 1* | **0.65 (0.44–0.92)** | 0.86 (0.57–1.24) |
| Model 2* | **0.65 (0.44–0.92)** | 0.89 (0.59–1.28) |
| **Terminal insomnia** | | |
| Crude model | 0.90 (0.63–1.23) | 1.00 (0.66–1.44) |
| Model 1* | 0.90 (0.63–1.24) | 0.99 (0.65–1.43) |
| Model 2* | 0.90 (0.63–1.24) | 1.02 (0.67–1.48) |
| **Short sleep duration** | | |
| Crude model | 1.01 (0.80–1.28) | 0.96 (0.76–1.23) |
| Model 1* | 1.04 (0.82–1.32) | 1.00 (0.78–1.27) |
| Model 2* | 1.04 (0.82–1.32) | 1.01 (0.79–1.29) |
| **Long sleep duration** | | |
| Crude model | 1.46 (0.84–2.35) | 1.39 (0.67–2.49) |
| Model 1* | 1.43 (0.82–2.31) | 1.37 (0.66–2.45) |
| Model 2* | 1.39 (0.79–2.27) | 1.34 (0.65–2.42) |
| **Sleep debt** | | |
| Crude model | 0.88 (0.70–1.10) | 1.13 (0.89–1.43) |
| Model 1* | 0.94 (0.74–1.19) | 1.26 (0.99–1.61) |
| Model 2* | 0.95 (0.75–1.20) | **1.30 (1.01–1.66)** |
| **Continuous sleep debt** | | |
| Crude model | 1.00 (0.93–1.07) | 1.05 (0.98–1.12) |
| Model 1* | 1.02 (0.95–1.09) | **1.08 (1.01–1.13)** |
| Model 2* | 1.02 (0.95–1.09) | **1.09 (1.02–1.14)** |

Notes: *Model 1 – adjusted for age, race/ethnicity, and level of education; Model 2 – model 1 + alcohol consumption, smoking, physical activity, and coffee consumption; †Model 2 also adjusted for menopause.

## DISCUSSION

After four years of follow-up, women with middle insomnia exhibited a lower risk of developing subclinical hypothyroidism, whereas sleep debt in men was associated with increased risk. Regarding insomnia and its subtypes, the observed protective effect of middle insomnia among women stood out. Generally, the literature does not support a protective role of insomnia against subclinical hypothyroidism. A recent cross-sectional study reported that individuals with sleep difficulties demonstrated a higher prevalence of hypothyroidism (odds ratio [OR] = 1.38, 95% CI: 1.14–1.68), including the subclinical form, compared to euthyroid individuals (33). A systematic review corroborates these findings, noting a positive relationship between poor sleep quality and hypothalamic-pituitary-thyroid axis dysfunction, although with some study heterogeneity (34). Similarly, Mendelian randomization analyses have found no causal association between genetic susceptibility to insomnia and the risk of hypothyroidism (35), making it biologically unlikely that fragmented sleep would protect thyroid function.

Furthermore, it cannot be ruled out that the finding reflects a chance effect or statistical fluctuations, especially as other insomnia subtypes did not show similar associations. Therefore, caution is warranted in interpreting results and replicating with objective sleep assessments and in other populations is necessary. Notably, an experimental crossover study showed that six weeks of sleep restriction to approximately 6.0 hours per night significantly reduced circulating TSH in women, but not men, with the most pronounced reduction in premenopausal women. Sleep restriction also modestly reduced FT4, with the largest effect size in postmenopausal women, suggesting that insufficient sleep disrupts the HPT axis more in women, possibly due to a more a pronounced proinflammatory response (36).

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Neither initial nor terminal insomnia was significantly associated with subclinical hypothyroidism, although their effect sizes also suggested a protective relationship. Initial and terminal insomnia may have less impact on thyroid hormones because they cause less sleep fragmentation compared to middle insomnia (36). The scarcity of studies assessing insomnia subtypes hampers a comprehensive interpretation. Still, a cross-sectional study on the relationship between sleep quality (assessed with the Pittsburgh Sleep Quality Index) and TSH levels showed that individuals with poor sleep quality and who took longer to fall asleep had higher odds (OR: 2.39, 95% CI: 1.44–3.98) of subclinical hypothyroidism (14). These results differ from our findings, as the comparisons are limited since the authors did not stratify by sex and used different measurement tools (14).

We found no statistically significant association between combined insomnia symptoms and subclinical hypothyroidism. To our knowledge, no comparable longitudinal studies exist. A cross-sectional study on hormone levels in the HPA and HPT axes in individuals with normal thyroid function and insomnia and in healthy controls failed to find a statistically significant difference in TSH levels (2.6 and 2.5 mIU/L, respectively) (20).

In the current study, insomnia symptoms (jointly or separately) were not associated significantly with subclinical hypothyroidism in men, possibly because they are less susceptible to sympathetic hyperexcitability from sleep fragmentation and thus experience fewer endocrine changes than women (37).

As for sleep debt, the results suggest an increased risk of subclinical hypothyroidism among men; nevertheless, this association should be interpreted cautiously given the borderline statistical significance of the results. Sleep debt is a stressor with documented effects on well-being and the endocrine system (38). A recent review showed that a single night of total sleep deprivation can increase TSH levels by up to 200% (39), whereas partial sleep deprivation produces more modest changes (39). Chronic partial sleep deprivation affects thyroid hormones which may cause a negative feedback response and decrease TSH level (39). A study of 32 healthy individuals (16 women) who were sleep deprived for one night noted higher TSH and FT4 levels in both sexes (18). Another interventional study in 118 individuals showed increased TSH and decreased FT4 after three consecutive days of partial sleep deprivation (4 hours/day) with normalization after four days of recovery sleep (17). These studies suggest that the duration of deprivation appears to alter FT4 levels differently.

Short or long sleep duration were not associated with subclinical hypothyroidism in either sex. Individual sleep needs vary, and sleep requirements often decline with age. Adults aged ≥40 appear resilient to shorter sleep durations, generally without hormonal disruption. Unlike our findings, a longitudinal and cross-sectional study using the Pittsburgh Sleep Quality Index found that short (<7 hours) and very short (<5 hours) sleep durations were linked to higher TSH levels (14). Conversely, another cross-sectional study found no higher odds of subclinical hypothyroidism among individuals sleeping <7 hours compared to subjects with normal sleep duration (7–8 hours) although long sleep (>8 hours) increased the odds of subclinical hypothyroidism by 91% (OR: 1.91; 95% CI: 1.03–3.53) (13).

Importantly, there are clear sex differences in sleep characteristics, as captured by both subjective scales or polysomnography and electroencephalography (40). Men and women interpret the quality of their sleep differently. For instance, women report more sleep problems and worse quality of sleep, presenting a higher risk of developing insomnia and thyroid disease than men (27,37,38). Despite women being more likely to complain of insomnia and men reporting short sleep duration more often (37,38), there is still no definitive explanation for why middle insomnia is only associated with subclinical hypothyroidism in women.

The strengths of this study include the large sample size, measurement of thyroid hormones at two time points using third-generation kits, which are more sensitive for detecting very low hormone levels, and the prospective cohort design, allowed for inferences regarding the exposure's effect on outcomes among individuals without baseline subclinical hypothyroidism. In addition, the availability of cohort data on medication use facilitated the exclusion of individuals

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with clinical thyroid diseases, as well as users of drugs that alter thyroid function or psychiatric medication. Despite these strengths, some limitations should be noted, particularly the use of subjective measures for sleep characteristics. Subjective sleep measures are highly susceptible to recall bias and misclassification. Nevertheless, subjective assessment remains the most common approach for measuring sleep characteristics in large cohorts, due to the high cost of objective measures, which are considered the gold standard. Furthermore, among ELSA-Brasil participants, the test-retest reliability of insomnia symptoms and self-reported sleep duration questions demonstrated substantial agreement (30). Lastly, the ELSA-Brasil study population has higher levels of education and income than Brazil's general population, as well as greater access to health services. For our study, this may limit only the generalizability of the findings.

Although the prospective design and control of potential confounders strengthen the robustness of the analysis, the inverse direction of the association suggests the possibility of residual bias or unmeasured factors, such as sleep apnea, which may have influenced the observed results. Future research should further investigate the physiological mechanisms underlying the sex-specific differences observed, particularly considering menopausal status as a potential effect modifier in the associations between sleep characteristics and thyroid function. Moreover, incorporating mechanistic biomarkers, such as serum cortisol, pro- and anti-inflammatory cytokines, and other markers of the HPT axis, could help elucidate the biological pathways linking sleep and hormonal regulation.

Middle insomnia appears to protect women from subclinical hypothyroidism, a finding that warrants further evaluation in future studies. In contrast, sleep debt seems to increase the risk of subclinical hypothyroidism, especially in men. Our study holds significant clinical relevance for public health. Most individuals with subclinical hypothyroidism progress to clinical hypothyroidism within four to five years. Given the uncertainty of signs and symptoms, all risk factors should be identified, mainly due to the increased cardiovascular risk.

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**Data availability:** data from the ELSA-Brasil Study is subject to restricted access.

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original

# The Brazilian Transition in Differentiated Thyroid Carcinoma Management: A 25-Year Nationwide Analysis of Declining High-Activity Radioiodine Use (2000-2024)

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## ABSTRACT

**Objective:** To analyze temporal trends in radioiodine (RAI) prescription patterns for differentiated thyroid carcinoma (DTC) in Brazil from 2000 to 2024, assessing adherence to evolving clinical guidelines and identifying opportunities for practice optimization. **Materials and methods:** This retrospective study utilized data from the Brazilian Unified Health System (Datasus) to evaluate RAI prescriptions, categorized by activity: low (30 and 50 mCi), high (100 and 150 mCi), and very high (200 and 250 mCi). Population-adjusted rates, procedure-adjusted ratios (RAI/oncologic thyroidectomies and RAI/new cases), and activity-level trends were analyzed. **Results:** Three distinct phases emerged: (1) 2000–2007, marked by increasing very high-activity RAI use (≥ 200 mCi); (2) 2008–2015, peak utilization with initial diversification (introduction of 30/50 mCi in 2014); and (3) 2016–2024, significant de-escalation, with high/very high-activity prescriptions declining by 34.2% and low-activity use increasing by 163%. By 2024, RAI distribution comprised 15.3% very high-, 67.1% high-, and 17.6% low-activity prescriptions. The RAI/new cases ratio fell sharply from 0.63 (2010) to 0.25 (2024), and RAI/oncologic thyroidectomies dropped from 1.22 (2013) to 0.70 (2024), reflecting more selective prescription. **Conclusion:** Brazilian medical practice has increasingly aligned with international DTC guidelines, showing a decline in RAI use and a shift toward low-to-high RAI activities. This suggests broader adoption of risk-adapted strategies. Notwithstanding, high-dose RAI still predominates, pointing that dissemination of standardized treatment protocols could further enhance DTC care within the Brazilian health system.

**Keywords**: Differentiated thyroid carcinoma; radioiodine; treatment

## INTRODUCTION

Differentiated thyroid carcinoma (DTC), comprising papillary, follicular and oncocytic subtypes, accounts for most of thyroid malignancies and was responsible for almost one million new cancer diagnoses worldwide in 2025 (1). The cornerstone of treatment for DTC is a multimodal approach, fundamentally centered on surgical resection of the thyroid gland and, when appropriate, cervical lymph node dissection. Radioiodine therapy (RAI) may then be indicated for ablation of remaining tissue, as adjuvant therapy in intermediate/high-risk cases, or as treatment in persistent/recurrent disease (2).

Recent randomized controlled trials have provided high-level evidence supporting the safety of omitting RAI in patients with low-risk DTC. The IoN (3) and ESTIMABL2 (4) trials demonstrated the non-inferiority of surgery without RAI therapy compared with RAI administration regarding recurrence and disease-free survival. In parallel, the 2025 American Thyroid Association (ATA) guidelines highlighted the potential toxicities associated with high RAI

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activities—including salivary gland dysfunction, lacrimal damage, risk of infertility, and secondary malignancies—and recommend careful patient selection and dose optimization to minimize harm (5). Together, these data support the de-escalation of RAI therapy, emphasizing the importance of aligning clinical practice with contemporary evidence to optimize patient outcomes while reducing unnecessary interventions.

A previous study by Schwengber and cols. (2020) provided a comprehensive analysis of RAI utilization patterns in Brazil from 2000 to 2018, revealing a concerning variability in prescription practices and a gradual, yet insufficient, decline in very high/high-activity RAI use (6). Since then, the indications for RAI prescription based on the patient's risk of recurrence have been emphasized. Nonetheless, emerging data on RAI-related cost-effectiveness have reinforced the need for its judicious use (7).

This study analyzes nationwide RAI prescription trends for DTC in Brazil from 2000 to 2024. By extending the timeline beyond our previous work, we seek to evaluate the current state of adherence to risk-adapted guidelines and identify persistent gaps between evidence and practice in the post-operative management of DTC.

## MATERIALS AND METHODS

This retrospective study examined patterns of RAI utilization for DTC management in Brazil from 2000 to 2024, utilizing data from the Brazilian Unified Health System (SUS). The primary data sources included the Department of Informatics of SUS (Datasus, http://datasus.saude.gov.br) from where we extracted annual counts of oncologic thyroidectomies and RAI prescriptions for DTC categorized by activity level: low (30 and 50 mCi), high (100 and 150 mCi), and very high (200 and 250 mCi), as detailed previously (6).

Population estimates for standardization were obtained from the Brazilian Institute of Geography and Statistics (IBGE, https://ibge.gov.br/), while annual projections of new DTC cases were derived from the Brazilian National Cancer Institute (INCA, https://www.inca.gov.br/) using established epidemiological methods.

Briefly, the methodology adopted by INCA for estimating cancer incidence in Brazil is primarily based on projecting data from high-quality Population-Based Cancer Registries (PBCRs) to cover the entire national territory. Since PBCRs do not cover all regions, statistical models, often using mean rates or regression techniques, are employed. These models correlate the available local incidence rates with sociodemographic and geographic indicators from uncapped areas. The projected incidence rates are then applied to the official population projections for the target year, generating the estimated number of new cases for each type of cancer nationwide. This approach enables a comprehensive and reasoned estimate, despite the absence of complete real-world data across all Brazilian regions (8).

The population estimates produced by IBGE are derived via demographic accounting methodology. This approach utilizes the most recent Population Census as a baseline and updates this figure annually by incorporating the components of population change: namely, births and deaths data from the civil registry system, and estimates of net migration, which are modeled using ancillary data sources such as school enrollments, health statistics, and prior demographic trends to project population counts for intercensal periods (9).

To analyze utilization patterns, we calculated population-adjusted rates (total RAI prescriptions per 100,000 inhabitants) and procedure-adjusted rates, including the ratio of RAI prescriptions to oncologic thyroidectomies and the ratio of RAI prescriptions to estimated new DTC cases. Datasus oncologic thyroidectomy data are available from 2008 onward.

Temporal trends in RAI prescription patterns were assessed using segmented (joinpoint) regression analysis applied to log-transformed annual national prescription counts (2000–2024). This approach estimates each segment's annual percent change (APC) and identifies statistically significant inflection points delineating distinct temporal trends. The optimal model was selected according to the Bayesian Information Criterion (BIC). Analyses were performed using R software (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria), and results were verified using the Joinpoint Trend Analysis Software (version 5.4.0, National Cancer Institute, Bethesda, MD, USA).

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The study protocol received ethical approval from the Research Ethics Committee of the *Hospital de Clínicas de Porto Alegre* (CAAE 29670919.9.0000.5327/GPPG 2019-0764) and was conducted in accordance with ethical standards for observational research. The authors verified the accuracy of all data analyses and methodological approaches.

## RESULTS

### Number of DTC RAI prescriptions in Brazil

Analysis of RAI use for DTC in Brazil (2000–2024) revealed significant temporal trends in three key metrics: population-adjusted prescriptions, prescriptions per oncologic thyroidectomy, and prescriptions per new DTC case (**Figure 1**).

Population-adjusted RAI prescriptions demonstrated a consistent upward trajectory from 2000 (0.44 per 100,000 inhabitants) to 2015 (2.30 per 100,000 inhabitants), followed by a gradual decline to 1.43 in 2020 during the COVID-19 pandemic, with subsequent recovery to 2.00 by 2024.

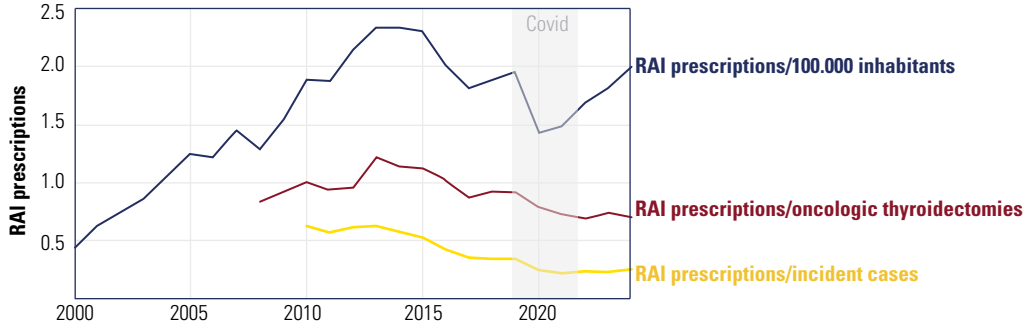
The most clinically informative metrics seems to be those adjusted for disease incidence: RAI prescriptions per oncologic thyroidectomy and per new DTC cases. The ratio of RAI prescriptions to oncologic thyroidectomies, available from 2008 onward, peaked at 1.22 in 2013 before decreasing to 0.70 in 2024 (42.6% reduction), indicating changing clinical practices in postoperative RAI administration. RAI prescriptions per new DTC cases showed a progressive decline from 0.63 in 2010 to 0.23-0.25 recently (2021-2024). As previously shown, the COVID-19 pandemic seems to have accelerated this trend, with the metric dropping sharply to 0.25 in 2020 and remaining at similarly reduced levels thereafter (10).

### RAI activities in Brazil

The analysis of RAI prescription data reveals significant evolution in treatment practices for DTC in Brazil over the 25-year study period. From 2000 to 2007, the early phase was characterized by exclusive use of activities ≥ 100 mCi, with total annual prescriptions increasing steadily from 767 to 2,747 (**Figure 2** and **Supplemental Table 1**). This period had a marked shift toward higher activities, with 200 mCi prescriptions growing 5.5-fold (from 179 to 980 prescriptions per year) and 150 mCi utilization increasing 4.5-fold (from 214 to 969 prescriptions per year).

Between 2008-2015, RAI use peaked while showing early signs of change. Total prescriptions climbed to a maximum of 4,647 in 2013, with very high-activity prescriptions (≥ 200 mCi) accounting for 1,011 in the same year. Notably, 250 mCi use peaked in 2009 (532 prescriptions), while 100 mCi became the most frequently prescribed activity by 2013 (1,886 prescriptions). In 2014, the introduction of low-activity options (30/50 mCi) resulted in 207 total prescriptions, signaling a paradigm shift in therapeutic practice.

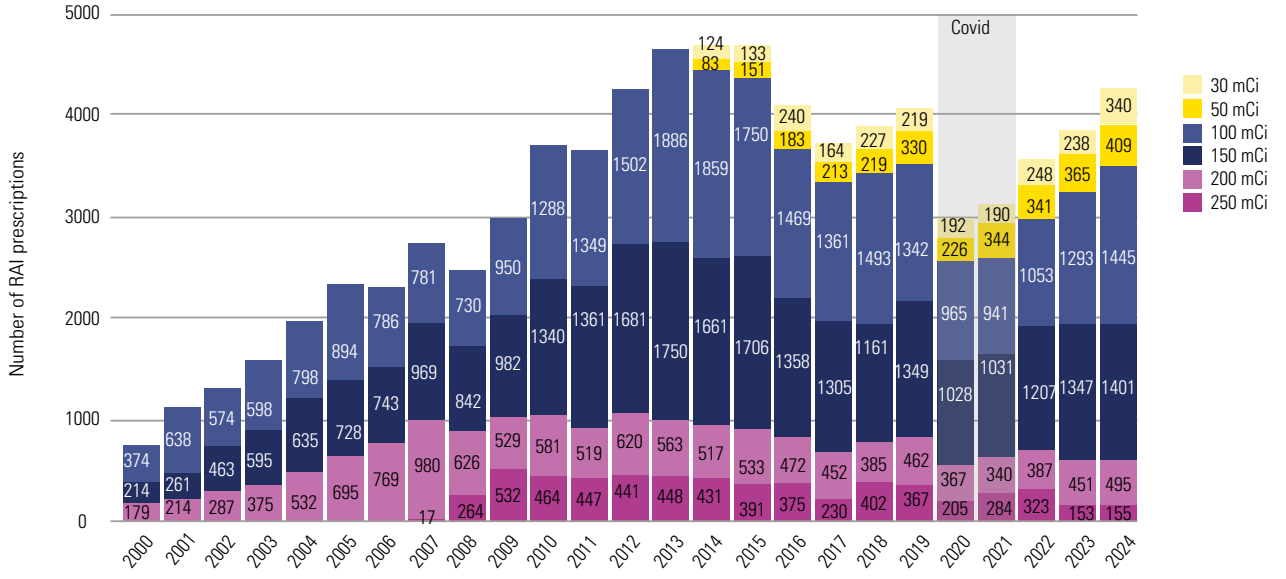
The most recent era (2016-2024) has been characterized by substantial de-escalation and diversification of RAI use. Very high-activity prescriptions (≥ 200 mCi) decreased by 35.7% from 2013 to 2024, while low-activity utilization demonstrated remarkable growth – 50 mCi prescriptions increased by 171% (from 151 in 2015 to 409 in 2024) and 30 mCi




Data sources: DATASUS, INCA and IBGE.

**Figure 1.** Population and thyroid cancer adjusted radioiodine (RAI) prescription trends for differentiated thyroid cancer (DTC), 2000–2024.

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Data source: DATASUS.

**Figure 2.** Radioiodine (RAI) activity prescriptions for differentiated thyroid cancer (DTC) in Brazil (2000–2024).

prescriptions rose by 156% (from 133 to 340) during the same period. The COVID-19 pandemic in 2020 temporarily disrupted this trend, causing a 28.3% single-year decline in total prescriptions, though post-pandemic recovery has maintained the trend toward lower RAI activities, which may be explained by the consolidation of guideline-based de-escalation of RAI and a still short post-pandemic observation period.

Segmented regression analysis objectively confirmed three distinct temporal phases in national RAI utilization. Two statistically significant joinpoints were identified in 2007 and 2015, corresponding to the onset of early diversification and subsequent de-escalation of RAI prescriptions. From 2000 to 2007, total RAI prescriptions increased markedly (APC = +17.2% per year, $p < 0.01$). Between 2008 and 2015, the rate of increase slowed but remained positive (APC = +4.1% per year, $p = 0.02$). From 2016 to 2024, a statistically significant decline was observed (APC = −3.9% per year, $p < 0.01$), consistent with the national adoption of selective, risk-adapted RAI practices. These findings quantitatively reinforce the descriptive three-phase narrative and demonstrate that the post-2015 reduction represents a true temporal inflection in Brazil's RAI prescribing behavior.

Current prescription patterns in 2024 reflect a balanced utilization across activity levels, with very high-activities (200/250 mCi) accounting for 15.3% of prescriptions, high-activities (100/150 mCi) comprising 67.1%, and low-activities (30/50 mCi) making up 17.6% of total utilization (**Figure 2**). This evolution likely reflects the progressive adoption of risk-adjusted strategies, with more varied RAI activities and reduced dependence on very high/high-dose regimens. The rise of low-activity use since 2014 reflects Brazil's alignment with international guidelines favoring more selective RAI application in DTC.

## DISCUSSION

Our nationwide analysis of RAI utilization patterns in Brazil from 2000 to 2024 reveals a significant transformation in DTC management, demonstrating the gradual adoption of up-to-date, evidence-based practices and persistent challenges in standardizing care. The trends reflect an important paradigm shift from routine to selective RAI use, though opportunities for further optimization remain.

Data from the United States and Europe illustrate a clear global trend toward de-escalating RAI for DTC, yet its adoption varies significantly by region (11,12). As observed in Brazil, the implementation of these

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protocols is often delayed, demonstrating that translating evidence-based guidelines into practice is highly dependent on local healthcare systems and context.

The most striking finding is the substantial decline in very high-activity RAI prescriptions (≥ 200 mCi), which decreased by 35.7% between 2013 and 2024, accompanied by a remarkable 163% average increase in low-activity (30-50 mCi) utilization during the same period. This evolution likely reflects the growing awareness of the thyroid cancer guidelines, reinforcing evidence of comparable outcomes with lower activities for low-risk DTC, while minimizing adverse effects and healthcare costs. This decline may also reflect the growing proportion of low-risk DTC cases recently, naturally reducing the indication for adjuvant RAI (13). The COVID-19 pandemic also led to an accelerated decline in RAI prescriptions, further reinforcing the viability of a more selective use of RAI therapy (10).

Our data reveal three distinct phases in Brazilian practice: (i) an early period (2000-2007) of rapidly increasing very high-activity utilization, (ii) a transitional phase (2008-2015) in which prescription patterns began diversifying, and (iii) the current era (2016-2024) characterized by progressive de-escalation. The introduction of low-activity options in 2014 marked a critical inflection point, with these regimens now comprising 17.6% of total prescriptions in 2024, which is a development that aligns with international trends toward individual patient risk-adapted therapy. These phases align closely with the emergence of evidence and guidelines advocating for more selective, risk adapted RAI therapy. The publication of 2009 American Thyroid Association (ATA) guidelines represented an initial paradigm shift, recommending against routine RAI for low-risk tumors (14). Subsequently randomized controlled trials in 2012, including the HiLo trial (UK) and the ESTIMABL1 trial (France), demonstrated non-inferiority of low-activity RAI (1.1 GBq or 30 mCi) compared to standard high-activity doses (3.7 GBq or 100 mCi) for remnant ablation in low-risk patients (15,16). These trials provided robust evidence supporting dose de-escalation. The 2015 ATA guidelines further reinforced this risk-stratified approach (2). The progressive adoption of these evidence-based recommendations in Brazil demonstrates successful translation of international clinical trial data and evolving consensus guidelines into local practice patterns.

Nevertheless, several challenges persist. The continued predominance of high-activities (67.1% of prescriptions) suggests potential over-treatment of low-risk patients, while the ongoing use of very high activities (15.3%) may reflect either appropriate management of high-risk cases or lingering adherence to outdated practices. These patterns likely stem from multiple factors, including regional variations in healthcare system structure and individual clinical practice (17).

The impact of reduced RAI use on oncologic outcomes remains a key consideration. Although this study cannot directly evaluate recurrence or mortality, long-term data from the HiLo and ESTIMABL1 trials suggest that low-dose RAI achieves similar ablation success and recurrence prevention compared to high-dose RAI in low- and intermediate-risk DTC, with no observed increase in recurrence or mortality (15,16). For high-risk DTC, however, RAI likely continues to provide a survival and recurrence benefit. A large multicenter prospective study of nearly 3,000 patients demonstrated improved overall survival and lower recurrence rates in high-risk patients receiving RAI (18), and national database analyses have shown that RAI remains protective for cancer-specific mortality primarily in high-risk subgroups (19).

The persistence of high-dose RAI (67.1% in 2024) may reflect past prescribing habits and heterogeneous adoption of guidelines across Brazilian regions. As shown in **Supplemental Table 2**, in the Brazilian Unified Health System (SUS), the remuneration for inpatient RAI therapy increases with administered activity, with distinct codes and higher payments for 100 to 250 mCi compared to lower doses (30 and 50 mCi), which are classified as ambulatory procedures with substantially lower reimbursement values. According to these data, for example, considering only the direct RAI costs, changing the prescription from 100 to 30 mCi represents a saving of R$ 628.20 per patient. Addressing these financial and behavioral drivers may assist a more uniform adoption of

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evidence-based, risk-adapted RAI therapy across Brazil. Notwithstanding, these economic factors were not assessed in this study.

The study's findings have important policy implications for SUS. The reduction in RAI/new case ratio (from 0.63 to 0.25) suggests opportunities for significant cost savings via continued optimization of RAI use. While our findings are descriptive, they highlight the need for a national DTC registry to evaluate the long-term impact of these practice changes and to guide future RAI utilization policies and research in Brazil.

Our findings should be interpreted considering two main limitations: the use of administrative data and ecological design. These preclude analysis of individual-level clinical factors (e.g., tumor stage) and regional practice variations. Further studies are needed to correlate these utilization trends with patient outcomes and assess the economic consequences of practice pattern changes.

In conclusion, our findings demonstrate substantial progress in aligning Brazilian DTC management with international standards, while highlighting remaining opportunities for improvement. The documented evolution toward more selective RAI use reflects successful knowledge translation, though continued efforts are needed to ensure equitable adoption of evidence-based practices across all regions and healthcare settings. The results underscore the importance of ongoing monitoring and quality improvement initiatives to optimize thyroid cancer care in the Brazilian Unified Health System.

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**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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## SUPPLEMENTARY MATERIALS

**Supplemental Table 1.** Number of radioiodine activities prescribed, and oncologic thyroidectomies performed in Brazil from 2000 to 2024

| Year | Radioiodine therapy for Differentiated Thyroid Cancer | | | | | | Oncologic Thyroidectomies |
|---|---|---|---|---|---|---|---|
| | 30 mCi | 50 mCi | 100 mCi | 150 mCi | 200 mCi | 250 mCi | All |
| 2000 | - | - | 374 | 214 | 179 | - | - |
| 2001 | - | - | 638 | 261 | 214 | - | - |
| 2002 | - | - | 574 | 463 | 287 | - | - |
| 2003 | - | - | 598 | 595 | 375 | - | - |
| 2004 | - | - | 798 | 635 | 532 | - | - |
| 2005 | - | - | 894 | 728 | 695 | - | - |
| 2006 | - | - | 786 | 743 | 769 | - | - |
| 2007 | - | - | 781 | 969 | 980 | 17 | - |
| 2008 | - | - | 730 | 842 | 626 | 264 | 2,938 |
| 2009 | - | - | 950 | 982 | 529 | 532 | 3,268 |
| 2010 | - | - | 1,288 | 1,340 | 581 | 464 | 3,665 |
| 2011 | - | - | 1,349 | 1,361 | 519 | 447 | 3,920 |
| 2012 | - | - | 1,502 | 1,681 | 620 | 441 | 4,424 |
| 2013 | - | - | 1,886 | 1,750 | 563 | 448 | 3,814 |
| 2014 | 124 | 83 | 1,859 | 1,661 | 517 | 431 | 4,094 |
| 2015 | 133 | 151 | 1,750 | 1,706 | 533 | 391 | 4,153 |
| 2016 | 240 | 183 | 1,469 | 1,358 | 472 | 375 | 4,030 |
| 2017 | 164 | 213 | 1,361 | 1,305 | 452 | 230 | 4,260 |
| 2018 | 227 | 219 | 1,493 | 1,161 | 385 | 402 | 4,210 |
| 2019 | 219 | 330 | 1,342 | 1,349 | 462 | 367 | 4,439 |
| 2020 | 192 | 226 | 965 | 1,028 | 367 | 205 | 3,785 |
| 2021 | 190 | 344 | 941 | 1,031 | 340 | 284 | 4,287 |
| 2022 | 248 | 341 | 1,053 | 1,207 | 387 | 323 | 5,109 |
| 2023 | 238 | 365 | 1,293 | 1,347 | 451 | 153 | 5,210 |
| 2024 | 340 | 409 | 1,445 | 1,401 | 495 | 155 | 6,034 |

Data source: TABNET/DATASUS (tabnet.datasus.gov.br)

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**Supplemental Table 2.** Unified Health System Thyroid Cancer Radioiodine Therapy Activities and Oncological Thyroidectomy Procedures Codes

| Procedure | Datasus Code(s) | Period of Code Validity | Reimbursement Values (in Brazilian reais) |
|---|---|---|---|
| Radioiodine therapy for DTC 30 mCi | 03.04.09.005-0 | From Feb/2014 to present | R$ 443.70 |
| Radioiodine therapy for DTC 50 mCi | 03.04.09.006-9 | From Feb/2014 to present | R$ 614.70 |
| Radioiodine therapy for DTC 100 mCi | 03.04.09.002-6<br>85.300.888 and 85.500.887 | From Jan/2008 to present<br>From Nov/1999 to Dec/2007 | R$ 1,071.90 |
| Radioiodine therapy for DTC 150 mCi | 03.04.09.001-8<br>85.300.900 and 85.500.909 | From Jan/2008 to present<br>From Nov/1999 to Dec/2007 | R$ 1,289.90 |
| Radioiodine therapy for DTC 200 mCi | 03.04.09.003-4<br>85.300.926 and 85.500.925 | From Jan/2008 to present<br>From Nov/1999 to Dec/2007 | R$ 1,471.32 |
| Radioiodine therapy for DTC 250 mCi | 03.04.09.004-2<br>03.03.12.001-0 | From Jul/2008 to present<br>From Jan/2008 to Jun/2008 | R$ 1,810.32 |
| Total thyroidectomy in oncology | 0416030270<br>0416030130 | From 2013 to present<br>From Aug/2008 to 2013 | R$ 2,836.30 |
| Total thyroidectomy with lymph node resection | 0416030122<br>0402010051 | From Jan/2008 to 2013<br>From Jan/2008 to present | R$ 767.77 |
| Transsternal thyroid tumor resection in oncology | 0416030360<br>0416030050 | From 2013 to present<br>From Jan/2008 to 2013 | R$ 4,186.64 |

Data source: DATASUS.
Data source: TABNET/DATASUS (tabnet.datasus.gov.br).
DTC: differentiated thyroid cancer.

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original

# Prognostic factors in the surgical treatment of non-functioning pituitary adenomas: a retrospective cohort study

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## ABSTRACT

**Objective:** This study aimed to analyze preoperative, intraoperative, and postoperative factors that affect the surgical prognosis of non-functioning pituitary adenomas (NFPA) and to determine postoperative complication rates. **Materials and methods:** We conducted a retrospective cohort study of patients with NFPA who underwent surgery between 1995 and 2024 at a tertiary public hospital in Brazil. Variables analyzed included tumor size, cavernous sinus invasion (Knosp classification), endocrinological status, preoperative clinical features, surgical complications, and outcomes. Statistical significance was set at $p \leq 0.05$. **Results:** Seventy-three patients were included, with a mean age of 53 years and a slight predominance of females. Tumors were classified as macroadenomas (59%) and giant adenomas (41%). The transsphenoidal approach was used in 81% of cases. Partial resection was achieved in 56%, subtotal in 16%, and total in 27%. Immediate postoperative complications included diabetes insipidus (30%), bleeding (11%), hydrocephalus (10%), ischemia (10%), meningitis (6%), and cerebrospinal fluid fistula (11%). Mortality was 9.6%, significantly associated with postoperative hydrocephalus, ischemia, and larger tumor size. Giant tumors were correlated with higher rates of preoperative neurological deficits and postoperative complications. Transcranial surgery was more frequently performed in cases of giant adenomas and was associated with increased rates of ischemia and neurological deficits. Tumor recurrence was observed in 33% of patients over a mean follow-up of 48 months. Tumor size and postoperative complications such as hydrocephalus and ischemia were associated with increased morbidity and mortality. **Conclusion:** Postoperative hydrocephalus, ischemia, and tumor size are key determinants of mortality in NFPA surgical treatment. Implementing preventive and management strategies targeting these complications could improve patient outcomes, albeit rigorous long-term follow-up is essential due to the high rates of recurrence and reoperation.

**Keywords:** Pituitary neoplasms; nonfunctioning pituitary adenoma; transsphenoidal surgerypostoperative complications; prognostic factors

## INTRODUCTION

Non-functioning pituitary adenomas (NFPA) account for 22%-54% of all pituitary adenomas and are characterized by the absence of clinical signs of hormonal hypersecretion (1). The main symptoms of NFPA result from mass effects, such as visual impairment from optic chiasm compression, headache (2,3), and apoplexy (4,5), as well as hormonal deficiencies caused by compression of the adenohypophysis (6,7). Because of their indolent growth and lack of overt endocrine hypersecretion, NFPAs are often diagnosed at a late stage, frequently when tumors have reached substantial size (8).

The preferred treatment for NFPA is transsphenoidal surgery via the endoscopic approach with microscopy, which offers higher rates of tumor resection (1,9,10). Multiple clinical, radiological, and surgical variables have been proposed as predictors of prognosis. Tumor size, cavernous sinus invasion, and extent of resection are consistently highlighted as primary

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determinants of recurrence risk. Giant non-functioning adenomas (>4 cm) pose greater surgical challenges, require specific approaches, and are associated with higher morbidity (11,12). Although gross total resection is associated with better tumor control, it may also increase the risk of new hypopituitarism or surgical complications. The postoperative period is crucial for patient quality of life, which emphasizes the need to evaluate factors influencing surgical prognosis and associated complications (13,14).

The surgical prognosis of pituitary adenomas is influenced by various clinical factors, including age, tumor size, invasiveness, hormonal activity, and the extent of resection. Transsphenoidal surgery remains the standard approach, with gross total resection resulting in improved outcomes and lower recurrence rates. Nevertheless, recurrence may still occur, particularly in non-functioning adenomas or those with cavernous sinus invasion (15). Although researchers have addressed outcomes after NFPA surgery (16,17), most have emphasized recurrence or long-term tumor control, with factors directly associated with surgical success being less clearly defined.

To address this gap, this retrospective cohort study aimed to analyze the main factors related to surgical success of non-functioning pituitary adenoma treatment. Specifically, we sought to identify preoperative, intraoperative, and postoperative predictors of complete resection and functional outcomes, as well as to determine the rate of surgical complications in relation to clinical and surgical variables.

## MATERIALS AND METHODS

### Study design and setting

This retrospective cohort study is reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (18). Data were obtained from the Neurosurgery Service at Hospital das Clínicas, Faculty of Medicine of Botucatu, a tertiary referral center in Brazil. The study was approved by the Research Ethics Committee of the same institution (CAAE no. 71548323.4.0000.5411). Given its retrospective nature, the requirement for informed consent was waived in accordance with national regulations.

### Participants

We reviewed the medical records of all patients diagnosed with NFPA and who underwent surgical treatment between 1995 and 2024.

### Variables

The following variables were extracted and analyzed:

- Preoperative imaging findings: tumor size and cavernous sinus invasion, with the latter classified according to the Knosp classification (19).
- Endocrinological assessment: including the presence of panhypopituitarism.
- Preoperative clinical presentation: with emphasis on visual loss.
- Surgical complications: including cerebrospinal fluid fistula, meningitis, diabetes insipidus, worsening of visual status, and bleeding in the tumor bed.
- Unfavorable surgical outcomes: defined as incomplete tumor resection or procedure-related mortality.

### Data sources and measurement

All data were obtained from institutional medical records. Data extraction was performed by trained researchers using a standardized protocol to ensure consistency.

### Bias and study size

As a retrospective study, potential sources of bias included variability in the completeness of medical records and the extended follow-up period. To mitigate this, only patients with complete preoperative, surgical, and postoperative data were included.

### Statistical analysis

Categorical variables were summarized as absolute and relative frequencies, and comparisons were made using the chi-square or Fisher's exact test, as appropriate. A $p$-value of ≤ 0.05 was considered statistically significant. Analyses were performed using Statistical Package for the Social Sciences (SPSS), version 24.0, for MacBook (IBM Corp., Armonk, NY, USA).

## RESULTS

### Participants and descriptive data

A total of 73 patients met the eligibility criteria and were included in the analysis (**Figure 1**). The mean age

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was 52.99 years (standard deviation [SD] ±13.41). Of these, 39 were women (53.4%) and 34 were men (46.6%). Regarding race, 60 patients (82.2%) were white, 10 (13.7%) were brown, and 3 (4.1%) were black. Comorbidities included systemic arterial hypertension in 27 patients (37.0%), diabetes mellitus in 8 (11.0%), dyslipidemia in 14 (19.2%), smoking in 16 (21.9%), and alcoholism in 6 (8.2%).

## Preoperative clinical characteristics

Preoperative neurological conditions included oculomotor nerve (cranial nerve III) paresis in 4 patients (5.5%), hydrocephalus in 4 (5.5%), mental confusion in 3 (4.1%), and headache in 28 (38.4%). Visual complaints were frequent, with hemianopsia reported in 49 patients (67.1%) and amaurosis in 16 (21.9%). Pituitary apoplexy and menstrual alterations were each observed in 10 patients (13.7%). **Figure 1** shows pituitary apoplexy on a T1-weighted magnetic resonance image.

## Preoperative endocrinological status

Endocrinological assessment revealed hyperprolactinemia (based on diluted prolactin measures) in 16 patients (21.9%), panhypopituitarism in 28 (38.4%), hypocortisolism in 13 (17.8%), hypogonadism in 18 (24.7%), and hypothyroidism in 25 (34.2%).

## Tumor characteristics

Of the cohort, 43 patients (58.9%) had macroadenomas and 30 (41.1%) had giant adenomas. Cavernous sinus invasion was graded by the Knosp classification: grade 1 in 8 tumors (11.0%), grade 2 in 16 (21.9%), grade 3a in 12 (16.4%), grade 3b in 2 (2.7%), and grade 4 in 18 (24.7%). **Figure 2** shows a macroadenoma, while **Figure 3** depicts a giant adenoma of the pituitary gland.

## Surgical approach and extent of resection

The primary surgical approach was transsphenoidal, performed in 59 patients (80.8%). Transcranial surgery was conducted in 14 cases (19.2%), comprising 6 pterional, 7 fronto-orbitozygomatic, and 1 transcallosal approach. The extent of tumor resection was partial in 41 patients (56.2%), subtotal in 12 (16.4%), and total in 20 (27.4%).

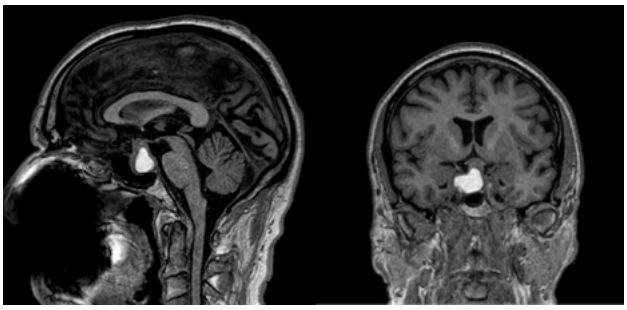


**Figure 1.** Sagittal and coronal views of T1 MRI without gadolinium showing an apoplexy of a pituitary macroadenoma.

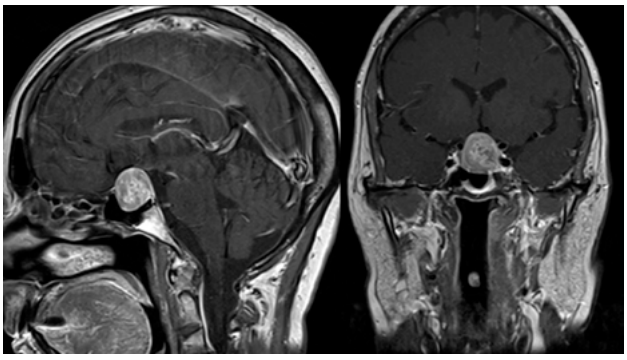


**Figure 2.** Sagittal and coronal views of T1 MRI with gadolinium showing a pituitary macroadenoma.

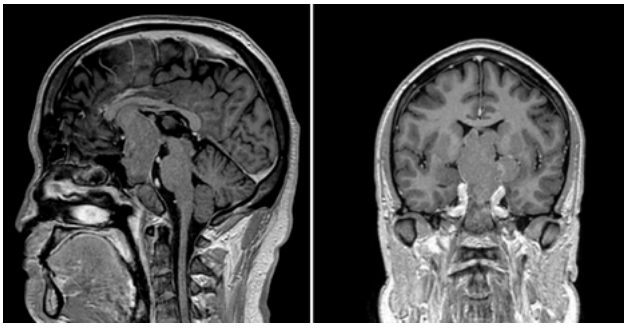


**Figure 3.** Sagittal and coronal views of T1 MRI with gadolinium showing a pituitary giant adenoma.

## Postoperative complications

Immediate postoperative complications included diabetes insipidus in 22 patients (30.1%), syndrome of inappropriate antidiuretic hormone secretion in 6 (8.2%), tumor bed bleeding in 8 (11.0%), hydrocephalus in 7 (9.6%), meningitis in 4 (5.5%), cerebral ischemia in 7 (9.6%), and cerebrospinal fluid fistula in 8 (11.0%). Persistent diabetes insipidus was observed in 12 patients (16.4%), and postoperative panhypopituitarism developed in 26 (35.6%).

## Functional outcomes and late deficits

Visual improvement was observed in 36 patients (49.3%). Late postoperative neurological deficits

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included 5 patients (6.8%) becoming bedridden, 2 (2.7%) experiencing visual deterioration, 2 (2.7%) with hemiparesis, and 2 (2.7%) developing dementia.

## Follow-up and long-term outcomes

During a mean follow-up of 48 months (range: 3–276 months), tumor recurrence occurred in 24 patients (32.9%), reoperation was required in 14 (19.2%), and radiotherapy was administered in 5 (6.8%). There were 7 deaths recorded, corresponding to a mortality rate of 9.6%. Table 1 systematically presents these results. Among significant statistical associations, mortality was associated with postoperative hydrocephalus ($p < 0.001$), postoperative ischemia ($p = 0.012$), and tumor size ($p = 0.017$), with higher mortality among patients with giant tumors. Tumor size significantly correlated with preoperative oculomotor paresis ($p = 0.05$), preoperative hydrocephalus ($p = 0.05$), and preoperative hyperprolactinemia ($p = 0.048$), being lower in giant adenomas compared to macroadenomas. Transcranial surgery was associated with giant tumors ($p = 0.015$), postoperative ischemia ($p = 0.022$), and postoperative deficits ($p = 0.031$). The occurrence of postoperative cerebrospinal fluid fistula was associated with meningitis ($p = 0.003$). Table 2 lists these significant statistical associations.

**Table 1.** General results

| Parameter | Result |
|---|---|
| Number of patients evaluated (n) | 73 |
| Mean age (years) | 52.99 ± 13.41 |
| Sex (n, %) | |
| Women | 39 (53.4%) |
| Men | 34 (46.6%) |
| Race (n, %) | |
| White | 60 (82.2%) |
| Brown | 10 (13.7%) |
| Black | 3 (4.1%) |
| Comorbidities (%) | |
| Hypertension | 37.0% |
| Diabetes mellitus | 11.0% |
| Dyslipidemia | 19.2% |
| Smoking | 21.9% |
| Alcoholism | 8.2% |
| Preoperative neurological conditions (%) | |
| Oculomotor nerve paresis | 5.5% |
| Hydrocephalus | 5.5% |
| Mental confusion | 4.1% |
| Headache | 38.4% |
| Visual complaints (%) | |
| Hemianopsia | 67.1% |
| Amaurosis | 21.9% |
| Other preoperative symptoms (%) | |
| Pituitary apoplexy | 13.7% |
| Menstrual alterations | 13.7% |
| Preoperative endocrinological status (%) | |
| Hyperprolactinemia | 21.9% |
| Pan-hypopituitarism | 38.4% |
| Hypercortisolism | 17.8% |
| Hypogonadism | 24.7% |
| Hypothyroidism | 34.2% |
| Tumor size classification (%) | |
| Macroadenomas | 58.9% |
| Giant adenomas | 41.1% |
| Cavernous sinus invasion (Knosp classification) (%) | |
| Grade 1 | 11.0% |
| Grade 2 | 21.9% |
| Grade 3a | 16.4% |
| Grade 3b | 2.7% |
| Grade 4 | 24.7% |
| Surgical approach (%) | |
| Transsphenoidal | 80.8% |
| Transcranial | 19.2% |
| Types of transcranial approaches (n) | |
| Pterional | 6 |
| Fronto-orbitozygomatic | 7 |
| Transcallosal | 1 |
| Tumor resection extent (%) | |
| Partial | 56.2% |
| Subtotal | 16.4% |
| Total | 27.4% |
| Immediate postoperative complications (%) | |
| Diabetes insipidus | 30.1% |
| Syndrome of inappropriate antidiuretic hormone secretion | 8.2% |
| Bleeding | 11.0% |
| Hydrocephalus | 9.6% |
| Meningitis | 5.5% |
| Ischemia | 9.6% |
| Fistula | 11.0% |
| Persistent complications (%) | |
| Persistent diabetes insipidus | 16.4% |
| Postoperative panhypopituitarism | 35.6% |
| Visual improvement (n, %) | 36, 49.3% |
| Late postoperative deficits (%) | |
| Bedridden | 6.8% |
| Visual worsening | 2.7% |
| Hemiparesis | 2.7% |
| Dementia | 2.7% |
| Late follow-up outcomes (%) | |
| Tumor recurrence | 32.9% |
| Reoperation | 19.2% |
| Radiotherapy | 6.8% |
| Mortality (n, %) | 7, 9.6% |
| Mean follow-up duration | 48 months (range: 3–276 months) |

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**Table 2.** Significant statistical associations

| Variable | Mortality | | *p*-value |
|---|---|---|---|
| | Mortality (yes) | Mortality (no) | |
| Hydrocephalus (postoperative) (yes) | 5 (71.4%) | 2 (28.6%) | < 0.001 |
| Hydrocephalus (postoperative) (no) | 2 (3.3%) | 59 (96.7%) | |
| Ischemia (postoperative) (yes) | 3 (50%) | 3 (50%) | 0.012 |
| Ischemia (postoperative) (no) | 4 (6.5%) | 58 (93.5%) | |
| Tumor size (giant adenomas) | 6 (21.4%) | 22 (78.6%) | 0.017 |
| Tumor size (macroadenomas) | 1 (2.5%) | 39 (97.5%) | |
| **Variable** | **Surgery type** | | ***p*-value** |
| | **Transsphenoidal** | **Transcranial** | |
| Tumor size (macroadenomas) | 39 (90.7%) | 4 (9.3%) | 0.015 |
| Tumor size (giant adenomas) | 20 (66.7%) | 10 (33.3%) | |
| Ischemia (no) | 56 (94.9%) | 10 (71.4%) | 0.022 |
| Ischemia (yes) | 3 (5.1%) | 4 (28.6%) | |
| Postoperative deficit (no) | 53 (89.85) | 9 (64.3%) | 0.031 |
| Postoperative deficit (yes) | 6 (10.15%) | 5 (35.7%) | |
| **Variable** | **Meningitis** | | ***p*-value** |
| | **Meningitis (no)** | **Meningitis (yes)** | |
| Cerebrospinal fluid fistula (no) | 64 (98.5%) | 1 (1.5%) | 0.003 |
| Cerebrospinal fluid fistula (yes) | 5 (62.5%) | 3 (37.5%) | |

## DISCUSSION

This study analyzed 73 patients with NFPA who underwent surgery, identifying key determinants of prognosis and outcomes. The cohort included 59% macroadenomas and 41% giant adenomas, with the transsphenoidal approach used in 81% of cases. Partial resection was most frequent (56%), and postoperative complications were common, including diabetes insipidus (30%), hydrocephalus (9.6%), ischemia (9.6%), cerebrospinal fluid fistula (11%), and meningitis (5.5%). The mortality rate was 9.6%, significantly associated with postoperative hydrocephalus, ischemia, and larger tumor size. Giant adenomas were linked to higher rates of preoperative neurological deficits and postoperative complications; transcranial surgery was also more frequent in these patients and associated with increased ischemia and neurological deficits. Tumor recurrence occurred in 33% of cases over an average follow-up of 48 months. These data underscore tumor size and severe postoperative complications as critical determinants of surgical prognosis in NFPA.

These results provide a comprehensive analysis of the demographic, clinical, neurological, endocrinological, and tumor characteristics, as well as surgical interventions, postoperative complications, and outcomes of patients with non-functioning pituitary macroadenomas. The findings corroborate the need for guidelines that emphasize preoperative endocrinological evaluation even in silent NFPA (8), ophthalmological assessment (20), complex surgical approaches when needed (21,22), and rigorous postoperative follow-up of these patients (23,24).

The results provide a comprehensive view of non-functioning pituitary macroadenomas, highlighting clinical and surgical patient characteristics, immediate and late complications, and outcomes, thereby enhancing our understanding of the condition and guiding clinical and surgical management. Evidence has pointed to high morbidity and mortality in NFPA surgery, evidencing prognostic factors such as cerebrovascular disease (25), prolonged glucocorticoid use (26), panhypopituitarism (27,28), and patient age (29). Females and younger patients (30), as well as tumor size (31), have also been associated with higher morbidity and mortality. Similarly, researchers have indicated predictors of postoperative evolution in these tumors regarding tumor recurrence (resection degree and Knosp classification) (32), cerebrospinal fluid fistula (tumor size and

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local invasion) (33), and visual recovery (integration of clinical and imaging findings) (34).

The occurrence of hydrocephalus has been reported in some studies (35,36), and in our series, postoperative hydrocephalus showed a strong association with mortality. Hydrocephalus may result from cerebrospinal fluid obstruction due to surgery or tumor mass itself, leading to increased intracranial pressure and severe neurological complications. This observation suggests that postoperative hydrocephalus identifies patients at higher risk of adverse outcomes, emphasizing the need for strict surveillance and prompt interventions to manage intracranial pressure and prevent fatal complications.

Postoperative cerebral ischemia was also significantly associated with mortality. Ischemia can result from vascular compromise during surgery, especially in cases with large tumors or cavernous sinus invasion, leading to serious neurological deficits and poorer prognosis (25). This finding highlights the importance of meticulous surgical planning and intraoperative management to minimize ischemic risk, as along with intensive neurological monitoring after surgery (12).

Mortality was higher in patients with giant adenomas compared to those with macroadenomas. Larger tumors present greater surgical complexity, more extensive invasion of adjacent structures (as seen in the high grades of Knosp cavernous sinus invasion classification), and increased risk of postoperative complications (e.g., hydrocephalus and ischemia). Tumor size, particularly with suprasellar extension, is a relevant prognostic factor, associating not only with mortality but also with additional severe complications (31,37,38).

Apart from mortality, tumor size was significantly associated with preoperative oculomotor nerve paresis ($p = 0.05$), preoperative hydrocephalus ($p = 0.05$), and preoperative hyperprolactinemia ($p = 0.048$). Notably, hyperprolactinemia, as reported in NFPA (39), occurred less frequently in giant adenomas than in macroadenomas, possibly due to differences in functional activity or pituitary tissue compression. These findings indicate that larger tumors exert a greater neurological and structural impact before surgery, reflecting their aggressiveness and extent.

Transcranial surgery was performed more frequently in giant tumors ($p = 0.015$) and was associated with a higher incidence of ischemia ($p = 0.022$) and postoperative deficits ($p = 0.031$). This reinforces the greater technical challenge and higher risks inherent in more invasive approaches, which are required for larger and more extensive tumors (22). The elevated risk of neurological and ischemic complications contributes to increased mortality and morbidity in this subgroup.

Postoperative cerebrospinal fluid fistula (33) was significantly associated with meningitis, a serious complication that can negatively impact recovery and increase mortality risk. This finding emphasizes the need for surgical techniques that minimize the incidence of fistulas and for stringent protocols to prevent and manage infections.

Statistically significant associations revealed that mortality in patients with non-functioning pituitary macroadenomas is strongly related to severe postoperative neurological complications such as hydrocephalus and ischemia and tumor size, which influences surgical complexity and the risk of such complications. This evidence indicates that the management of these patients must prioritize careful risk assessment, intensive postoperative monitoring to detect complications such as hydrocephalus and ischemia early, and strategies to minimize harm, especially in cases of giant tumors. Additionally, the association between cerebrospinal fluid fistula and meningitis reinforces the importance of preventing infectious complications, which significantly worsen prognosis and increases mortality. Given the high rates of tumor recurrence and reoperation, long-term follow-up must be integral to patient management (23,24,32). Conservative treatment of NFPA is reserved for small, asymptomatic tumors that must be closely monitored through imaging and endocrinological controls (40,41). After all, the smaller the tumor, the better the surgical outcome.

The main limitations of this study include its retrospective, single-center design, which may introduce selection bias and limit generalizability. Additionally, the relatively small sample size of 73 patients over an extended period (1995–2024) may have affected the statistical power for detecting certain associations.

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Heterogeneity in surgical approaches and advances in techniques over nearly three decades may have also impacted outcomes and were not fully controlled for. Some postoperative complications and long-term outcomes may have also been underreported due to variable follow-up durations (ranging from 3 to 276 months). Collectively, these factors may have restricted the ability to draw definitive conclusions regarding prognostic factors in non-functioning pituitary adenoma surgery.

The long-term follow-up (mean, 48 months; range: 3–276 months) allowed detailed assessment of tumor recurrence and late complications, which is critical given the indolent nature and late diagnosis of NFPAs. This study's comprehensive evaluation of clinical, endocrinological, radiological, and surgical factors over nearly three decades at a tertiary referral center provides valuable institutional data on surgical outcomes and risk factors in a Brazilian population, which may have unique epidemiological characteristics. The inclusion of a relatively large and demographically diverse cohort with detailed complication assessment reinforces the value of this report as it addresses knowledge gaps in perioperative predictors of surgical success and mortality in NFPA patients.

Clinically, identifying tumor size and severe postoperative complications such as hydrocephalus and ischemia as key predictors of mortality can improve preoperative risk stratification. These findings support intensifying perioperative monitoring, especially in patients with giant adenomas, to rapidly detect and address complications (e.g., hydrocephalus and ischemia), potentially reducing morbidity and mortality. Preventive measures targeting cerebrospinal fluid fistula formation and meningitis should be emphasized in surgical and postoperative care protocols. Early diagnosis through endocrinological and ophthalmological evaluation may facilitate detection of smaller tumors, which are associated with better surgical outcomes, advocating for improved screening strategies. Moreover, our findings underscore the need for individualized surgical planning, balancing gross total resection against the risk of hypopituitarism and complications, especially when considering transcranial approaches for large tumors. Long-term follow-up is essential due to high recurrence and reoperation rates, influencing postoperative surveillance guidelines. In summary, this study advances understanding of NFPA by detailing how tumor size and critical postoperative complications influence mortality and surgical outcomes, and underscores refined perioperative risk assessment, preventive strategies for complications, and early detection to improve clinical management and patient prognosis.

Regarding management protocols for complications, our approach to cerebrospinal fluid fistula involved prompt identification through clinical and imaging evaluation, followed by initial conservative measures, including bed rest and head elevation. If conservative management failed, surgical repair was performed using multilayer reconstruction techniques with autologous tissue grafts, with lumbar drainage reserved for persistent cases. Postoperative hydrocephalus was managed by close neurological monitoring and imaging, with ventricular drainage or ventriculoperitoneal shunting employed in patients with symptomatic hydrocephalus or increased intracranial pressure. Cerebral ischemia was addressed via intensive neurological monitoring and supportive care, including optimization of cerebral perfusion and prevention of secondary insults, while surgical planning aimed to minimize vascular injury, particularly in cases with cavernous sinus invasion. Diabetes insipidus was managed with fluid and electrolyte monitoring, desmopressin as needed, and patient education, with persistent diabetes insipidus cases followed long-term with endocrinological support.

Over the years, preventive strategies have included adopting endoscopic transsphenoidal surgery (used in 81% of cases) due to its superior visualization, aiding tumor resection and skull base reconstruction, complemented by microsurgical techniques. To prevent cerebrospinal fluid fistula leaks, multilayer closure methods were employed, including vascularized nasoseptal flaps when feasible, fat grafts, and synthetic dural substitutes. Prophylactic lumbar drainage was selectively applied in high-risk cases with extensive dural defects or intraoperative cerebrospinal fluid fistula leaks. Rigorous intraoperative hemostasis and careful vascular management aimed to reduce ischemic complications. Postoperative monitoring

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protocols have been established to identify early signs of hydrocephalus and diabetes insipidus.

Lessons learned and recommendations for future practice stress the importance of early diagnosis through comprehensive endocrinological and ophthalmological evaluation, enabling earlier treatment of smaller tumors and potentially reducing complication rates. Since giant adenomas requiring transcranial approaches were associated with more ischemic and neurological deficits, minimally invasive endoscopic approaches should be prioritized whenever possible. Advances in reconstruction techniques, including adoption of vascularized flaps and meticulous multilayer reconstruction, have become central to reducing cerebrospinal fluid fistula and subsequent meningitis. Intensive postoperative monitoring with standardized protocols including neuroimaging and neurological assessments is critical for the early detection and management of hydrocephalus and ischemia, thereby decreasing mortality. Multidisciplinary care involving neurosurgeons, endocrinologists, and otolaryngologists is essential for optimizing patient outcomes. Although the retrospective nature and evolving surgical techniques over the 29-year period may have influenced complication rates, these insights form the basis for ongoing refinement of our protocols. Hence, we aim to incorporate these management approaches, preventive strategies, and lessons learned to provide a more comprehensive and instructive discussion for the readership.

In conclusion, this study provides a solid foundation for understanding factors influencing mortality in non-functioning pituitary macroadenomas, highlighting postoperative hydrocephalus, ischemia, and tumor size as key elements. Strategies focused on preventing and managing these complications may contribute to reducing mortality and improving clinical outcomes in these patients. Early diagnosis of these tumors through endocrinological and ophthalmological diagnosis confirmed by imaging exams enables detection of smaller tumors and more favorable surgical treatment results.

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# Comparison of bioimpedance and anthropometric estimates of body fat in patients with mild autonomous cortisol secretion and nonfunctional adrenal incidentalomas

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## ABSTRACT

**Objective:** This study aimed to compare body composition measurements between patients with nonfunctional adrenal incidentalomas (NFAI) and mild autonomous cortisol secretion (MACS) using bioelectrical impedance analysis (BIA) and anthropometric methods. **Subjects and methods:** This cross-sectional study included patients diagnosed with MACS or NFAI. Body composition was assessed using BIA, anthropometric measurements, and the Durnin and Womersley (DW) method. Correlation and Bland-Altman analyses were performed to assess the relationship and agreement between the DW method and BIA. **Results:** Fifty-seven patients (32 with MACS and 25 with NFAI) were included; those with MACS were older ($p = 0.004$). Post-dexamethasone suppression test cortisol levels ($p < 0.001$) and the incidence of bilateral tumors ($p = 0.017$) were higher in MACS patients. No significant differences in body composition parameters were observed between the MACS and NFAI groups. A strong correlation was observed between BIA- and DW-derived fat mass in MACS patients ($r = 0.890$, $p < 0.001$). Bland-Altman analysis revealed a slight mean bias for body fat mass of −0.4 kg (limits of agreement: −9.14–8.34 kg) and for body fat percentage of −0.83% (limits of agreement: −11.32–9.66%) between methods. **Conclusion:** A robust correlation and acceptable agreement was demonstrated between the DW method and BIA for estimating body fat. The DW equation may provide a practical and low-cost alternative for assessing body composition in MACS and NFAI cohorts. Limitations include the lack of a healthy control group and the inability to validate BIA and anthropometric estimates against gold-standard imaging techniques, potentially introducing accuracy bias.

**Keywords:** Adrenal incidentaloma; mild autonomous cortisol secretion; body composition; bioelectrical impedance analysis; nonfunctional adrenal incidentaloma

## INTRODUCTION

Adrenal incidentaloma (AI) refers to incidentally detected masses in the adrenal glands during cross-sectional radiological imaging performed for conditions unrelated to the adrenal glands. The prevalence of AI ranges from 1.2% to 8.7%, with rates increasing with age, reaching up to 10% in individuals aged >70 years. (1-3). Nonfunctional adrenal incidentalomas (NFAIs) account for 40–70% of adrenal adenomas, whereas 30–50% consist of adenomas with mild autonomous cortisol secretion (MACS) (1,4,5). According to the 2023 ESE/ENAT guidelines, in patients without overt signs and symptoms of Cushing's syndrome, a morning serum cortisol level exceeding 1.8 µg/dL (>50 nmol/L) after an overnight 1 mg dexamethasone suppression test (DST) is considered indicative of MACS (1). The increased frequency of cardiometabolic complications in Cushing's syndrome is well established (6). Although the

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association in patients with MACS is not entirely clear, recent studies suggest that chronic cortisol exposure may contribute to an increased risk of diabetes mellitus, hypertension, dyslipidemia, obesity, and mortality (7).

In individuals with MACS, increased abdominal adiposity is associated with metabolic comorbidities, cardiovascular diseases, and mortality risk (4,8). The gold-standard methods for assessing abdominal fat are computed tomography and magnetic resonance imaging (9). Bioelectrical impedance analysis (BIA) is a convenient, cost-effective, and easily applicable method for measuring body composition (9); however, its reliability has varied across studies (9-13). Previous studies have evaluated body composition in endocrine disorders using computed tomography, the gold standard (4). Nevertheless, both computed tomography and dual-energy X-ray absorptiometry are limited by cost and potential radiation exposure (9). Therefore, our study aimed to investigate how reliably anthropometric measurements reflect visceral adiposity derived from BIA in patients with MACS and NFAI and explore their potential as practical and accessible alternatives in routine endocrinology practice.

## SUBJECTS AND METHODS

### Study population

We conducted a cross-sectional study of body fat distribution ratios obtained through various methods in patients with MACS and NFAI. After ethics committee approval, informed consent was obtained, and patients managed in the endocrinology clinic of a tertiary university hospital between December 1, 2022, and May 31, 2023, were included. Hospital records from January 1, 2019, to May 31, 2023, were screened for patients aged ≥18 years with the diagnosis code "E27.9 Adrenal gland disease, unspecified," identifying 45 MACS patients. Of these, 32 were contacted by phone and agreed to participate. The NFAI group comprised newly admitted patients. Inclusion criteria were patients with adrenal masses identified on abdominal CT within the previous year. Biochemical and radiological data were retrieved from hospital records. Patients had previously undergone a 1 mg DST, with fasting plasma cortisol levels >1.8 mcg/dL at 8:00 am. denoting MACS. Exclusion criteria were active malignancy, adrenocortical carcinoma, myelolipoma, alcoholism, use of medications affecting dexamethasone metabolism, and history of unilateral or bilateral adrenalectomy. Pheochromocytoma and primary hyperaldosteronism were excluded biochemically and clinically.

### Body composition measurements

Waist, hip, and mid-arm circumferences, as well as skinfold thicknesses at the triceps, subscapular, abdominal, and suprailiac sites, were measured for all participants. Skinfold thicknesses were assessed with a manual caliper; other measurements used a measuring tape. Body fat distribution and lean body mass (LBM) were calculated using the Durnin and Womersley (DW) equation (Online calculator: Durnin and Womersley Calculator). Body composition was also assessed via BIA using the TANITA MC-780 Black. BIA measurements were performed between 8:00 and 10:00 am after at least 8 hours of fasting. Participants stood barefoot, ensuring proper placement of hands and feet on the device sensors. Body mass index (BMI), lean mass, fat mass, fat mass percentage, and muscle mass were automatically calculated.

### Ethical considerations

This study was approved by the local ethics committee (approval no.: 2022-17/31).

### Statistical analysis

Data were expressed as mean ± standard deviation, median with interquartile range (IQR), or frequency and percentage for categorical variables. Pearson's chi-square test was used to compare categorical variables. The Shapiro-Wilk test assessed normality of continuous variables. Patients were grouped as MACS or NFAI. Continuous variables were analyzed by independent samples t-test or the Mann-Whitney U test, as appropriate. The correlations between overnight 1 mg DST values and body composition measurements were evaluated using Pearson's correlation analysis. Correlations between body composition measures obtained by the DW method and BIA were also assessed. Agreement between the DW method and BIA measurements was additionally evaluated using Bland–Altman analysis. Statistical analyses were conducted using the IBM SPSS v. 29

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(Armonk, USA) software and $p < 0.05$ for statistical significance. Correlation results were visualized using R v. 4.3.2 (2023-10-31 ucrt).

## RESULTS

### Participants and comorbidities

Fifty-seven patients (mean age 58.5 ± 9 years; 45 females, 78.9%) with adrenal adenomas were included. Of these, 32 (56%) were classified as MACS and 25 (44%) as NFAI; NFAI patients were significantly younger than MACS patients (54.6 ± 6.7 vs. 61.5 ± 9.5 years; $p = 0.004$). Hypertension was more frequent in the MACS group (24/32; 75%), and no significant differences were noted in diabetes mellitus or dyslipidemia rates between groups. Cardiovascular disease was present in 5 patients (15.6%) with MACS, while no NFAI patients had cardiovascular disease, although this difference was not statistically significant ($p = 0.061$) (Table 1).

### Biochemical and radiological parameters

The overnight 1 mg DST results were significantly higher in the MACS group compared to the NFAI group (2.8 [IQR 2.3–3.6] vs. 1.1 [IQR 0.7–1.4], $p < 0.001$), whereas adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEAS) levels were lower in the MACS group. Other biochemical parameters were similar between groups (Table 2).

Bilateral tumors were detected in 15 (46.9%) MACS patients, significantly more often than in the NFAI group, with only 3 (12%) patients having bilateral tumors ($p = 0.017$). The largest tumor size was similar between groups: 27.5 mm (IQR 20.5–32.7) in MACS and 22 mm (IQR 13–30.5) in NFAI patients ($p = 0.053$) (Table 3).

### Body composition measurements

Waist circumference, hip circumference, mid-arm circumference, waist-to-hip ratio, and waist-to-height ratio did not differ significantly between groups. Similarly, there were no significant differences in body fat distribution or LBM values obtained using either the DW equation or the BIA method (Table 4).

**Table 1.** Demographic data and comorbid diseases

| Variables | Total (n = 57) | MACS (n = 32) | NFA (n = 25) | *p* |
|---|---|---|---|---|
| Age (year) | 58.5 ± 9 | 61.5 ± 9.5 | 54.6 ± 6.7 | **0.003** |
| Sex | | | | |
| Female, n (%) | 45 (78.9) | 24 (75) | 21 (84) | 0.52 |
| Male, n (%) | 12 (21.1) | 8 (25) | 4 (16) | |
| Height (cm) | 162.6 ± 8.2 | 161.1 ± 8.4 | 164.6 ± 7.8 | 0.116 |
| Weight (kg) | 81.4 ± 15.9 | 81.4 ± 16.2 | 81.5 ± 15.8 | 0.973 |
| BMI (kg/m$^2$) | 30.7 ± 5.2 | 31.4 ± 5.7 | 29.9 ± 4.4 | 0.315 |
| BMI classification | | | | |
| Normal, n (%) | 7 (12.3) | 4 (12.5) | 3 (12) | 0.982 |
| Overweight, n (%) | 22 (38.6) | 12 (37.5) | 10 (40) | |
| Obesity, n (%) | 28 (49.1) | 16 (50) | 12 (48) | |
| Current smoker, n (%) | 20 (35.1) | 10 (31.3) | 10 (40) | 0.35 |
| Alcohol, n (%) | 1 (1.8) | 1 (3.1) | 0 | |
| Comorbidities | | | | |
| Hypertension, n (%) | 31 (54.4) | 24 (75) | 7 (28) | **0.001** |
| Diabetes mellitus, n (%) | 14 (14) | 9 (28.1) | 5 (20) | 0.691 |
| Dyslipidemia, n (%) | 10 (17.5) | 7 (21.9) | 3 (12) | 0.487 |
| Cardiovascular disease, n (%) | 5 (8.8) | 5 (15.6) | 0 | 0.061 |
| Hypothyroidism, n (%) | 14 (24.6) | 7 (21.9) | 7 (6.1) | 0.824 |

MACS: mild autonomous cortisol secretion; NFA: nonfunctional adenoma; BMI: body mass index.

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**Table 2.** Biochemical parameters of the participants

| Variables | Total (n = 57) | MACS (n = 32) | NFA (n = 25) | *p* |
|---|---|---|---|---|
| 1-mg DST (μg/dL) | 2.1 (1.2–3) | 2.8 (2.3–3.6) | 1.1 (0.7–1.4) | **<0.001** |
| 24-hour urine free cortisol (mg/d) (n = 22) | 176 (45.4–407) | 176 (45.4–407) | – | |
| ACTH (pg/mL) | 13 (6.1–17.8) | 10 (6–14) | 16 (11.5–20) | **0.007** |
| DHEA-S (μg/dL) | 68.2 (50.3–120.8) | 52.6 (35.2–105.3) | 85.7 (68.5–138.6) | **0.035** |
| Fasting glucose (mg/dL) | 96 (88.5–114) | 94.5 (85.5–113) | 99 (89.5–120.5) | 0.338 |
| HbA1c (%) | 5.7 (5.4–6.1) | 5.8 (5.6–6.2) | 5.7 (5.3–6.1) | 0.329 |
| AST (U/L) | 17 (15–22) | 18 (15–23) | 17 (15–20.5) | 0.714 |
| ALT (U/L) | 18.4 ± 7.1 | 18.6 ± 7.6 | 18.2 ± 6.5 | 0.856 |
| Creatinine (mg/dL) | 0.73 (0.68–0.89) | 0.75 (0.7–1) | 0.71 (0.64–0.84) | 0.06 |
| Sodium (mmol/L) | 139 (137–140.5) | 139 (137–140) | 140 (138–141) | 0.329 |
| Potassium (mmol/L) | 4.4 ± 0.4 | 4.4 ± 0.4 | 4.5 ± 0.5 | 0.705 |
| Total cholesterol (mg/dL) | 213.7 ± 42.2 | 208.4 ± 44.6 | 221.6 ± 38 | 0.273 |
| HDL (mg/dL) | 54.7 ± 11.2 | 52.5 ± 11.8 | 58 ± 9.5 | 0.081 |
| LDL (mg/dL) | 131.6 ± 37.4 | 129.5 ± 39.4 | 134.9 ± 34.7 | 0.618 |
| Triglycerides (mg/dL) | 117.5 (88.5–173) | 115 (95–170) | 126 (77.5 – 201.5) | 0.926 |
| TSH (mU/L) | 1.3 (0.7–1.9) | 1.24 (0.63–1.61) | 1.39 (0.78–2.11) | 0.364 |
| Free T4 (ng/dL) | 1.01 (0.89–1.09) | 1.02 ± 0.14 | 1.02 ± 0.16 | 0.883 |

MACS: mild autonomous cortisol secretion; NFA: nonfunctional adenoma; DST: dexamethasone suppression test; ACTH: adrenocorticotropic hormone; DHEA-S: dehydroepiandrosterone sulfate; HbA1c: hemoglobin A1c; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TSH: thyroid-stimulating hormone; Free T4: free thyroxine.

**Table 3.** Radiological characteristics of adrenal adenomas

| Variables | Total (n = 57) | MACS (n = 32) | NFA (n = 25) | *p* |
|---|---|---|---|---|
| Bilateral, n (%) | 18 (31.6) | 15 (46.9) | 3 (12) | **0.017** |
| Unilateral, n (%) | 39 (68.4) | 17 (53.1) | 22 (88) | |
| Left, n (%) | 26 (45.6) | 12 (37.5) | 14 (56) | |
| Right, n (%) | 13 (22.8) | 5 (15.6) | 8 (32) | |
| Tumor size | | | | |
| Largest (mm) | 26 (19–32) | 27.5 (20.5–32.7) | 22 (13–30.5) | 0.053 |
| Left (mm) | 22.4 ± 8.6 | 23.3 ± 7.8 | 21 ± 10 | 0.39 |
| Right (mm) | 25 (19–34) | 24 (19.2–35) | 27 (17–34) | 0.967 |
| Tumor density | | | | |
| Non-contrast (HU) (n = 38) | -5 (-10 ± 5.5) | -5 (-13 ± 1) | -4 (-8 ± 14) | 0.123 |
| Contrast-enhanced (n = 5) | | | | |
| Early phase (HU) | 65.2 ± 30.5 | 38.5 ± 17.7 | 83 ± 22.9 | 0.106 |
| Late phase (HU) | 25 ± 21.9 | 23 ± 35 | 26 ± 18 | 0.894 |

MACS: mild autonomous cortisol secretion; NFA: nonfunctional adenoma; HU: Hounsfield unit.

## Comparison of body composition measurements

Correlations for selected body composition parameters across all methods are shown in **Figure 1**. In the MACS group, there were very strong correlations between body fat distribution (kg) measured by the DW method and BIA (Spearman's $r$ = 0.890, $p$ < 0.001).

In the overall cohort, Bland–Altman analysis showed minimal mean bias between BIA and the DW equation for body fat mass (−0.4 kg) and LBM (+0.4 kg), with limits of agreement of approximately ±9 kg. Similar results were observed in the MACS group, indicating agreement at the group level. By contrast, the NFAI group showed a slight systematic bias (−1.34 kg

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**Table 4.** Body composition measurements of participants

| Variables | Total (n = 57) | MACS (n = 32) | NFA (n = 25) | p |
|---|---|---|---|---|
| Anthropometric indicators | | | | |
| Waist circumference (cm) | 102.7 ± 12.9 | 104.9 ± 12.3 | 99.9 ± 13.5 | 0.163 |
| Hip circumference (cm) | 109.9 ± 10.7 | 109.7 ± 11.1 | 110 ± 10.4 | 0.917 |
| Mid-arm circumference (cm) | 30 (28–32) | 30.5 (28–32.2) | 30 (28.5–32) | 0.946 |
| Waist-to-hip ratio (cm/cm) | 0.93 ± 0.09 | 0.96 ± 0.1 | 0.91 ± 0.08 | 0.414 |
| Waist-to-height ratio (cm/cm) | 0.63 ± 0.08 | 0.65 ± 0.08 | 0.61 ± 0.07 | 0.883 |
| Skinfold thickness | | | | |
| Biceps (mm) | 10.3 ± 3.9 | 10.6 ± 3.8 | 10 ± 4.1 | 0.624 |
| Triceps (mm) | 15.4 ± 5.2 | 16.1 ± 5.4 | 14.6 ± 4.9 | 0.317 |
| Subscapular (mm) | 21.6 ± 6.4 | 20.5 ± 5.9 | 22.9 ± 6.8 | 0.17 |
| Suprailiac (mm) | 16.4 ± 6.2 | 16 ± 5.8 | 16.9 ± 6.6 | 0.601 |
| Body composition | | | | |
| Durnin and Womersley's equations | | | | |
| Body fat (%) | 35.7 (32.7–37.5) | 35.6 (32.4–37.9) | 35.6 (32.6–37.5) | 0.939 |
| Body fat mass (kg) | 28 ± 8 | 27.9 ± 8.7 | 28.1 ± 7.2 | 0.95 |
| Lean body mass (kg) | 53.3 ± 10.4 | 53.2 ± 10 | 53.4 ± 10.9 | 0.935 |
| Bioelectrical impedance analysis | | | | |
| Body fat (%) | 33.3 ± 7.7 | 34.1 ± 8.8 | 32.2 ± 5.9 | 0.35 |
| Body fat mass (kg) | 27.6 ± 9.7 | 28.2 ± 10.4 | 26.7 ± 8.9 | 0.566 |
| Lean body mass (kg) | 51.9 (47.5–58.5) | 50.6 (44.9–56.5) | 52.1 (49.6–59.1) | 0.563 |

MACS: mild autonomous cortisol secretion; NFA: nonfunctional adenoma.

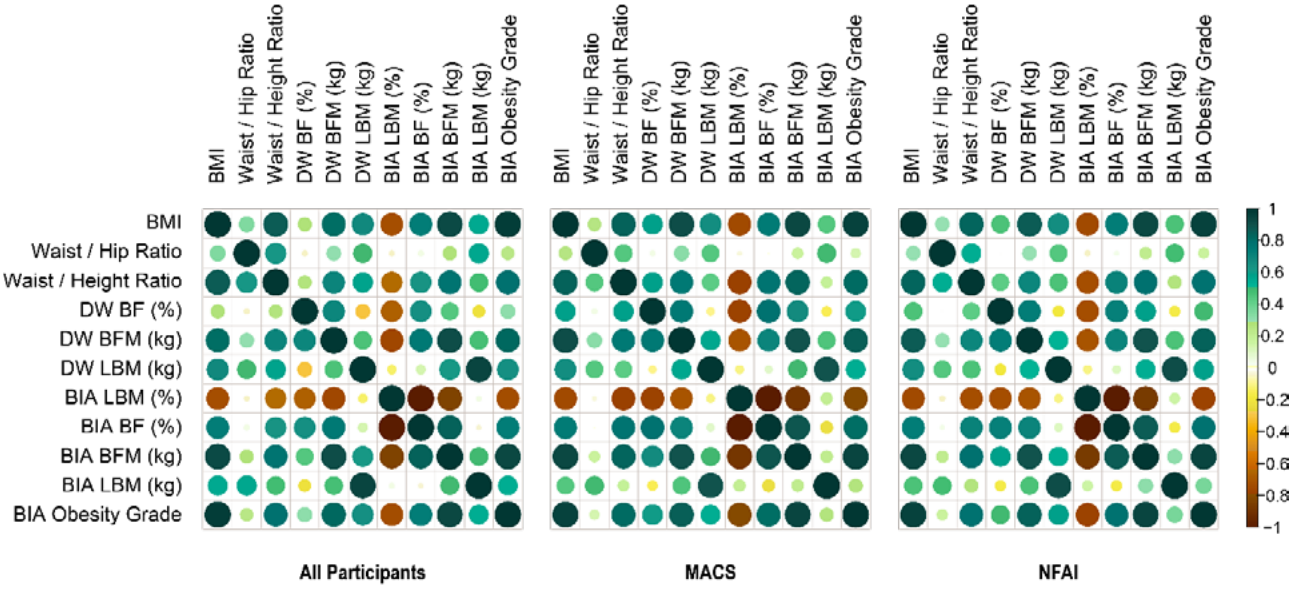



**Figure 1.** Correlations of body composition measurements. There was a positive and significant correlation in all patients and between BMI and DW BFM (kg) ($r = 0.861$, $p < 0.001$), BIA BFM (kg) ($r = 0.933$, $p < 0.001$), and BIA body fat percentage (BF%) ($r = 0.748$, $p < 0.001$). Positive correlations were also observed between BIA BFM (kg) and DW BFM (kg) in all patients, as well as in the MACS and NFAI groups ($r = 0.891$, $r = 0.890$, $r = 0.902$, $p < 0.001$, respectively). BMI: Body mass index; DW: Durnin-Womersley equation; BF: body fat; BFM: body fat mass; LBM: lean body mass; BIA: bioelectrical impedance analysis; MACS: mild autonomous cortisol secretion; NFAI: nonfunctional adrenal incidentaloma.

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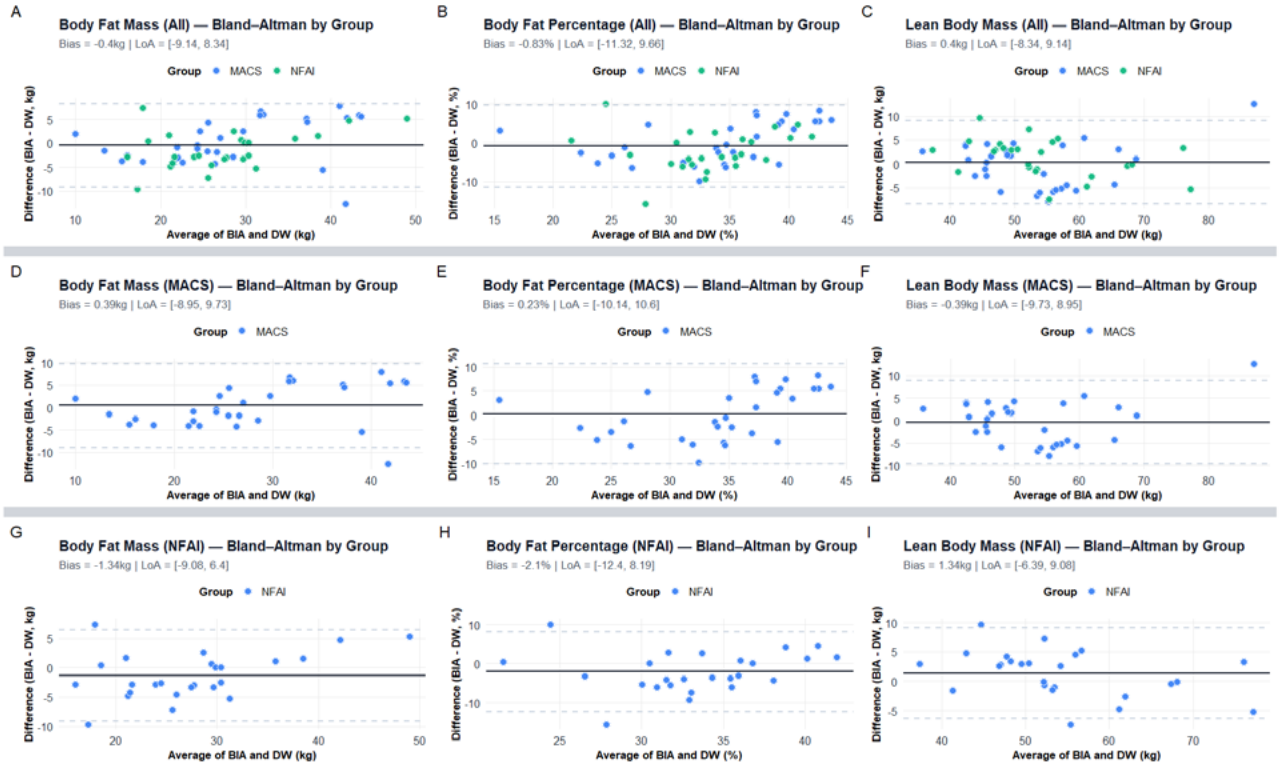



**Figure 2.** Bland-Altman plots of body composition parameters. Comparisons of bioelectrical impedance analysis (BIA) and the Durnin-Womersley (DW) equation for estimating body composition parameters are depicted. Each plot displays the difference between the two methods (BIA − DW) plotted against their mean, with the solid line indicating the mean bias and dashed lines showing the 95% limits of agreement (LoA). Panels **A–C** show data for all patients; panels **D–F** for those with mild autonomous cortisol secretion (MACS); and panels **G–I** for patients with nonfunctional adrenal incidentaloma (NFAI). Minimal mean bias was observed between BIA and DW across parameters, although the relatively wide LoA suggested moderate inter-individual variability. In the NFAI group, BIA slightly overestimated body fat compared to DW. BIA: bioelectrical impedance analysis; DW: Durnin-Womersley equation; BFM: body fat mass; LBM: lean body mass; MACS: mild autonomous cortisol secretion; NFAI: nonfunctional adrenal incidentaloma; LoA: limits of agreement.

for body fat mass and −2.1% for body fat), suggesting that BIA may overestimate body fat relative to the DW method (**Figure 2**). Despite acceptable agreement at the group level, substantial individual-level variability (within ±9–12%) indicates that BIA and DW may not be particularly useful for individual patient monitoring in routine clinical practice.

### Comparison of biochemical parameters with body composition measurements

In the MACS group, the overnight 1 mg DST was significantly correlated with body fat mass percentage by the DW method ($r$ = −0.43, $p$ = 0.019), whereas no such correlation was observed in the NFAI group. DHEAS levels showed positive correlations exclusively in the NFAI group: with lean body mass (kg) obtained via BIA ($r$ = 0.79, $p$ = 0.004), body fat mass (kg) obtained via the DW method ($r$ = 0.67, $p$ = 0.023), and LBM (kg) measured using the DW method ($r$ = 0.65, $p$ = 0.029).

## DISCUSSION

No significant differences were identified between the MACS and NFAI groups regarding anthropometric measurements, skinfold thicknesses, or body composition measurements obtained using the DW equation and BIA. Some studies have reported an inverse relationship between post-DST cortisol levels and LBM in women, as measured by BIA, while this relationship was not observed in men. Additionally, LBM was similar between NFAI and MACS patients (10). In one study comparing BIA with dual-energy X-ray absorptiometry, the former underestimated total fat mass compared to the latter in the same individuals (11). Although research indicates a high correlation between BIA and other methods of body composition

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analysis, BIA may overestimate LBM and underestimate fat mass, particularly in obese individuals (9). Considering that approximately half of our patient group consisted of obese individuals, this may explain some of the observed differences. BIA measurements can also be influenced by factors such as age, sex, ethnicity, and medication use (9). Previous studies have demonstrated a weak correlation between visceral fat mass measurements obtained by BIA and those obtained with computed tomography or magnetic resonance imaging (12,13).

Some studies have found higher waist-hip ratios in NFAI patients; however, in our measurements, there was no significant difference between groups (14,15). Garrapa and cols. (16) reported a positive correlation between waist circumference, waist-hip ratio, and abdominal fat mass. In the present study, a robust correlation was identified between body fat mass measurements obtained using BIA and the DW method, whereas such a correlation was not observed for LBM. This finding underscores the potential utility of skinfold thickness and anthropometric measurements as cost-effective, simple, and practical alternatives for monitoring body fat mass, particularly in settings where access to BIA devices is limited. Consistent with the literature, our study observed that patients in the MACS group were significantly older and predominantly female (5,14-17). This may be attributed to the fact that MACS patients are often postmenopausal women diagnosed during this period. Additionally, the positive linear relationship between age and overnight DST results may contribute to this observation (18).

This study demonstrated that there are no significant differences in body composition between NFAI and MACS patients when assessed using anthropometric measurements, the DW equation incorporating various skinfold thicknesses, or the BIA method. When these measurements were compared both across all patients and within the NFAI and MACS groups, significant correlations between methods were observed. Beyond correlational findings, Bland-Altman plots were utilized to evaluate the agreement between BIA and DW methods. The analysis confirmed a substantial degree of inter-method concordance, particularly evident in the MACS cohort. Conversely, a slight systematic bias was observed in the NFAI cohort, which indicated that BIA consistently provided higher estimates of body fat compared to the DW method. Nevertheless, the degree of individual variability suggested that despite showing overall consistent at the group level, these methods may not be sufficiently interchangeable for individual patient monitoring. These findings emphasize that correlation alone is insufficient to establish clinical interchangeability and underscore the necessity of further validation studies utilizing gold-standard imaging modalities (i.e., computed tomography and dual-energy X-ray absorptiometry) (11-13).

In accordance with the observations reported by Podbregar and cols. (17), we found no significant disparities in BMI measurements between groups. The study conducted by Olsen and cols. (18) reported a negative association between BMI values below 30 kg/m$^2$ and DST results, whereas no such relationship was identified for BMI values above 30 kg/m$^2$. In our study, approximately half of the participants had BMI values above 30 kg/m$^2$.

In patients with overt Cushing's syndrome, there is an increased risk of comorbid conditions such as hypertension, diabetes mellitus, and dyslipidemia. However, the association of these comorbidities with MACS remains uncertain (7). Some researchers have identified a higher prevalence of comorbid conditions in MACS patients compared to NFAI patients. Furthermore, improvements in these comorbidities, including those observed after adrenalectomy, have been reported in MACS patients (5-7,14,19). Similar to our findings, several studies did not report consistent or significant differences in the prevalence of complications such as diabetes mellitus and dyslipidemia (4,20). These differences may be attributed to the saturation of the 11β-HSD2 enzyme in the kidney by cortisol, leading to activation of mineralocorticoid receptors by elevated cortisol levels, subsequently increasing blood pressure. Other potential mechanisms include enhanced beta-adrenergic receptor sensitivity to catecholamines, elevated endothelin-1 levels, or inter-individual variation in glucocorticoid receptor polymorphisms (6,21). Additionally, the presence of certain comorbidities at similar frequencies in the

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NFAI group, although not at diagnostic levels, may suggest the possibility of mild cortisol secretion, as supported by prior studies (19).

As anticipated, in subjects exhibiting autonomous cortisol secretion independent of the hypothalamic-pituitary-adrenal axis, ACTH and DHEAS levels were suppressed and post-DST cortisol levels elevated (10,17,20,22). This can be explained by the suppression of ACTH resulting from autonomous cortisol release, which, in turn, leads to reduced DHEAS secretion (23).

In our study, although no significant difference in adenoma size was observed between groups, bilateral adenomas were more frequent in MACS patients, consistent with previous reports. Some studies have shown that adrenal adenoma size is significantly larger in MACS patients and is positively correlated with cortisol levels (10,17,20,24). Bleier and cols. (24) reported that an adenoma size smaller than approximately 14 mm had high enough sensitivity to exclude the possibility of MACS. Conversely, Araujo-Castro and cols. (25) indicated that a size of ≥25 mm had sensitivity and specificity rates up to 70% in favor of MACS. Additionally, bilateral adenomas were associated with higher cortisol levels and lower DHEAS levels (3,26,27). Nevertheless, some reports have indicated that adenoma size and laterality (unilateral or bilateral) do not provide significantly differentiate between MACS and NFAI (5,20,25). The lack of differences may not be solely attributable to cortisol secretion but could also be influenced by the secretion of other bioactive metabolites (25). Moreover, although the BclI polymorphism of the *NR3C1* (glucocorticoid receptor) gene does not appear to affect parameters such as adenoma size, cortisol levels, or BMI, the N363S polymorphism has been reported to be associated with larger tumor sizes in some patients (21). Furthermore, elevated insulin resistance may stimulate the proliferation of adrenocortical cells, potentially contributing to increased adenoma sizes (3).

Despite our promising findings, this study has several limitations. First, we were unable to perform computed tomography or magnetic resonance imaging, which are the gold-standard methods for body composition analysis, due to software limitations. Second, the accuracy of BIA measurements may have been compromised by confounding variables such as hydration status, ambient temperature, and concomitant medication use. Third, the overrepresentation of obese patients in the cohort may have resulted in an underestimation of fat tissue measurements derived from BIA. Fourth, although approximate age matching was attempted during data collection, several participants with incomplete anthropometric and biochemical data were necessarily excluded from the final analysis to ensure data integrity. Furthermore, because recruitment efforts were primarily concentrated on patients with MACS, the resulting cohort of NFAI patients was smaller and slightly older on average, leading to group heterogeneity that may have introduced interpretative bias. Fifth, the absence of a healthy control group limited the comparative context and may have provided valuable additional interpretative insight into our findings. Lastly, the relatively lower number of NFAI patients than MACS patients is acknowledged, reflecting the study's primary focus on MACS during the research period. Future studies should include larger, age- and sex-matched cohorts and use gold-standard imaging modalities.

In conclusion, we demonstrated that there were no significant differences in body composition measurements obtained using BIA between NFAI and MACS patients with AIs. Furthermore, we identified a strong correlation between body fat mass measurements obtained via BIA and the DW method. This suggests that simpler, more accessible, and cost-effective methods beyond BIA analysis may be utilized for body fat mass measurements or follow-up.

**Authos' contributions:** all authors made significant contributions to this study. Ensar Aydemir conceptualized the study, defined the research question and hypothesis, collected the necessary data, performed the statistical analysis, and drafted the initial manuscript. Yasemin Ünsal was instrumental in designing the data collection strategies. Coşkun Ateş and Semiha Yaşar Türk both actively conducted interviews with study participants. Soner Cander provided mentorship to junior researchers and played a key role in interpreting the analysis results and drawing conclusions. Özen Öz Gül diligently reviewed and corrected the language, clarity, and overall readability of the manuscript. Canan Ersoy was responsible for providing necessary materials and offered crucial supervision throughout the project. Lastly, Erdinç Ertürk contributed significantly to the conceptualization of the study, defined the research question and hypothesis, provided overall supervision, and made important revisions to the manuscript. All authors have read and approved the final version of the manuscript.

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**Disclosure:** no potential conflict of interest relevant to this article was reported.

**Data availability:** The data that support the findings of this study are available within the article. Due to the sensitive nature of the patient data and ethical restrictions from the hospital system, the raw data are not publicly available but are accessible to qualified researchers upon reasonable request, subject to institutional approval and data usage agreements.

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invited review

# Cushing's disease across the lifespan: a case-based clinical review

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## ABSTRACT

Cushing's disease (CD), caused by an adrenocorticotropin-secreting pituitary adenoma, is a rare but severe endocrine disorder associated with high cardiometabolic morbidity and mortality. Diagnosis is challenging as many symptoms are nonspecific, and biochemical or imaging results may be inconclusive, contributing to substantial diagnostic delay. Although the core features of CD are consistent across the lifespan, certain clinical manifestations of chronic hypercortisolism vary from childhood through older age, reflecting differences in growth, puberty, metabolism, and comorbidity burden. In children, impaired growth coupled with weight gain is most prominent, whereas adolescents often present with pubertal disturbances and psychological or academic difficulties. Adults typically exhibit the classic Cushingoid features such as round face, plethora, and central obesity, along with metabolic and reproductive complications. Older individuals typically present with frailty, sarcopenia, fractures, and cognitive decline. Age also influences the interpretation of endocrine tests, the accuracy of pituitary magnetic resonance imaging, the role of inferior petrosal sinus sampling, perioperative risks, and the long-term impact of remission or persistent disease. Given this context, this narrative review used five representative clinical vignettes (pediatric, adolescent, adult female, adult male, and elderly) to illustrate how the presentation, diagnostic evaluation, and management of CD vary across the lifespan. Each case was paired with a structured synthesis of current evidence, highlighting both shared principles and age-specific nuances essential for timely diagnosis, appropriate treatment selection, and effective long-term multidisciplinary care. Understanding age-related differences is crucial to improving outcomes and reducing the substantial morbidity and mortality associated with CD throughout the lifespan.

**Keywords:** Pituitary; cortisol; children; adults; elderly; individualized care

## INTRODUCTION

Cushing's disease (CD) is caused by chronic cortisol excess resulting from an adrenocorticotropin (ACTH)-secreting pituitary adenoma (1,2). With a global incidence of approximately 0.24 (95% confidence interval [CI] 0.15–0.33) cases per 100,000 person-years (3) and a substantial diagnostic delay (4), CD remains a challenging condition to recognize. Uncontrolled hypercortisolism has profound multisystem consequences, leading to increased cardiometabolic morbidity and mortality if not rapidly treated (1).

Although the core features of CD are similar at all ages, each age group exhibits characteristic clinical differences reflecting their developmental stage (**Figure 1**). In young children, growth failure and weight gain predominate, while adolescents often experience pubertal disturbances, weight gain, and menstrual changes (5,6). Among adults, metabolic and reproductive complications are common, whereas older individuals frequently develop frailty,

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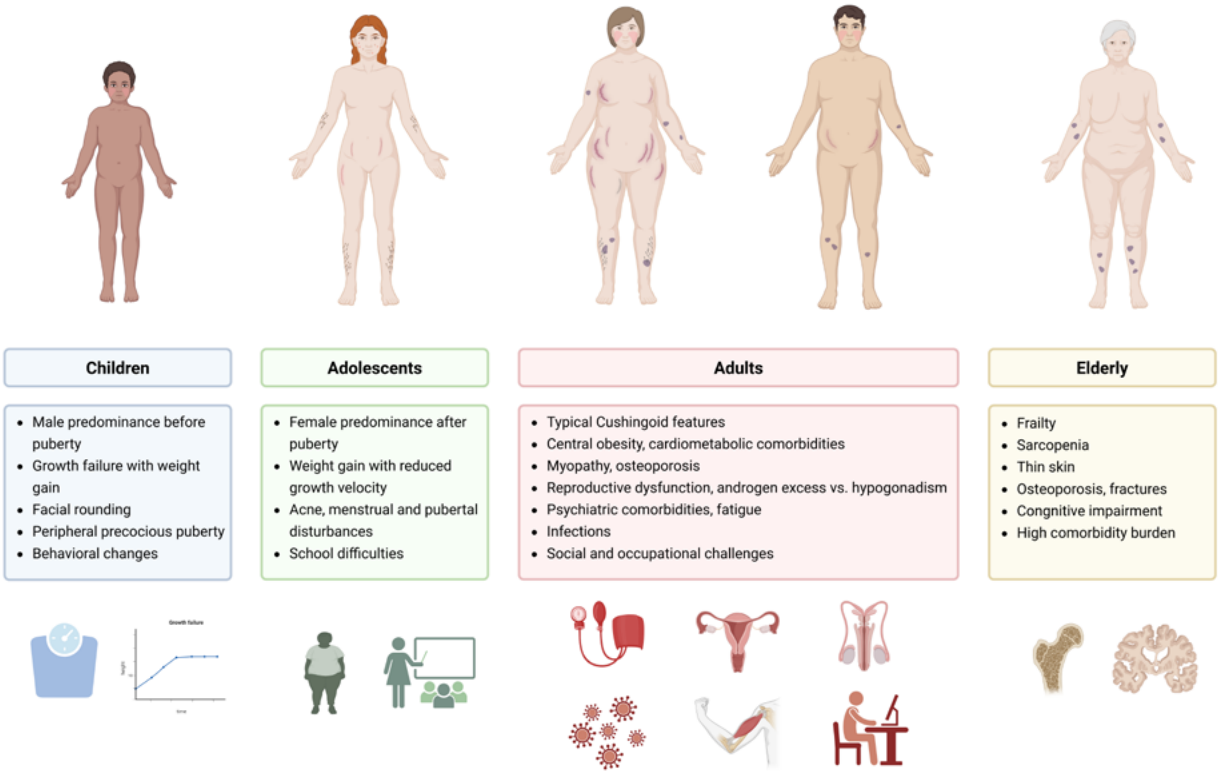



**Figure 1.** Key clinical features of Cushing's syndrome across the lifespan. Created with biorender.

sarcopenia, fractures, or cognitive decline, which may be misattributed to normal aging or comorbidities (1,7). Recognizing these differences is crucial for interpreting diagnostic tests and selecting appropriate treatment strategies. For example, dynamic test interpretation differs in children, fertility considerations are key for reproductive-age patients, and risk-benefit assessments require particular caution in older adults. Long-term complications and recurrence risk also differ across the lifespan, highlighting the need for age-tailored management (1,5).

This narrative review provides a structured, case-based overview of CD across the age spectrum — from childhood through adolescence, adulthood, and older age. For each age group, we present a representative clinical vignette followed by a concise synthesis of current evidence regarding etiology, clinical and biochemical presentation, diagnostic strategies (Table 1), and therapeutic considerations (Table 2). By highlighting both shared and age-specific patterns of CD, we aim to support clinicians in recognizing and managing this complex condition at every stage of life.

## INFANTS AND PREPUBERTAL CHILDREN (0–10 YEARS)

### Case vignette

A 6-year-old boy with 18 months of growth deceleration, central weight gain, facial rounding, and new-onset hypertension. His parents reported mood swings and decreased academic performance, prompting an ongoing investigation for autism. On examination, his height was -1.6 standard deviation score (SDS), weight +1.5 SDS, and BMI +3.4 SDS (Figure 2). Biochemical testing showed repeatedly elevated 24-hour urinary free cortisol (24h-UFC) and inadequate suppression of morning serum cortisol on low-dose dexamethasone. ACTH levels were inappropriately normal to high. Pituitary magnetic resonance imaging (MRI) revealed a left-sided 4-mm microadenoma. Given the concordant MRI findings and the rarity of ectopic ACTH secretion in this age group, the multidisciplinary team proceeded directly to surgery without further confirmatory testing. Transsphenoidal surgery confirmed an ACTH-secreting microadenoma; postoperative glucocorticoid replacement was required. On follow-up,

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**Table 1.** Diagnostic considerations according to lifespan

| Domain | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|
| Subtype frequency (14,63,69,70)<br>Pituitary<br>Adrenal<br>Ectopic | ~70–80%<br>Predominant in very young children<br>Very rare | ~70–80%<br>~15–20%<br>Very rare | ~60–70%<br>~20–30%<br>~5–10% | ~69%<br>~25%<br>~6% |
| Key diagnostic challenges | Physiologic variability of cortisol<br>Difficulties in cooperation for testing | Overlap with normal puberty (acne, mood, weight changes), non-neoplastic hypercortisolism | Wide comorbidity spectrum (e.g., metabolic syndrome, depression) can mask early disease | Symptoms may be misattributed to aging<br>Multimorbidity and polypharmacy complicate interpretation |
| Considerations for dynamic testing | Weight-specific cortisol cut-offs<br>24h-UFC correction for body surface area | Weight-specific cortisol cut-offs<br>Need to distinguish CD from non-neoplastic hypercortisolism | Higher specificity of LNSC and LDDST | Reduced renal function can affect 24h-UFC<br>Exogenous glucocorticoid therapy can complicate diagnosis |
| Pituitary imaging | Microadenomas often <3-4 mm<br>MRI-negative in ~40-50% | Microadenomas often <3-4 mm<br>MRI-negative in ~40-50% | Higher MRI detection rates<br>Microadenomas more frequent than macroadenomas | Cerebral atrophy may reduce contrast resolution |
| Use of IPSS | Selective use in MRI-negative or discordant biochemistry | Selective use in MRI-negative or discordant biochemistry | Standard practice when imaging is negative or adenoma < 6-10 mm | Higher procedural risk depending on comorbidities but often still indicated |

CD: Cushing's disease; IPSS: inferior petrosal sinus sampling; LDDST: low dose (1 mg) dexamethasone suppression test; LNSC: late night salivary cortisol; MRI: magnet resonance imaging; 24h-UFC: 24h urinary free cortisol.

**Table 2.** Therapeutic considerations according to lifespan

| Domain | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|
| Surgical considerations | Requires experienced pediatric pituitary surgeon | Requires experienced pediatric pituitary surgeon | Established first-line therapy | Higher perioperative risk, individualized decision-making |
| Medical therapy | Reserved for persistent/ recurrent disease | Reserved for persistent/ recurrent disease | Standard second-line options (steroidogenesis inhibitors, pituitary-directed drugs)<br>Individual choice depending on severity, comorbidities, and gender | Polypharmacy and comorbidities guide choice and dosing |
| Long-term issues | Catch-up growth<br>Pubertal progression<br>Bone health | Psychosocial support<br>School performance<br>Fertility | Cardiometabolic<br>Psychological<br>Social and occupational impact<br>Risk of recurrence | Frailty<br>Cognitive decline<br>Falls and fracture prevention |

hypertension resolved, and both growth velocity and mood improved.

## Mini-review

Cushing's disease is rare in childhood, with an incidence of approximately 0.5 new cases per million children per year, roughly 10% of the adult rate (5). Corticotroph microadenomas are the most common pituitary tumors in childhood, comprising ~55% of adenomas in ages 0–11 and ~30% in ages 12–17 (5). Cushing's disease is exceedingly rare below the age of 5, in whom adrenal causes predominate (8). Marked male predominance (~70%) is observed before puberty, with boys demonstrating higher ACTH concentrations and more aggressive features (5). Growth failure with simultaneous weight gain is the hallmark symptom, with height SDS decreasing while body mass index (BMI) increases (6) (**Figure 2**). Unlike adults, children with CD rarely have myopathy, sleep, memory disturbances, or skin striae (9). Current pediatric guidelines recommend screening only when unexplained weight gain is accompanied by decreased height velocity or height percentile, given the high diagnostic sensitivity and specificity of this pattern (5).

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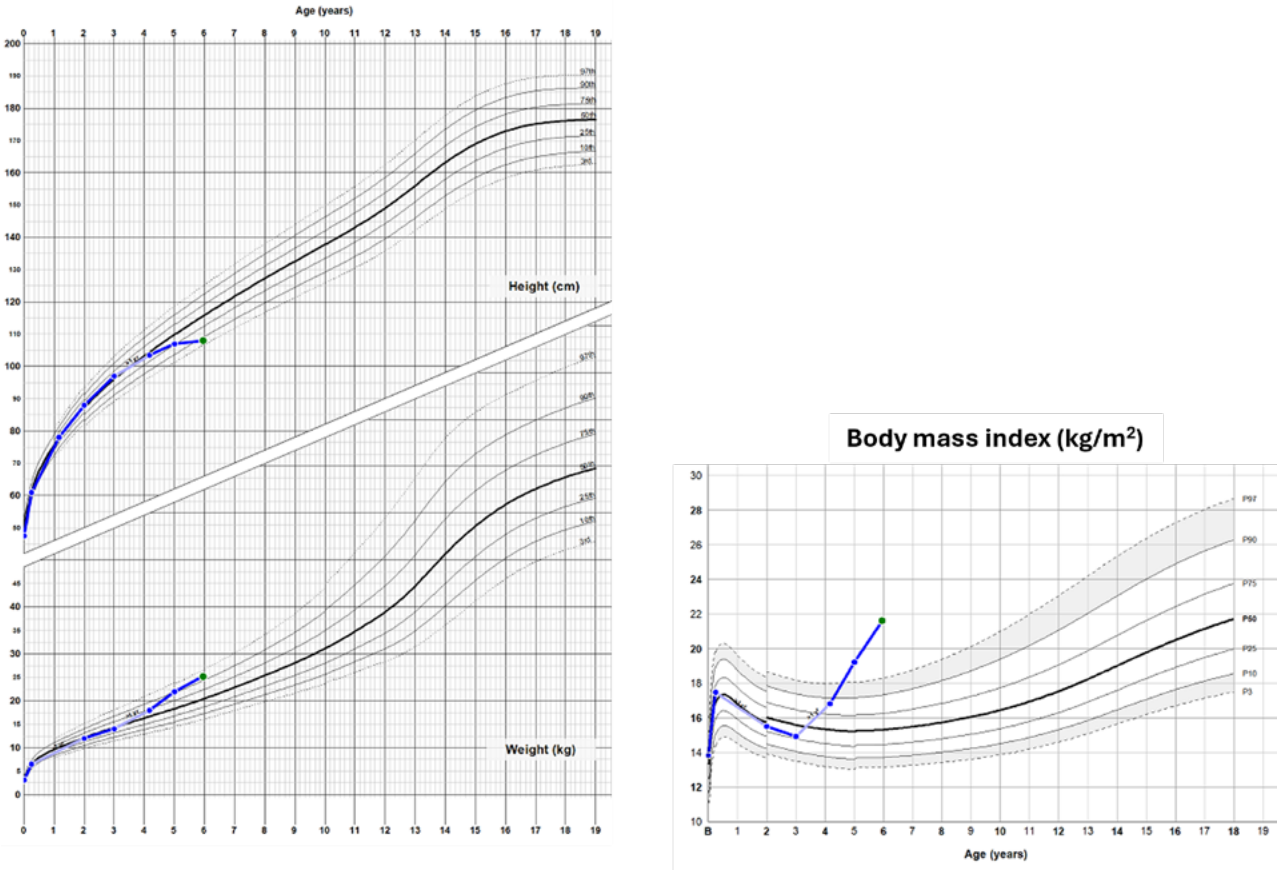



**Figure 2.** Growth charts of a 6-year-old boy with Cushing's disease showing growth failure with simultaneous weight gain and increased BMI.

Biochemical confirmation should include the 24h-UFC (adjusted for body surface area), low-dose (typically 1 mg) dexamethasone suppression test (LDDST), and late-night serum or salivary cortisol (10,11).

In children and adolescents, Cushing's syndrome can be reliably excluded by two normal 24h-UFC results along with a normal LDDST. A midnight sleeping serum cortisol <1.8 μg/dL (<50 nmol/L) also excludes CD with 94–100% sensitivity and 100% specificity in pediatric patients, though hospital sampling is required (5). Late-night salivary cortisol (LNSC) may substitute for the midnight serum test (12,13), with sensitivity 93–100%, and specificity 95–100% (5); however, its use is limited by the absence of well-defined age- and assay-specific reference intervals (5). MRI identifies only 50–63% of pediatric corticotroph adenomas due to their very small size. If MRI is negative, inferior petrosal sinus sampling (IPSS) is strongly recommended, confirming a central ACTH source with nearly 100% sensitivity in experienced centers. A central-to-peripheral ACTH gradient ≥3 following corticotropin-releasing hormone (CRH) or desmopressin stimulation confirms CD, and lateralization accuracy varies from 58–87%, depending on the series (5).

First-line treatment is selective transsphenoidal adenomectomy performed by an experienced pediatric neurosurgeon (14). Second-line radiotherapy provides disease control in most children within 12–18 months but carries long-term risks of hypopituitarism (5,15,16). Steroidogenesis inhibitors (metyrapone, ketoconazole) are also regarded as second-line therapy, usually employed short-term to stabilize severe hypercortisolism or bridge to surgery. In children, long-term metyrapone suppository treatment has been associated with clinical and biochemical improvement without reported developmental adverse effects (17). Osilodrostat's safety and pharmacological profile are currently being assessed in a small phase II study (NCT03708900), representing the first evaluation of this agent in pediatric patients (<18 years), with completion expected in 2025 (18). With specialized care, prognosis for pediatric CD is favorable. Early

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biochemical remission is associated with identifiable microadenomas and postoperative cortisol <1 µg/dL (28 nmol/L).

Long-term remission is highest in younger children and in those without cavernous sinus invasion (19). Nevertheless, even after remission, children with CD may experience persistent reductions in quality of life and neurocognitive functioning. Longitudinal research demonstrates residual impairments in attention, memory, and psychosocial adjustment after remission, suggesting that hypercortisolism during critical developmental periods may have lasting effects (20–23). Lifelong follow-up is necessary to address these neurocognitive sequelae as well as the risk of recurrence (6–40%), pubertal disturbances, and growth impairment (5). In children and adolescents, growth hormone (GH) testing should be performed 3–6 months post-remission, as early detection and treatment of GH deficiency are critical for optimizing catch-up growth and achieving target adult height. GH replacement in confirmed cases generally improves growth outcomes, albeit obesity may persist (24).

## PUBERTY/ADOLESCENCE (11-18 YEARS)

### Case vignette

A 15-year-old girl was referred for evaluation of obesity and menstrual irregularity. Over the previous two years, she had gained 15 kg and developed severe acne, purple striae, and depressive symptoms. Her height had plateaued, and she reported being bullied at school. Laboratory tests showed impaired glucose tolerance, elevated LNSC, increased 24h-UFC, and failure to suppress cortisol on LDDST. ACTH was elevated, with pituitary MRI showing a suspected left-sided 6-mm microadenoma. She underwent transsphenoidal surgery and developed transient postoperative corticotrope insufficiency. Over the subsequent year, she lost 7 kg, and her menses returned.

### Mini-review

The typical age of onset for pediatric-onset CD is 12–14 years, corresponding with puberty (8). The marked male predominance observed before puberty becomes less pronounced in mid- to late adolescence (5). Diagnosing Cushing's syndrome in adolescents is particularly challenging, as many teenagers have overlapping features such as weight gain, acne, and mood changes. Distinguishing true CD from non-neoplastic hypercortisolism associated with obesity, depression, or chronic stress is therefore essential (6). In adolescents, the hallmark is weight gain with reduced growth velocity, often accompanied by changes in facial appearance, truncal obesity, violaceous striae, acne, virilization or menstrual disturbance, and psychological or academic difficulties (8). Pubertal timing may be abnormal in either direction: delayed puberty due to cortisol-induced hypogonadotropic hypogonadism, or, less commonly, androgen-driven premature adrenarche or peripheral precocious puberty resulting from ACTH-stimulated adrenal androgen excess (6,25).

Biochemical confirmation follows pediatric protocols: repeated 24h-UFC (adjusted for body surface area), assessment of diurnal cortisol rhythm (midnight serum or LNSC), and LDDST, interpreted against age- and assay-specific cut-offs after excluding exogenous glucocorticoid exposure (5). High-resolution pituitary MRI is recommended, although corticotroph microadenomas are often very small and may not be visualized in up to ~40–50% of pediatric CD (5). In adolescents with biochemically confirmed ACTH-dependent hypercortisolism and negative imaging, IPSS at specialty centers is recommended to confirm a pituitary source and, cautiously, assist with lateralization (5). Transsphenoidal surgery by an experienced pediatric neurosurgeon remains first-line therapy, offering high remission rates and low postoperative hypopituitarism (5).

Radiotherapy and medical therapy are reserved for persistent or recurrent disease, while bilateral adrenalectomy is considered solely for severe, refractory hypercortisolism or life-threatening situations (5). As with pediatric CD, lifelong follow-up is essential, as achieving biochemical remission does not equate to complete recovery. Management must extend beyond biochemical control to address catch-up growth, normalization of puberty and fertility, bone health, and the substantial psychosocial burden related to body image, mood, and academic performance (5,22,23,26). Ideally, these complex needs should be addressed

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within a structured multidisciplinary transition pathway to adult endocrine care (5,8).

## ADULT WOMEN

### Case vignette

A 32-year-old woman presented with progressive weight gain, oligomenorrhea, hirsutism, easy bruising, and uncontrolled hypertension. She reported two years of infertility and had been diagnosed with "polycystic ovary syndrome". An X-ray performed for chronic back pain revealed an atraumatic L1 vertebral fracture. Biochemical evaluation showed a 24h-UFC six times the upper limit of normal, elevated LNSC, and non-suppressed morning serum cortisol after LDDST. ACTH was elevated, and pituitary MRI revealed a 6-mm right-sided microadenoma. IPSS confirmed a central-to-peripheral ACTH gradient ≥ 3 after desmopressin stimulation with right-sided lateralization. She underwent successful selective transsphenoidal adenomectomy but developed postoperative glucocorticoid withdrawal syndrome characterized by severe fatigue and myalgias. Glucocorticoid replacement was temporarily increased to supraphysiological doses and was then gradually tapered. She also received thromboprophylaxis with low-molecular-weight heparin, physiotherapy, and bisphosphonates. Over the following year, she experienced substantial clinical improvement, including restoration of regular menstrual cycles.

### Mini-review

In young and middle-aged women, CD is most commonly caused by ACTH-secreting pituitary microadenomas (1,2). Clinically, patients typically present with classic features of hypercortisolism including central obesity, facial plethora, easy bruising, menstrual irregularities, hirsutism, mood changes, osteoporosis, and cardiometabolic comorbidities (1). However, many of these signs are nonspecific and overlap with lifestyle-related conditions such as metabolic syndrome (27). Several scoring systems have been proposed to enhance pretest probability (28,29), but despite high specificity, their low sensitivity in external validation limits clinical utility (30).

Current guidelines recommend screening adults with age-inappropriate comorbidities (e.g., early osteoporosis or hypertension), those with multiple or progressive features of Cushing's syndrome, and individuals with adrenal incidentalomas consistent with adenoma (11,31). Diagnostic evaluation relies on three first-line tests: LDDST, LNSC, and 24h-UFC, with at least two abnormal results needed to confirm the diagnosis (10,11). Notably, repeated testing (2–3 times) with 24h-UFC and LNSC may be required to detect or exclude Cushing's syndrome, as cortisol concentrations demonstrate substantial inter- and intraindividual variation (32,33); the most extreme anomalies are seen in rarer, cyclic Cushing's syndrome cases (34,35). High-resolution 3.0 and 7.0 Tesla MRI improves lesion detection, yet imaging remains negative or inconclusive in up to 27% of cases (36). In complex scenarios, molecular PET imaging may help localize the causative lesion (37,38).

In ACTH-dependent hypercortisolism, a pituitary macroadenoma confirms CD. If no lesion or a microadenoma-like lesion <6 mm is seen (findings occurring in up to 20% of the general population), further evaluation is required (39). IPSS remains the gold standard for distinguishing pituitary from ectopic ACTH secretion (10). The primary goals of CD treatment are remission of hypercortisolism, management of comorbidities and cardiovascular risk, restoration of hypothalamic-pituitary-adrenal axis function, preservation of fertility and pituitary function, and improvement of visual deficits in cases of suprasellar macroadenomas (40). Transsphenoidal surgery is the first-line treatment, while persistent or recurrent disease may require repeat surgery, radiotherapy, or medical therapies (10). These comprise steroidogenesis inhibitors (e.g., ketoconazole, levoketoconazole, metyrapone, and osilodrostat), pituitary-directed agents (e.g., pasireotide or off-label, cabergoline, with variable efficacy) (17), and, in selected scenarios, glucocorticoid receptor antagonists such as mifepristone (41–43). Bilateral adrenalectomy remains a definitive option for refractory disease or when rapid control of hypercortisolism is required (10). Postoperative management requires structured surveillance, as many patients experience prolonged morbidity even after biochemical remission. In the immediate postoperative period, morning cortisol and ACTH measurements are

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essential to confirm remission and guide glucocorticoid replacement (10).

Thromboprophylaxis using low-molecular-weight heparin is recommended (unless contraindicated) from diagnosis and continued for up to three months after biochemical cure, due to the sustained hypercoagulable state (44). During the first six months, clinical reassessment should address weight, blood pressure, mood, muscle strength, and fatigue, alongside evaluation of metabolic status and pituitary hormone axes (10,45). Glucocorticoid replacement must be tapered in line with hypothalamic-pituitary-adrenal axis recovery, with initially supraphysiological doses in the first weeks after surgery to manage glucocorticoid withdrawal symptoms (46). Lifelong follow-up is required, including annual biochemical screening for recurrence (LNSC or LDDST), and targeted management of cardiometabolic risk, bone fragility, cognitive dysfunction, and quality of life (10,45). Bone mineral density testing every 1–2 years is advised. Recurrence risk is highest within the first five years but persists lifelong, necessitating ongoing multidisciplinary follow-up (39,45).

Hypercortisolism substantially impacts social and occupational functioning: Large Scandinavian registry studies demonstrated reduced employment, increased sick leave and disability pensions up to six years before diagnosis and long after remission, with 20%–25% of patients requiring disability support (47,48). Long-term care should therefore address not only somatic health but also support functional recovery and reintegration into daily and occupational activities.

### Pregnancy in CD

Special considerations are required in certain circumstances such as pregnancy. Cushing's syndrome during pregnancy is rare but carries significant maternal (hypertension, diabetes, preeclampsia, venous thromboembolism) and fetal risks (miscarriage, preterm birth, growth restriction) (49–51). Diagnosis is challenging due to physiological cortisol increases in pregnancy. The most reliable tests are 24h-UFC (≥ 3× ULN) and midnight serum cortisol, with MRI (without gadolinium) for imaging (50). Second-trimester surgery is the preferred treatment for both adrenal Cushing's syndrome and CD. When surgery is not an option, metyrapone is the medical therapy of choice, although cabergoline may also be considered for CD (17). Pasireotide, levoketoconazole, and osilodrostat are contraindicated in pregnancy, with no safety data available. Mitotane is contraindicated due to teratogenicity and prolonged tissue persistence, requiring delayed conception after treatment. Mifepristone is contraindicated because of its antiprogestogenic effects, and there are no pregnancy data for relacorilant (17). Patients require intensive monitoring during pregnancy and structured cardiometabolic follow-up postpartum (49,50).

## ADULT MEN

### Case vignette

A 45-year-old man presented with resistant hypertension, new-onset type 2 diabetes, muscle weakness, and low libido. He reported easy fatigability and irritability. Physical examination revealed truncal obesity and mild facial plethora. Morning testosterone was low, with suppressed LH/FSH. Hypercortisolism was confirmed by elevated 24h-UFC, loss of salivary cortisol diurnal rhythm, and failure of morning serum cortisol suppression on LDDST. ACTH was elevated. MRI showed an 8-mm pituitary adenoma, and a desmopressin test confirmed a pituitary origin. He underwent transsphenoidal surgery. Postoperative adrenal insufficiency was managed with glucocorticoid replacement, with gradual recovery of testosterone levels.

### Mini-review

Cushing's disease is less common in men than women (female-to-male ratio 4:1) (52), but when it does occur, it may manifest with more pronounced clinical features including striae, hypertension, myopathy, osteoporosis, and kidney stones (53,54). Moreover, secondary hypogonadism (manifested as low libido, erectile dysfunction, and reduced testosterone concentrations) affects 75%–93% of men at diagnosis of Cushing's syndrome (55–58), and can serve as a surrogate marker of glucocorticoid excess (55). Diagnostic evaluation of CD follows the same algorithm as in women and

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therapeutic recommendations are also similar, with transsphenoidal surgery as first-line treatment (10,11). Most men recover from secondary hypogonadism during the first year after successful surgery (55–58). Thus, testosterone replacement may not be necessary at the time of Cushing's syndrome diagnosis if surgery is planned; however, continued postoperative surveillance is essential for early detection of persistent or new hypogonadism after treatment (e.g., postsurgical panhypopituitarism), with testosterone therapy guided by symptoms, biochemical assessment, and current guidelines (59). In adults with CD, GH testing should typically be delayed for 1–2 years after remission, as GH secretion often recovers spontaneously over time (24). GH replacement may improve body composition, lipid profile, bone density, and quality of life, but requires individualized decision-making given potential adverse effects on glucose metabolism (24). The choice of steroidogenesis inhibitor should consider local availability and potential side effects, as hypogonadism may worsen with ketoconazole use (41). Further management should focus on impaired erythropoiesis (55,60), persistent cardiovascular risk, myopathy, osteoporosis, mental health, and structured rehabilitation for return to work and social reintegration.

## ELDERLY PATIENTS (≥ 65 YEARS)

### Case vignette

A 73-year-old woman was referred from geriatrics for refractory hypertension, recurrent fractures, sarcopenia with frequent falls, and poorly controlled diabetes. She was described as "frail," exhibiting cognitive slowing and depression. Clinical examination revealed central adiposity, thin skin, multiple ecchymoses, and proximal myopathy. Bone densitometry showed osteoporosis with a T-score of -3.2. Hypercortisolism was confirmed by elevated LNSC and 24h-UFC. ACTH was elevated. MRI showed a 12-mm pituitary macroadenoma. Despite high surgical risk and progressive clinical decline, she underwent carefully planned transsphenoidal surgery. Postoperatively, mild hypercortisolism persisted due to residual tumor. Medical therapy with steroidogenesis inhibitors was initiated but discontinued because of side effects. The patient received bisphosphonates, physiotherapy, and psychiatric counseling.

### Mini-review

In older adults, the true prevalence of CD is likely underestimated, as many symptoms overlap with normal aging and common comorbidities. CD becomes less common as adrenal-dependent Cushing's syndrome increases with age (7,61,62). In ERCUSYN, only ~10% of all Cushing's syndrome patients and 8-9% of CD patients were ≥65 years (63). Aging also reduces the typical female predominance, with around 60% of elderly patients with CD being female. Older patients more often present with macroadenomas, including extrasellar lesions (~17%), while microadenomas are less frequent (~39%) (63). The clinical picture is less typical and is more driven by comorbidities. Compared with younger patients, elderly patients with Cushing's syndrome report less weight gain and show fewer skin changes and hyperandrogenism-related signs such as hirsutism, hair loss, and reduced libido (7,63). In contrast, hypertension, diabetes, myopathy, fractures, cardiovascular disease, and thrombosis are more frequent (63).

Cognitive decline is also common, and may only partially improve after treatment, with age and prolonged cortisol exposure contributing to persistent memory deficits (64,65). Depression appears to be less frequently reported in the elderly (63), likely due to underrecognition, as late-life depression often presents with predominantly somatic rather than mood symptoms. 24h-UFC supports the diagnosis of CD slightly less often in older than younger patients (~87% vs ~95%), and renal impairment, polypharmacy, and chronic illness may further complicate interpretation (7,63). Consequently, a high index of suspicion is required in older patients with new or worsening hypertension, diabetes, fractures, or unexplained muscle weakness, even in the absence of overt Cushingoid features. Invasive diagnostic and therapeutic procedures should be weighed cautiously in very frail patients, balancing potential benefit and risk. Management requires an individualized approach that considers surgical risk, life expectancy, and symptom burden. Transsphenoidal surgery remains first-line therapy but is used more selectively with age.

In ERCUSYN, ~31% of elderly CD patients never underwent surgery (vs ~11% of younger patients),

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with first-line medical therapy and/or radiotherapy chosen more frequently (63). In expert centers, elderly patients had acceptable complication rates but experienced lower remission after TSS (≈52% vs 65%) and shorter disease-free survival (63). To our knowledge, no large studies specifically address medical therapy in elderly CD patients; available data are from adult cohorts encompassing a broad age range, with outcomes rarely reported separately for older individuals. In clinical practice, agents such as metyrapone are generally considered effective in older patients (17), but safety and tolerability must be assessed individually given comorbidities and polypharmacy. Overall mortality is higher in the elderly, and older age is an independent predictor of death, mainly due to vascular events, infections, and tumor progression (63). Ongoing management should prioritize functional status, prevention of falls and fractures, cardiovascular risk reduction, and optimization of quality of life.

## CONCLUSIONS

Cushing's disease presents and progresses differently across the lifespan, leading to age-specific diagnostic challenges and requiring individualized therapeutic approaches. Symptoms frequently overlap with common developmental or age-related conditions, contributing to delays in diagnosis. Careful attention to age-related clinical features and test interpretation is crucial for accurate evaluation and tailored management. Mortality risk in CD varies with age but remains closely linked to persistent hypercortisolism and its systemic complications.

Mortality is generally low in pediatric Cushing's syndrome (≈2.5% over 2–4 years)most often related to sepsis (66) but children and adolescents remain susceptible to long-term cardiometabolic consequences that may persist into adulthood. In adults, excess mortality is well documented and is primarily attributable to cardiovascular disease, infections, and persistent hypercortisolism. In a nationwide cohort study with a median follow-up of 10.6 years, 18% of patients with CD died compared to 9% of matched controls (HR 2.1), with markedly worse outcomes in those who did not achieve remission (67). Mortality risk persists even among patients in remission, as observed in large contemporary cohorts (68), underscoring the importance of long-term management of comorbidities. Across all age groups, such management should be coordinated within a multidisciplinary framework that reflects the patient's developmental stage, comorbidities, and long-term risk profile. Recognizing these age-dependent distinctions is essential to support earlier detection and improve outcomes across the lifespan.

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invited review

# Hypophosphatemic rickets: diagnosis and treatment

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## ABSTRACT

Hypophosphatemic rickets (HR) represents a heterogeneous group of disorders characterized by renal phosphate wasting, impaired bone mineralization, and skeletal deformities. This narrative review provides an overview of phosphate homeostasis and the molecular mechanisms underlying HR, focusing on the role of Fibroblast Growth Factor 23 (FGF23) in regulating renal phosphate use and vitamin D metabolism. The clinical, biochemical, and genetic features of both FGF23-dependent and -independent forms of HR are discussed, including X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets (ARHR), tumor-induced osteomalacia (TIO), hereditary hypophosphatemic rickets with hypercalciuria (HHRH), and Fanconi syndrome. Advances in understanding these mechanisms have led to the development of targeted therapies, such as burosumab, which are redefining the clinical management of affected individuals.

**Keywords:** Burosumab; C-type natriuretic peptide; fibroblast growth factor 23; hereditary hypophosphatemic rickets with hypercalcuria; X-linked hypophosphatemia

## INTRODUCTION

Phosphate is a crucial mineral for skeletal development and metabolic health, playing a role in bone mineralization, cellular energy metabolism, and enzymatic activity (1,2). Phosphate homeostasis is maintained through a tightly regulated relationship between intestinal absorption (3), renal reabsorption, and storage as hydroxyapatite by bone remodeling (4,5). Fibroblast growth factor 23 (FGF23) acts as a key regulator (6,7). Disruption of this balance leads to hypo- and hyperphosphatemic disorders, as reviewed by us (8) and others (9,10). The focus of this review will be on hypophosphatemic rickets (HR), a group of disorders characterized by impaired bone mineralization due to impaired phosphate availability. HR disorders can be divided into disorders of phosphate absorption from the diet in the gut and due to chronic renal phosphate wasting, which in turn can be FGF23-dependent, Parathyroid Hormone (PTH)-dependent, and FGF23/PTH-independent, each with specific genetic and pathophysiological mechanisms (7,11,12). Differentiating HR subtypes is important for targeted and effective management strategies. This manuscript is structured as a narrative review. Rather than employing a formal systematic review of methodology, we conducted a focused, non-systematic synthesis of the literature to integrate novel mechanistic, clinical, and therapeutic insights across the spectrum of hypophosphatemic disorders. Emphasis was placed on studies elucidating hormonal regulation, genetic mechanisms, diagnostic algorithms, and emerging targeted therapies.

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## PHOSPHATE HOMEOSTASIS AND THE ROLE OF FGF23

Phosphate balance in the body is strictly regulated through coordinated actions in the intestine, kidney, and bone (13). FGF23 acts as a key regulator of renal phosphate excretion by inhibiting the renal sodium-dependent phosphate cotransporters NPT2a (encoded by *SLC34A1*) and NPT2c (encoded by *SLC34A3*) (14). FGF23 also suppresses the synthesis of calcitriol in the proximal tubules (15,16). Calcitriol refers to 1,25-dihydroxyvitamin D3 however, both

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ergocalciferol (D2) and cholecalciferol (D3) can be activated in the kidneys to the active hormone that regulates calcium (17). For the purpose of this review 1,25-dihydroxyvitamin D and calcitriol are used interchangably. By inhibiting calcitriol FGF23 reduces intestinal phosphate absorption, restoring elevated serum phosphate levels toward normal (18). Canonical action via Fibroblast growth receptor 1c (FGFR1c) requires klotho (KL) and heparan sulfate for the formation of quaternary complexes FGF23:KL:FGFR1c:HS 2:2:2:2, which is responsible for phosphate excretion in the kidneys (19,20). In addition, recent crystal structure studies suggest FGF23 may signal in a 1:2:1:1 asymmetric quaternary complex with FGFR3 or 4 (21) or even in a KL-independent manner in a tissue-specific fashion, which is responsible for non-canonical actions of FGF23 on calcitriol synthesis and cardiac remodeling (22) (**Figure 1**). PTH also inhibits NPT2a and NPT2c (23), however, because of its primary role in calcium homeostasis, it differs from FGF23 and stimulates calcitriol synthesis to restore low blood calcium levels toward normal (24). Calcitriol and phosphate, in turn, stimulate gene expression and secretion of bioactive FGF23 (25). Dietary phosphate is absorbed in the small intestine via the sodium-phosphate cotransporter NPT2b (30%) and via paracellular transport (70%) (3). Calcitriol enhances intestinal phosphate uptake by increasing the abundance of NPT2b at the apical membrane and by transcriptionally activating *SLC34A2*, the gene encoding NPT2b. This occurs through direct binding of the vitamin D receptor (VDR)/retinoid X receptor (RXR) heterodimer to vitamin D response elements within the *SLC34A2* promoter (26). Calcitriol induces expression of tight-junction components that facilitate paracellular calcium movement, thereby amplifying overall absorptive capacity (27). Conversely, paracellular transport of phosphate in the gut appears to be calcitriol-independent as discussed in the next chapter (28).

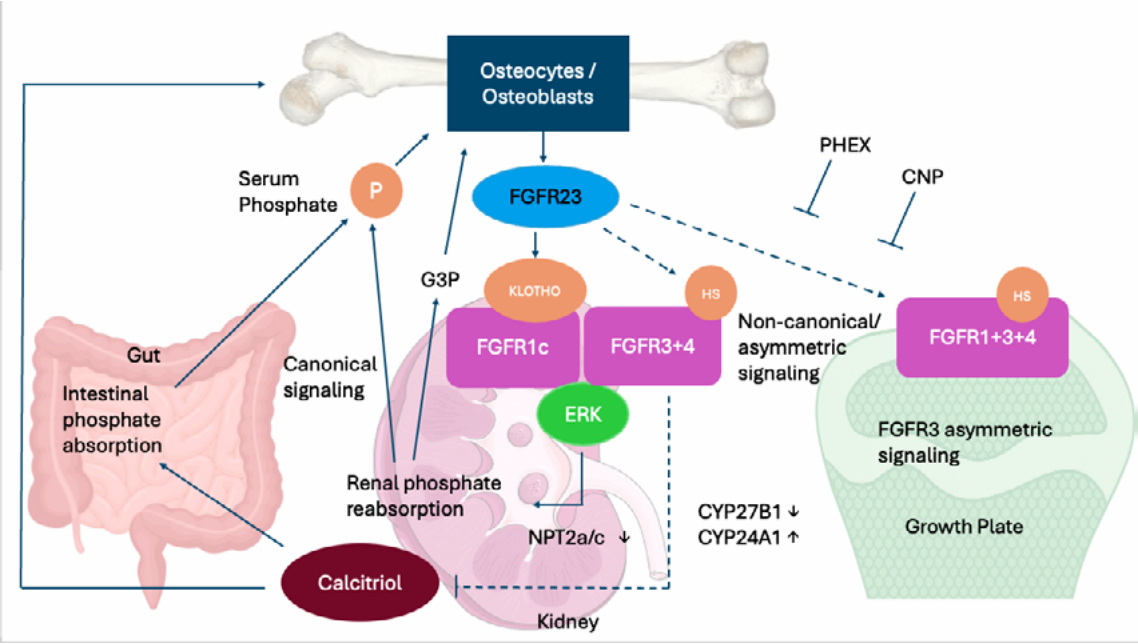



**Figure 1. Integrated regulation of phosphate homeostasis by FGF23 through canonical and non-canonical signaling pathways.** Osteocytes and osteoblasts secrete FGF23, which circulates to the kidney and growth plates of growing children. In the kidney, canonical signaling occurs through the formation of an FGF23-KLOTHO-FGFR1c-Heparin sulfate (HS) complex, activating ERK signaling to suppress NPT2a/NPT2c-mediated renal phosphate reabsorption. Non-canonical signaling via FGFR3 or 4 to decrease CYP27B1 while increasing CYP24A1, leads to reduced calcitriol synthesis. Lower calcitriol levels diminish intestinal phosphate absorption. FGF23 may also signal through non-canonical/asymmetric FGFR3-dependent pathways at the growth plates of growing children, which are independent of KLOTHO and modulated by HS. These pathways influence chondrocyte differentiation and may contribute to skeletal abnormalities in hypophosphatemic disorders. PHEX and C-type natriuretic peptide (CNP) act as modulators that oppose excessive FGF23 signaling in the growth plate. Serum phosphate feeds back on osteocytes to regulate FGF23 production, completing the homeostatic loop. G3P denotes glycerol-3-phosphate. Solid lines represent canonical, αKlotho-dependent endocrine signaling supported by genetic, biochemical, and structural evidence (20). Broken lines represent non-canonical, sometimes αKlotho-independent pathways (22).

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The phosphate-independent features of XLH resemble the *FGFR3* activation disorders (29), achondroplasia and hypochondroplasia and are likely caused by excess FGF23 activity at sites other than the kidney (22). Hyp mouse fetuses, a model of XLH, are not exposed to an hypophosphatemic environment *in utero*, but rather extremely high levels of FGF23 (30). The classic findings in newborn Hyp mice include craniofacial abnormalities and pelvic malformation, rather than rickets, and cannot be due to low ambient phosphate. This finding parallels the clinical observation in human children, in whom radiographic evidence of rickets (impaired mineralization of growth plate cartilage) is not evident until several months of age, after a prolonged exposure to hypophosphatemia, which is generally not apparent until several weeks of age. Conversely, their appearance at birth is similar to that of children affected with achondroplasia and hypochondroplasia; overlapping features include the head shape indicative of aberrant fusion of sutures of cranial bones, Chiari malformation, coxa vara, short stature, and prominent lumbar lordosis among others (8). Importantly, clinical studies in children and young adults with XLH have demonstrated preserved or increased bone mineral density (BMD), particularly at the lumbar spine and proximal femur (31). These findings occur early in life and cannot be attributed solely to enthesopathy or degenerative changes, which typically emerge later in adulthood. Rather, they likely reflect intrinsic alterations in bone modeling and mineralization associated with excess FGF23 signaling, aberrant FGFR3 pathway activation or PHEX deficiency (29,32). This observation further supports the concept that certain skeletal features of XLH are independent of hypophosphatemia.

Dysregulation of FGF23, whether through excessive secretion (6) or resistance to degradation (7), can lead to phosphate-wasting disorders, such as FGF23-dependent hypophosphatemic rickets. In contrast, loss of function of NPT2a and NPT2c transporters causes FGF23-independent hypophosphatemic rickets (12,33). Comprehending the molecular mechanisms of phosphate metabolism is essential for diagnosing and managing these conditions, as discussed in the following sections.

## DISORDERS OF PHOSPHATE ABSORPTION FROM THE DIET IN THE GUT

Phosphate absorption in the gastrointestinal tract occurs primarily in the small intestine, with both active transcellular transport and passive paracellular diffusion contributing to uptake (3). Approximately 30% of dietary phosphate is absorbed through the sodium-dependent phosphate cotransporter NPT2b (*SLC34A2*) located on the apical membrane of enterocytes in the jejunum and ileum (3). NPT2b activity is strongly regulated by calcitriol, which enhances transporter expression and increases active phosphate uptake (17).

Most phosphate absorption occurs via paracellular transport (~70%), which is dependent on electrochemical gradients and tight junction proteins, such as claudins (28). Sodium/hydrogen exchanger 3 (NHE3) indirectly contributes by modulating luminal pH and sodium flux, which influence phosphate permeability (28,34). Experimental data show that this pathway can be inhibited pharmacologically, such as Tenapanor, an NHE3 inhibitor developed for irritable bowel syndrome with constipation, which reduces intestinal phosphate uptake by decreasing paracellular phosphate permeability. It has also received approval in Canada and the United States for the management of hyperphosphatemia in patients with chronic kidney disease undergoing dialysis (28).

Several dietary and hormonal factors regulate intestinal phosphate absorption. Calcitriol is the primary stimulator, upregulating NPT2b and enhancing overall uptake (3,17). FGF23 indirectly reduces phosphate absorption by suppressing renal production of calcitriol, therefore decreasing NPT2b expression (15). Likewise, parathyroid hormone indirectly stimulates gut phosphate transport through it's positive regulation of calcitriol synthesis (35).

## GENETIC DISORDERS OF INTESTINAL PHOSPHATE ABSORPTION

Primary genetic causes of impaired intestinal phosphate absorption are rare but provide insight into phosphate physiology. Loss-of-function variants in *SLC34A2* (which encodes NPT2b) in humans cause pulmonary alveolar microlithiasis, but intestinal

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phosphate malabsorption does not appear to be a significant problem in these individuals (36). Animal studies, however, suggest that NPT2b ablation in the intestine alters systemic phosphate balance, especially under conditions of high dietary phosphate intake (3).

## ACQUIRED CAUSES OF IMPAIRED PHOSPHATE ABSORPTION

Acquired conditions are more commonly responsible for impaired intestinal phosphate absorption and can significantly contribute to hypophosphatemia. Vitamin D deficiency is the most frequent cause, as it diminishes 25-hydroxyvitamin D (25-OHD) levels, the substrate for calcitriol production, and as a result impairs active intestinal phosphate uptake (37). Importantly, calcitriol levels are not invariably low in vitamin D deficiency; they may be normal or even transiently elevated due to secondary hyperparathyroidism stimulating renal 1α-hydroxylase activity (38), particularly in early or moderate deficiency states. This mechanism is particularly relevant in nutritional rickets and may clinically overlap with hereditary HR. Dietary factors can also reduce intestinal phosphate availability, including excessive intake of phosphate binders such as calcium, sevelamer, or lanthanum (39,40), as well as diets high in phytate-rich foods like whole grains or legumes, which contain poorly absorbable organic phosphate (41). Several medications interfere with intestinal phosphate handling. For example, niacin therapy has been reported to reduce phosphate absorption by blocking Npt2b (42), while the NHE3 inhibitor Tenapanor lowers paracellular phosphate permeability (28,34). In addition, infants receiving specialized elemental formulas such as Neocate™ have been shown to develop phosphate deficiency and hypophosphatemic rickets, likely due to reduced intestinal phosphate bioavailability inherent to these preparations (43). Recognition of this complication has prompted reformulations of Neocate™.

## CLINICAL IMPLICATIONS

Although disorders of intestinal phosphate absorption are less common than renal phosphate wasting syndromes, they are an important consideration in the differential diagnosis of hypophosphatemic rickets. Key distinguishing features include low urinary phosphate excretion (reflecting intact renal conservation) and improvement with dietary supplementation or vitamin D repletion (38). In practice, patients with vitamin D deficiency, malabsorption, or exclusive elemental formula feeding should be carefully evaluated for concurrent phosphate deficiency to avoid misdiagnosis as renal HR (43).

## DISORDERS OF RENAL PHOSPHATE WASTING

For clinical diagnostic purposes, these disorders are best classified into FGF23-dependent, PTH-dependent, and FGF23/PTH-independent disorders (6,12). Because PTH-dependent phosphate wasting disorders are primarily calcium disorders, we will focus in this review on FGF23-dependent and FGF23/PTH-independent renal phosphate wasting disorders. PTH-dependent hypophosphatemic states include primary hyperparathyroidism, secondary hyperparathyroidism and tertiary hyperparathyroidism, which may be due to vitamin D deficiency or chronic kidney disease (44), in which elevated PTH promotes renal phosphate wasting via downregulation of proximal tubular phosphate transport (45).

## FGF23-DEPENDENT HYPOPHOSPHATEMIC RICKETS

FGF23-dependent hypophosphatemic rickets refers to a group of disorders characterized by overproduction or reduced degradation of FGF23, resulting in renal phosphate wasting and reduced calcitriol levels (6). Chronic hypophosphatemia impairs bone mineralization and causes bowing and short stature in children and stress fractures in adults (6,7). The most common forms of FGF23-dependent hypophosphatemic rickets include X-linked hypophosphatemia (XLH) (11), autosomal dominant hypophosphatemic rickets (ADHR) (7,46), autosomal recessive hypophosphatemic rickets (ARHR) (47), and tumor-induced osteomalacia (TIO) (6).

### X-linked hypophosphatemia (XLH)

X-Linked Hypophosphatemia (XLH) is the most prevalent form of hereditary hypophosphatemic rickets, caused by loss-of-function variants in the

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*PHEX* (phosphate-regulating endopeptidase homolog X-linked) gene (11). The PHEX protein is a membrane-associated metalloprotease expressed in osteocytes and odontoblasts. Primary biochemical studies demonstrate that *PHEX* has protease activity and can cleave mineralization-inhibiting ASARM peptides, supporting a physiological enzymatic role in regulating extracellular matrix mineralization (48). Loss-of-function variants in *PHEX* are associated with increased FGF23 expression in bone and renal phosphate wasting (25) and decreased vitamin D activation (15). XLH typically presents with rickets, progressive lower limb deformities, stress fractures and short stature, which are generally directly attributed to hypophosphatemia (49). Additional features include craniosynostosis and dental abscesses during childhood (50) and premature osteoarthritis and enthesopathies in adulthood (51), the mechanism of which is less clear and may in part be mediated by non-canonical signaling of FGF23 via *FGFR3* (38,52). At the biochemical level, patients exhibit low serum phosphate, elevated FGF23 levels, normal to low calcitriol, normal or elevated alkaline phosphatase and normal or elevated parathyroid hormone (PTH) levels (11,25,53). Diagnosis is based on these biochemical markers, characteristic clinical findings, and radiographic evidence of rickets (54), and confirmed by genetic testing for *PHEX* loss-of-function variants (11).

Conventional and burosumab therapy for XLH will be discussed below; however, specific issues in the disorder include improving dental hygiene, treating dental abscesses (55), and surgical correction of deformities in children (37). Sometimes growth hormone therapy is considered (56) as discussed below. Adults often require dedicated orthopedic care for pre-mature osteoarthritis and enthesopathies (57).

## Autosomal dominant hypophosphatemic rickets (ADHR)

Another FGF23-dependent subtype is autosomal dominant hypophosphatemic rickets (ADHR), which presents with variable expressivity and often delayed onset. ADHR is caused by missense variants in *FGF23* that affect arginine residues at positions 176 or 179 within the RXXR subtilisin/furin protease recognition site (7) These variants render FGF23 resistant to intracellular proteolytic cleavage, resulting in stabilization of intact biologically active FGF23 (58). However, resistance to proteolytic processing alone is insufficient to sustain FGF23 transcription which is tightly regulated in systemic factors, including serum phosphate levels (59). Chronic hypophosphatemia normally suppresses *FGF23* gene expression, even when the encoded protein is cleavage-resistant. Therefore, it is believed that additional environmental or physiological triggers are required for manifestation in ADHR.

Iron deficiency is the most well-characterized such trigger (60). Iron deficiency increases FGF23 transcription, but in healthy individuals intracellular proteolytic cleavage prevents accumulation of intact biologically active FGF23. In FGF23 variants causing ADHR render the RXXR subtilisin, furin cleavage site resistant to proteolysis, leading to increased production and reduced degradation of intact FGF23 protein, precipitating renal phosphate wasting and symptomatic hypophosphatemia (61). The requirement for iron deficiency as trigger explains the variable penetrance and fluctuating clinical course observed in ADHR (62).

Clinically, ADHR presents with less severe extremity deformities, muscle weakness, bone pain, and dental issues (7). Biochemical abnormalities include elevated FGF23, low serum phosphate, and normal to low calcitriol (6). Diagnosis is confirmed through genetic testing (7).

Treatment strategies for ADHR are like those for XLH, as specified below. In cases of iron deficiency, iron replacement therapy has been shown to reduce FGF23 levels and improve phosphate homeostasis (60).

## Autosomal recessive hypophosphatemic rickets (ARHR)

ARHR is a rare form of hereditary rickets, caused by *DMP1* (ARHR1) (46) or *ENPP1* (ARHR2) (47) loss-of-function variants. Both genes suppress FGF23 expression by mechanism not well understood, and loss-of-function variants in these genes lead to excessive FGF23 secretion (59) and renal phosphate wasting (63). Patients exhibit skeletal deformities, short

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stature, and dental abnormalities, such as those seen in XLH (46).

Diagnosis is based on biochemical markers, including low phosphate, high FGF23, and low to normal calcitriol (46,64). In addition, there may be characteristic clinical features associated with ENPP1 enzyme deficiency, which causes generalized arterial calcification of infancy (GACI) and osteoporosis in adults and the diagnosis is confirmed by genetic testing (65). Treatment consists of phosphate and vitamin D supplementation. There is ongoing research on targeted therapies, such as ENPP1-Fc enzyme replacement therapy (66).

## Tumor-induced osteomalacia (TIO)

In contrast to inherited forms, tumor-induced osteomalacia (TIO) represents an acquired FGF23-dependent disorder caused by mesenchymal tumors that secrete excessive amounts of FGF23. This leads to severe phosphate wasting and osteomalacia (6). Although described in children, TIO typically presents in adulthood with progressive bone pain, muscle weakness, and bone fractures (67). Biochemically, patients exhibit profound hypophosphatemia, elevated FGF23 levels, and suppressed calcitriol (6).

Diagnosis involves measuring serum FGF23 by venous sampling and localizing the tumor with functional imaging techniques, such as 68Ga-DOTATATE PET/CT scanning (68). Genetic testing of resected tumors in 60% of cases reveals somatic genetic rearrangements of *FGF1* or *FGFR1* with the fibronectin gene (FN1) (69), and in a single case the rearrangement of *NIPBL-BEND2* (70), which are thought to drive tumor-specific overproduction of FGF23.

## Current therapy

Treatment for FGF23-dependent hypophosphatemic rickets, including XLH, ADHR, and ARHR, has historically involved oral phosphate supplementation in combination with active vitamin D analogs such as calcitriol (31). This approach supports skeletal mineralization and improves rickets and osteomalacia of affected individuals, however, it is limited by the requirement for frequent dosing and side effects, including the development of secondary hyperparathyroidism and nephrocalcinosis (71).

Over time, prolonged secondary hyperparathyroidism may progress to tertiary hyperparathyroidism, characterized by autonomous PTH secretion resulting in hypercalcemia (72).

Medical management with active vitamin D analogs, calcimimetics (e.g., cinacalcet), or adjustment of phosphate dosing may improve secondary hyperparathyroidism, however, parathyroidectomy may be required in patients with tertiary hyperparathyroidism, particularly when hypercalcemia contributes to nephrocalcinosis (73).

Burosumab, a monoclonal antibody that inhibits FGF23, represents a significant advancement in the management of these conditions (74). Approved initially for both children and adults with XLH and, more recently, for tumor-induced osteomalacia (TIO) (75), burosumab directly targets the pathophysiology of FGF23 excess, leading to improved phosphate homeostasis, linear growth, physical function, and quality of life (76). Despite its benefits, limitations include high cost, limited availability in certain countries, and lack of long-term safety data. Moreover, it is not recommended for use in patients with severe kidney dysfunction and has not been fully validated for FGF23-mediated hypophosphatemic rickets subtypes other than XLH and TIO. Therefore, treatment must be individualized, and more research is needed to expand access and optimize outcomes across all forms of FGF23-dependant hypophosphatemia. In tumor-induced osteomalacia (TIO), the standard treatment is complete surgical resection of the responsible phosphaturic mesenchymal tumor, which typically results in rapid normalization of FGF23 levels and correction of hypophosphatemia (67). In unresectable cases, conventional therapy or burosumab may be used for symptomatic management (76) and chemotherapeutic approaches are needed for rare cases with metastatic TIO (77).

Special consideration should be given to treatment during transitional periods, particularly from late childhood to adolescence and from adolescence to adulthood in patients with genetic forms of hypophosphatemic rickets. During adolescence, puberty increases skeletal growth velocity, which may exacerbate deformities and functional impairment,

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necessitating careful adjustment of phosphate or burosumab dosing. Transition to adult care is another critical period, as medication adherence often declines and long-term complications such as enthesopathy, osteoarthritis, dental disease, and persistent bone pain may emerge despite apparent radiographic healing. Adult treatment goals shift from promoting linear growth to optimizing functional outcomes, minimizing pain, preventing fractures, and reducing extraskeletal complications. Structured transition programs and coordinated multidisciplinary follow-up are therefore recommended to ensure continuity of care and prevent disease progression across the lifespan (78).

### Emerging therapies and future directions for FGF23-dependent HR

The development of burosumab has shown that neutralizing FGF23 can markedly improve outcomes in XLH and TIO (74). Parallel strategies could include modulation of FGF23 cleavage and stability, such as pharmacologic agents that enhance proteolysis or prevent pathological stabilization of intact FGF23, with the goal of reducing hormone activity (79). But FGF23 neutralizing therapy leaves certain aspects of the phenotype unaddressed, namely the dental, craniofacial and chondrodysplastic features of XLH and tumor recurrence in TIO (75). Several investigational approaches aim to broaden the therapeutic landscape. Selective FGFR inhibitors, such as infigratinib, are being tested for TIO by targeting signaling downstream of FGF23 (77). Trametinib is a MEK-inhibitor shown to improve hypophosphatemia in patients with cutaneous nevus syndrome (80) and it may be suitable to treat other forms of FGF23-dependant hypophosphatemia as well.

C-type natriuretic peptide (CNP) analogs like vosoritide are being evaluated for their ability to improve the craniofacial and chondrodysplasia in patients with XLH, which may also improve adult height (81).

Gene-directed approaches aimed at correcting underlying genetic defects remain in preclinical stages. For example, *PHEX* replacement or correction of protease-resistant *FGF23* variants represent long-term prospects to restore normal phosphate metabolism at the molecular level.

## FGF23-INDEPENDENT HYPOPHOSPHATEMIC RICKETS

While FGF23-dependent mechanisms are responsible for most cases of hypophosphatemic rickets, a smaller group of disorders arises from FGF23-independent defects in phosphate utilization (82). These conditions directly impair renal phosphate reabsorption or vitamin D metabolism (18).

In contrast to FGF23-dependent forms, FGF23-independent hypophosphatemic rickets is characterized by intrinsic defects in renal phosphate transport or proximal tubular function rather than excess FGF23 activity (64). The most prominent conditions include hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (64), Fanconi syndrome (2), and other rare genetic causes (33).

### Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)

The best-known FGF23-independent disorder is hereditary hypophosphatemic rickets with hypercalciuria (HHRH), which is characterized by high-normal or frankly elevated blood calcium levels and hypercalciuria (83). HHRH is caused by loss-of-function variants in *SLC34A3*, which encodes the NPT2c transporter in the proximal tubules of the kidney (64). Loss of NPT2c leads to isolated renal phosphate wasting, resulting in hypophosphatemia, increased proximal tubular 1-alpha hydroxylase (encoded by *CYP27B1*) and reduced 24-hydroxylase (encoded by *CYP24A1*) activity, which may be a consequence of appropriately suppressed FGF23 levels (18). This causes elevated calcitriol, suppression of PTH, enhanced intestinal and decreased distal tubular calcium absorption, and hypercalciuria. Elevated calcitriol levels are a notable difference between HHRH and FGF23-related disorders, where calcitriol levels are typically low or inappropriately normal (6).

Clinically, patients present with rickets, nephrocalcinosis, and kidney stones in the first decade of life (83). Diagnosis is based on biochemical findings (low phosphate, high calcitriol, hypercalciuria, normal or low FGF23) and confirmed by genetic testing. Treatment includes phosphate supplementation alone. Unlike FGF23-related HR, active vitamin D analogs are not indicated given the already elevated $1,25(OH)_2D$ levels.

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## Fanconi syndrome and other causes of phosphate wasting

Fanconi Syndrome represents a generalized dysfunction of the proximal renal tubules, resulting in impaired reabsorption of multiple solutes, including phosphate, glucose, amino acids, and bicarbonate (2,84). Unlike other forms of HR, which primarily affect phosphate metabolism, Fanconi syndrome presents with a broader spectrum of metabolic derangements, including glycosuria, aminoaciduria, and non-gap metabolic acidosis. The condition may be congenital or acquired, with diverse etiologies including genetic, drug-induced, and systemic disorders.

Genetic causes of Fanconi syndrome present early in life, leading to progressive renal dysfunction, growth retardation, and metabolic bone disease. One well-characterized genetic form is Dent's disease, an X-linked recessive disorder caused by loss-of-function variants in the *CLCN5* or *OCRL* genes (85). Both impair proximal tubular endosomal trafficking and protein reabsorption. Patients with Dent's disease exhibit low molecular weight proteinuria, hypercalciuria, nephrocalcinosis and progressive renal failure, as well as phosphate wasting and rickets (86).

Cystinosis is another major genetic cause, an autosomal recessive lysosomal storage disorder caused by pathogenic variants in the *CTNS* gene, which encodes the lysosomal cystine transporter (87). Cystine accumulation within lysosomes lead to tubular damage and dysfunction, manifesting as Fanconi syndrome in infancy. Without treatment, cystinosis progresses to end-stage renal disease (ESRD) (88).

Acquired Fanconi syndrome can result from various environmental, pharmacological, and systemic insults that damage the proximal tubules. Drug-induced nephrotoxicity is a common etiology, specifically from agents like valproic acid, and expired tetracyclines. Also Tenofovir Disoproxil Fumarate (TDF), a common antiretroviral for HIV, has been strongly associated with proximal tubulopathy, characterized by phosphate wasting, proteinuria, and progressive kidney injury (89).

Heavy metal poisoning, specifically with lead, cadmium, or mercury, can induce proximal tubule dysfunction, leading to Fanconi syndrome. Chronic exposure to such metals is typically occupational or environmental, with long-term consequences including osteomalacia, anemia, and nephropathy (90).

Systemic conditions such as multiple myeloma (91), amyloidosis (92) and autoimmune disorders (like Sjögren's syndrome) (93) can further contribute to acquired Fanconi syndrome. Such conditions may cause protein accumulation or inflammatory damage within renal tubules (94).

Patients with Fanconi syndrome exhibit profound metabolic disturbances, often presenting with polyuria, polydipsia, rickets, growth failure, and muscle weakness due to chronic electrolyte imbalances. A diagnostic workup includes serum biochemical markers, urinary studies, imaging, and bone studies.

Treatment of Fanconi syndrome focuses on correcting metabolic abnormalities and managing the underlying cause. Phosphate and bicarbonate supplementation are common therapies, often used in conjunction with specific treatments for genetic or acquired conditions (84). In cases of cystinosis, cysteamine therapy significantly delays renal decline (87). Withdrawal of nephrotoxic agents is essential for drug-induced cases (2).

## Emerging therapies and future directions for FGF23-independent HR

For disorders caused by impaired renal phosphate transport or proximal tubulopathies, therapeutic innovation is focusing on transporter modulation and replacement and strategies to mitigate the pathophysiology. Proof-of-concept studies of ENPP1-Fc enzyme replacement suggest benefit for ENPP1 deficiency-associated rickets and arterial calcification by restoring extracellular pyrophosphate levels (66,95), and analogous pyrophosphate-based strategies may be relevant for mitigating renal calcification in patient with HHRH, as suggested by experimental studies in NPT2a-deficient mouse models (96). Additionally, case reports indicate that fluconazole (97) or rifampin (98) may lower excessive $1,25(OH)_2D$ and mitigate hypercalciuria in disorders characterized by excessive calcitriol, including *SLC34A3*-related HHRH. Advances in targeted delivery of gene therapies for *SLC34A3*, *CLCN5*, or *CTNS* could eventually enable

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curative strategies using specialized adeno-associated viruses (66) or CAR-T cells (99) to deliver the therapeutic. Progress in biomarker discovery and standardized functional assays will also be crucial to shorten the diagnostic pathway and align patients with the most appropriate therapy early in the disease course.

## HORMONAL ADAPTATIONS IN HYPOPHOSPHATEMIC DISORDERS

Accurate interpretation of serum levels of PTH, FGF23, 1, 25 dihydroxyvitamin D (1, 25(OH2)D) requires a clear understanding of expected hormonal adaptations. Recognizing typical patterns is critical for distinguishing primary disorders of parathyroid hormone excess, vitamin D deficiency, FGF23-mediated phosphate wasting, and intrinsic renal phosphate transport defects.

In primary hyperparathyroidism, elevated PTH drives renal phosphate wasting and increases calcitriol synthesis (13). Serum phosphate is typically low or low-normal, FGF23 may be secondarily elevated, and $1,25(OH)_2D$ levels are often high-normal or elevated due to PTH stimulation of CYP27B1 and inhibition of CYP24A1 (44).

In secondary hyperparathyroidism due to vitamin D deficiency, low 25-(OH)D results in reduced calcitriol production, impaired intestinal calcium absorption, resulting in compensatory elevation of PTH. Serum phosphate may be low due to PTH-mediated phosphaturia, FGF23 levels are generally low or normal low, and $1,25(OH)_2D$ is low or normal (37).

In FGF23-dependent hypophosphatemias such as XLH, excess intact FGF23 leads to renal phosphate wasting (6) and suppression of calcitriol synthesis (16). Serum phosphate is low, FGF23 is elevated, $1,25(OH)_2D$ is low or normal, and PTH is typically normal or mildly elevated secondary to calcitriol deficiency and chronic phosphate therapy.

In FGF23-independent disorders, such as HHRH, renal phosphate wasting occurs in the setting of low or suppressed FGF23. Reduced phosphate and suppressed FGF23 stimulate increased calcitriol production, leading to elevated $1,25(OH)_2D$ levels, enhanced intestinal calcium absorption, suppressed PTH, and hypercalcuria (83).

## DIAGNOSTIC APPROACH TO HYPOPHOSPHATEMIC RICKETS

The diagnostic evaluation of HR requires a comprehensive, stepwise approach to differentiate between intestinal and renal causes, which are further classified into FGF23-dependent and -independent renal causes. Due to the range of genetic, acquired, and metabolic disorders that can contribute to phosphate wasting, a structured assessment of biochemical markers, urinary studies, imaging findings, and genetic testing is required.

Measurement of circulating FGF23 plays a central role in differentiating FGF23-dependent from FGF23-independent hypophosphatemic disorders; however, interpretation requires understanding of available assay methodologies. Two main types of immunoassays are used in clinical practice: C-terminal assays and intact FGF23 assays. C-terminal assays detect both intact biologically active FGF23 and inactive or inhibitory C-terminal cleavage fragments, whereas intact assays specifically quantify the full-length active hormone (79).

In conditions such as iron deficiency or inflammation, FGF23 transcription may be upregulated with concurrent increased proteolytic cleavage (7). In these settings, C-terminal FGF23 levels may be elevated while intact FGF23 remains normal, potentially leading to diagnostic ambiguity. Conversely, in FGF23-dependent hypophosphatemic rickets (e.g., XLH, ADHR, TIO), intact FGF23 is inappropriately elevated relative to serum phosphate levels (6).

Assay standardization remains limited, and reference ranges differ between platforms, making interpretation assay-specific. Furthermore, FGF23 results should always be interpreted in conjunction with serum phosphate, calcitriol, urinary phosphate handling, and the clinical context.

Stepwise diagnostic evaluation

- Urinary studies
  - **Tubular reabsorption of phosphate (TRP):** calculated using paired fasting serum and second-morning void urine samples, whereas calcium excretion is typically determined from 24-hour urine collections when clinically indicated (18). Fasting samples are

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preferred to minimize postprandial variation. Urinary calcium excretion: elevated in FGF23-independent, normal in most FGF23-dependent HR.

- **Urinary glucose, amino acids, β2-microglobulin:** elevated in Fanconi syndrome, indicating generalized proximal tubular dysfunction.

- Serum biochemical markers
  - **Serum phosphate:** low in all forms of HR.
  - **Serum calcium** is typically normal; however, it may be low in patients with FGF23-dependent HR (37).
  - **Calcitriol:** normal to low in FGF23-dependent rickets, elevated in FGF23-independent forms (16).
  - **PTH:** usually normal, may be elevated (secondary hyperparathyroidism) in FGF23-dependent HR.
  - **Serum FGF23:** elevated in FGF23-dependent HR, normal to low in FGF23-independent forms.
- Genetic testing
  - **Genetic panels** using whole exome or whole genome sequencing offered by commercial or academic providers now screen several known genes for pathogenic variants (see **Table 1**).
- Imaging studies
  - **Skeletal radiographs:** used in children to show characteristic signs of rickets (metaphyseal widening, fraying, bowing deformities).
  - **Dual-energy X-ray absorptiometry (DXA):** used in adults and adolescents with closed growth plates to quantify reduced bone mineral density (BMD).
  - **Functional imaging** (e.g., $^{68}$Ga-DOTATATE PET/CT) can identify the re-sponsible phosphaturic mesenchymal tumor in patients with TIO by detecting somatostatin receptor expression (69).

## CLINICAL IMPLICATIONS

In addition to maintaining a high degree of clinical vigilance to the possibility of HR in patients presenting with unexplained rickets, bone pain, fractures, short stature, or dental abnormalities, especially when serum phosphate is low and alkaline phosphatase is elevated, a stepwise approach that incorporates biochemical assessment, FGF23 quantification, imaging, and genetic testing is recommended (82). Treatment should be guided by etiology, patient age, symptom severity, and risk of complications. Regular follow-up and multidisciplinary management are key to achieving optimal outcomes and minimizing long-term effects.

**Table 1.** Renal hypophosphatemic rickets: for clinical diagnostic purposes, these disorders are best classified into FGF23-dependent and FGF23-independent disorders

| Disorder | Inheritance | Gene Defect | Serum Phosphate | FGF23 | PTH | 25-OHD | 1,25 $(OH)_2D$ | Urinary Calcium | Key Features |
|---|---|---|---|---|---|---|---|---|---|
| Primary Hyperparathyroidism | Autosomal Dominant | *CAR, AP2S1, GNAS11* | ↓ / low-normal | ↑ / N | ↑↑ | N | ↑ | ↑ | Hypercalcemia, PTH-driven phosphaturia |
| Vitamin D Deficiency (Secondary HPT) | Autosomal Dominant or Recessive, acquired | *VDR, CYP27B1* | ↓ | ↓ / N | ↑ | ↓ | ↓ / N | ↓ / N | Secondary hyperparathyroidism |
| XLH | X-linked dominant | *PHEX* | ↓↓ | ↑↑ | ↑ / N | N | ↓ / N | N | Dental abscesses, craniosynostosis |
| ADHR | Autosomal Dominant | *FGF23* | ↓↓ | ↑ (CR) | ↑ / N | N | ↓ / N | N | Iron deficiency triggers onset |
| ARHR | Autosomal Recessive | *DMP1, ENPP1* | ↓↓ | ↑ | ↑ / N | N | ↓ / N | N | May overlap with GACI (*ENPP1*) |
| TIO | Sporadic, somatic | *FN1–FGFR1 / FN1–FGF1* | ↓ | ↑ | N | N | ↓ | N | Tumor localized by DOTATATE PET/CT |
| HHRH | Autosomal Recessive | *SLC34A3* | ↓↓ | ↓ / N | ↓ | N | ↑↑ | ↑↑ | Hypercalciuria, kidney stones |
| Fanconi Syndrome | Autosomal Recessive, X-linked, acquired | *CTNS, CLCN5, OCRL*, etc. | ↓ | N | N | N | Variable | Variable | Multi-solute wasting |

N: normal; GACI: generalized arterial calcification of infancy.

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## CONCLUSIONS

Hypophosphatemic rickets (HR) represents a diverse group of disorders unified by defective phosphate homeostasis, which ultimately impairs skeletal mineralization and leads to significant clinical consequences. The differentiation between FGF23-dependent and FGF23-independent etiologies is crucial in determining the optimal diagnostic and therapeutic approach, considering their distinct pathophysiological mechanisms, inheritance patterns, and treatment responses. Among FGF23-mediated disorders, X-linked hypophosphatemia (XLH) remains the most prevalent, while tumor-induced osteomalacia (TIO) is the most common acquired cause in adult-onset cases. Genetic defects in renal phosphate transporters characterize FGF23-independent forms, such as hereditary hypophosphatemic rickets with hypercalciuria (HHRH).

Early recognition and differentiation among HR subtypes are important for timely intervention, minimizing skeletal deformities, and improving patient quality of life. In addition to conventional therapy with phosphate ± active vitamin D analogs, the introduction of targeted therapies with burosumab has improved care in XLH and other FGF23-dependent forms of HR.

Despite these advances, several needs remain unmet. Standardized diagnostic algorithms, improved access to genetic screening, and long-term outcome data, particularly for novel therapies, such as fluconazole, rifampin, infigratinib, trametinib, and vosoritide, are necessary to optimize care. Research into gene editing technologies and FGF23 pathway modulators may hold curative potential for certain HR subtypes in the future. A collaborative effort across multiple clinical modalities is essential to advancing understanding and treatment of hypophosphatemic rickets.

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case report

# A novel activating somatic mutation in *EPAS1*, coding for HIF-2α, in a patient with a paraganglioma and sickle cell disease

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## ABSTRACT

Pheochromocytomas and paragangliomas (collectively referred as PPGLs) are highly heritable neoplasms arise from chromaffin cells of neural crest tissues; 40% of patients with PPGLs harbour germline pathogenic variants (PV), which up to 45% of patients exhibit somatic mutations in similar susceptibility genes. Endothelial PAS domain-containing protein-1 [also known as hypoxia inducible factor-2α, HIF-2α] is encoded by *EPAS1*, and along with other hypoxia-inducible factors (HIFs) acts as a key mediator in the cellular response to hypoxia. Gain-of-function mutations in *EPAS1* have been linked to the Pacak-Zhuang syndrome, congenital cyanotic heart disease and sickle cell anaemia. Hypoxia due to chronic anaemia and/or associated nephropathy in patients with sickle cell disease (SCD) may increase the expression of genes related to HIFs, thereby increasing susceptibility to the development of PPGLs. We describe a case of young female with a history of sickle cell anaemia and sickle cell nephropathy who was found to have a para-aortic mass. Histology confirmed the diagnosis of a paraganglioma. She did not exhibit somatic mutations of the common predisposition genes but demonstrated a likely pathogenic activating somatic *EPAS1* variant mutation. This case illustrates the predisposition of patients with SCD to PPGLs due to somatic *EPAS1* mutations, and should increase awareness of such tumours in these patients.

**Keywords:** *EPAS1* gene; HIF-2α; sickle cell disease; paraganglioma; hypoxia inducible factor

## INTRODUCTION

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumours originating from chromaffin cells derived from the neural crest, and are notable for their high metastatic potential. Approximately 40% of PPGLs are associated with germline pathogenic variant (PVs) in one of more than 20 genes, including *VHL*, *RET*, *SDHx*, *MAX*, *FH*, *NF1* and *TMEM127* (1). More recently, somatic PVs in similar genes have been identified in 30-45% of PPGLs (2,3). The *EPAS-1* gene, which encodes endothelial PAS domain-containing protein-1 (also known Hypoxia Inducible Factor-2α (*HIF-2α*) (4), plays a critical role in oxygen sensing and tumorigenesis. Mosaic gain-of-function mutations in *EPAS1* have been described in the Pacak-Zhuang syndrome, a disorder characterised by congenital erythrocytosis, somatostatinomas and multifocal PPGLs, as well as retinal abnormalities (5). In addition, somatic *EPAS1* mutations have been reported in patients with sporadic PPGLs (6,7).

Sickle cell disease (SCD) is a chronic haemoglobinopathy associated with intermittent and sustained

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hypoxia, resulting from recurrent vaso-occlusion and anaemia. It has been proposed that chronic hypoxia in SCD may promote the development of PPGLs through the acquisition of pathogenic somatic *EPAS1* variants (8). We report a case of a somatic *EPAS1* mutation in a patient with a paraganglioma and sickle cell anaemia, supporting a potential link between chronic hypoxic states and tumorigenesis.

## CASE PRESENTATION

A 33-year-old Afro-Caribbean lady presented to the emergency department with a history of left flank pain. She had no history of episodic flushing, palpitations, sweating or headache. She had moved to the UK from Nigeria approximately one year prior to her presentation. On examination, her blood pressure was 120/77 mmHg, pulse rate of 80 beats per minute. She had a history of sickle cell anaemia since childhood, with frequent hospital admissions associated with painful vaso-occlusive crises as a child, with a brief pause in her 20s followed by further episodes of vaso-occlusive crises. She also had suffered with several episodes of an 'acute chest syndrome', a life-threatening complication of sickle cell disease characterised by fever, cough, chest pain and new lung infiltrates on a chest X-ray, requiring blood transfusions. Due to her chronic and active disease, she also required exchange transfusion prior to any surgical intervention. She was started on hydroxyurea in Nigeria, but she took this only inconsistently. She had no pregnancies in the past, no sickle cell retinopathy and no skin ulcers. Her urine protein/creatinine ratio was 385.7 mg/mmol (normal range: < 15 mg/mmol for non-pregnant adults). Ultrasound of her kidneys was unremarkable.

Her blood test results revealed a haemoglobin of 73 g/L (normal range for women: 115-165 g/L), MCV (mean corpuscular volume) 91.4 fL (80-100 fL), white cell count 11.4 x $10^9$/L (normal range: 4.00 to 11.00 x $10^9$/L), urea 9.1 mmol/L (normal range: 2.5 to 7.8 mmol/L), creatinine 109 mmol/L, with an eGFR of 58 ml/min (normal value: > 80 mL/min).

She was restarted on hydroxyurea 1 gm once a day with a view to prevent further vaso-occlusive crises, ampicillin for infection prophylaxis, and ramipril 1.25 mg for renal protection. However, her routine radiological investigations revealed a right-sided soft tissue mass posterior to the inferior vena cava (IVC). Functional imaging with Fluoro-18-deoxy-glucose Positron Emission Tomography ($^{18}$FDG-PET) scanning and Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-octreotate ($^{68}$Ga-DOTATATE) scanning revealed the mass to be intensely FDG-avid, but DOTATATE non-avid (**Figure 1**).

Histologically, the retrocaval soft tissue core biopsy revealed extensive infiltration of fibrous tissue by a neoplasm composed of confluent irregular trabecular structures. Tumour cells had abundant amphophilic cytoplasm and small to medium-sized, round or oval nuclei without visible nucleoli. Immunohistochemistry revealed the tumour cells to be strongly and diffusely positive for chromogranin, synaptophysin, S100, GATA3, and SDHB-positive. The epithelial marker MNF116 was negative. The Ki-67 proliferation index was < 1%. All immunohistochemical findings confirmed the diagnosis of a paraganglioma (**Figure 2**).

Somatic mutation analysis identified a likely pathogenic variant in *EPAS1* (Exon 12, c.1594T>G, p.Tyr532Asp) with a variant allele frequency (VAF) of 39.9%, which was subsequently validated by Sanger sequencing (**Supplementary Figure 1**). According to ClinGen/CGC/VICC guidelines for somatic variant oncogenicity, the variant scores 6 points, and thus is considered probably oncogenic when the score is between 6 and 9 (9). Furthermore, previous functional studies have demonstrated that mutations affecting residues 530–532 impair prolyl hydroxylation of the HIF-2$\alpha$ protein, thereby disrupting its oxygen-dependent degradation. This evidence supported the pathogenic character of the variant.

She was referred for the surgical removal of this mass; however, due to her being asymptomatic, she declined the surgery and decided to be followed up and monitored for the mass in a surveillance clinic. Further diagnostic work up with plasma metanephrines and assessment of her genetic germline mutation studies was advised, but she opted not to pursue these further.

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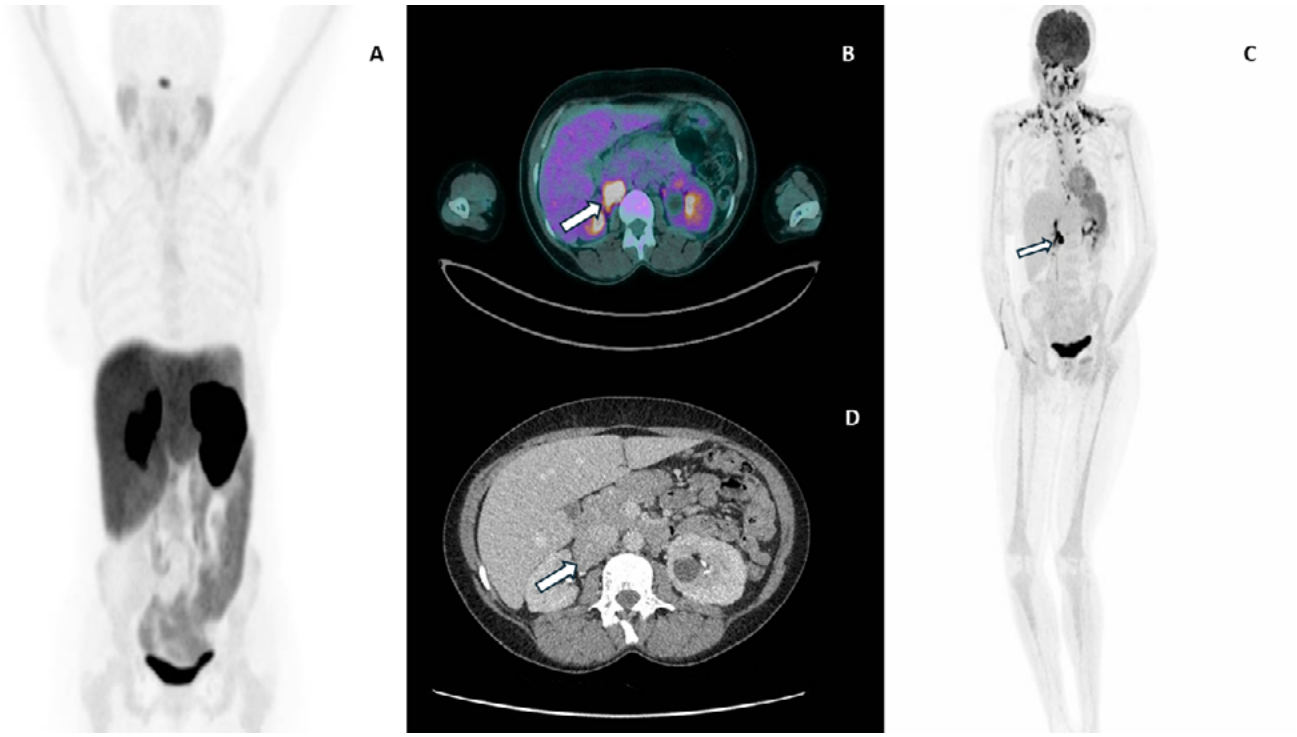



**Figure 1.** (**A**) $^{68}$Ga-DOTATATE scan shows no DOTATATE avid mass; (**B, C**) $^{18}$FDG-PET scan shows an intensely FDG-avid soft tissue mass on the right side (white arrow); **D**: CT abdomen and pelvis show a 26 x 34 mm mass adjacent to right renal vein, duodenum and inferior vena cava.

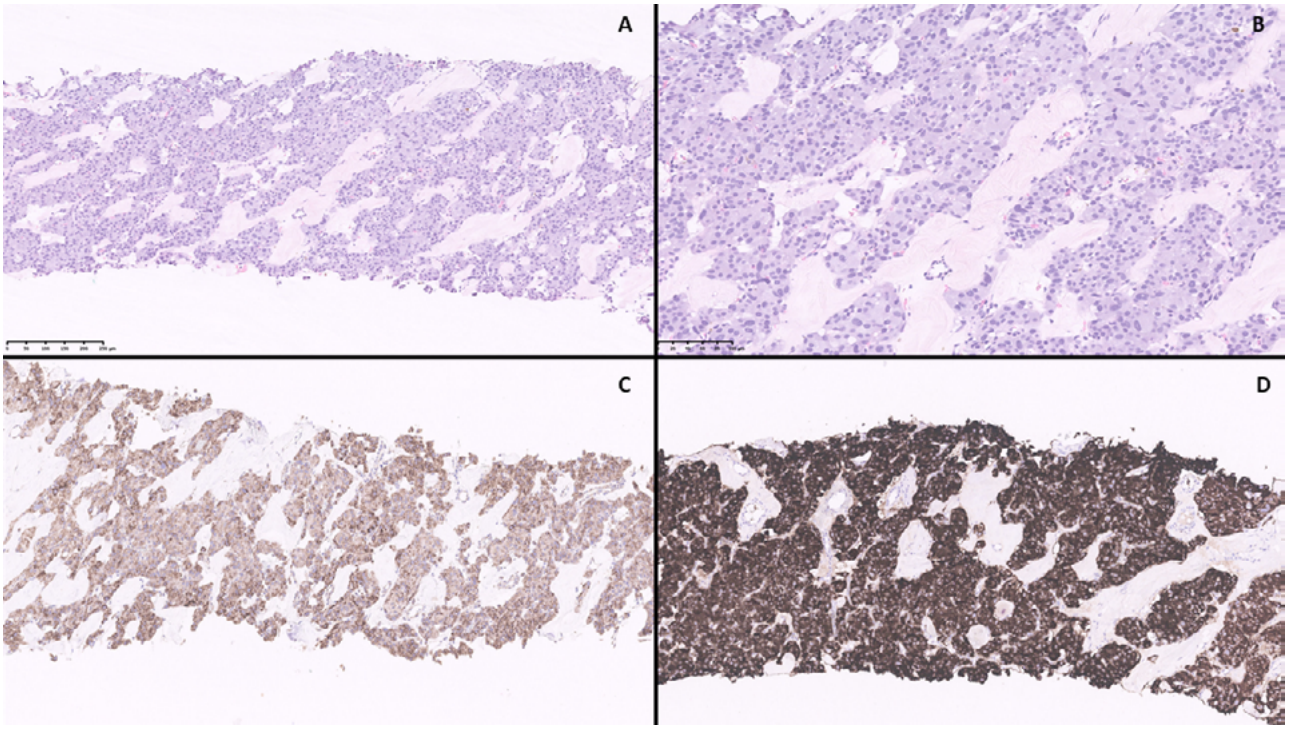



**Figure 2.** (**A**) H&E-stained section (x10) showing extensive infiltration by neoplastic cells. (**B**) H&E-stained section (x20) showing thick trabeculae of epithelioid cells divided by fibrous bands. (**C**) Immunohistochemistry (IHC) staining (x10) showing diffusely retained, cytoplasmic positivity for *SDHB*. (**D**) Immunohistochemistry (IHC) staining (x10) showing diffuse positivity for chromogranin A.

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## DISCUSSION

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumours of neural crest origin, arising from chromaffin tissues throughout the body. Their prevalence increases with age, with the highest incidence observed in individuals aged 60-79 years (10). Paragangliomas most often arise along the thoracic and abdominal sympathetic chains, with abdominal paragangliomas accounting for up to 50% of all cases (11). Rarely, they may be found in other locations such as the urinary bladder (12). Up to 25% of paragangliomas are functional, characterised by episodic secretion of catecholamines, including adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine.

This present case illustrates a clinically relevant association between sickle cell disease and *EPAS1*-mutated paragangliomas, supporting the emerging hypothesis that chronic hypoxia exerts selection pressure favouring gain-of-function mutations in the HIF-2α (*EPAS1*) pathway.

The cellular response to hypoxia is predominantly mediated by hypoxia-inducible factors (HIFs), transcriptional complexes which serve as master regulators of oxygen homeostasis (13). Endothelial PAS domain-containing protein 1 (*EPAS1*), also known as Hypoxia-Inducible Factor 2-alpha (*HIF-2α*), is an *HIF-α* subunit of the transcription complex HIF-2. The stability of HIF-α subunits is oxygen-dependent. At normal oxygen concentrations, prolyl hydroxylation of HIF-α at specific proline residues creates a binding site for Von-Hippel-Lindau (VHL) tumour suppressor protein, subjecting the subunit to proteasomal degradation (8,14). During hypoxic conditions, this prolyl hydroxylase activity is inhibited, allowing stable HIFα subunits to form HIF-dimer complexes (8,14). Failure of prolyl hydroxylation activity due to gain-of-function mutations at prolyl residues will result in accumulation of HIF, leading to persistent activation of hypoxia-responsive genes in normoxic conditions. HIF activity promotes angiogenesis and cell proliferation (8).

The somatic *EPAS1* variant identified in our patient (c.1594T>G, p.Tyr532Asp) affects position 532 on exon 12. This mutation has not, to our knowledge, been previously reported. Previously functional studies have shown that mutations in residues 530-532 disrupt prolyl hydroxylation of the HIF-2α protein (10). With this variant lying at the critical hydroxylation site, this likely leads to increased stabilisation of HIF-2α and HIF activity, and therefore the Tyr532Asp mutation is almost certainly pathogenic by the same mechanism. The 39.9% variant allele frequency in our case suggests significant clonal expansion, consistent with the driver role of *EPAS1* mutations in PPGL tumorigenesis.

Somatic *EPAS1* mutations exist in patients with isolated PPGL (10), but have also been identified in association with erythrocytosis (10). In the Pacak-Zhuang syndrome, somatic mosaicism of PVs in *EPAS1* appears to cause PPGLs alongside polycythaemia, with or without somatostatinomas (5). However, while this condition is rare, recent studies have established a compelling link between chronic hypoxic conditions and *EPAS1*-mutated PPGLs (5–8). The prevalence of PPGLs in patients with cyanotic congenital heart disease is up to four times higher than in the normal population (15). In patients with cyanotic congenital heart disease with PPGLs, Vaidya and cols. reported a strikingly high 80% *EPAS1* mutation prevalence among PGLs developing in these patients, with a 94% positive mutation rate in a subsequent Japanese series. These findings support the hypothesis that chronic hypoxaemia may foster a selective environment that promotes the clonal expansion of cells harbouring gain-of-function mutations in *EPAS1* (16,17). This principle may also apply in SCD: White and cols. identified four patients with SCD and PPGL, all of whom had an *EPAS1* mutation (8). In a more recent study involving 40 patients with *EPAS1*-mutated paragangliomas, haemoglobin disorders were identified in 59% of cases where electrophoresis data were available. Of these, 6 patients had SCD, 5 had sickle cell trait, and two were heterozygous for haemoglobin C disease (18). These findings further support the mechanistic hypothesis that sickle cell haemoglobinopathy carriers have an increased susceptibility to developing *EPAS1*-driven PPGLs, although the actual prevalence in SCD, a common disorder in many parts of the world, is unknown.

Clinically, these *EPAS-1*-mutated PPGLs in SCD/sickle cell carriers occur in younger patients with a

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female predominance (8). They are usually indolent, but the tumours do carry metastatic potential (19). Our patient's presentation aligns closely with these established patterns. The patient was a young female aged 33 years, the tumour was in the retroperitoneum and demonstrated typical histological features including a low proliferative index (Ki-67 < 1%) and retained *SDHB* expression. Current published studies and cases of *EPAS-1* mutation-related PPGL are summarised in **Tables 1** and **2**. While surgical treatment would be treatment of choice in these cases, some patients might want to avoid major surgical intervention, as in our case. However, knowledge of this somatic mutation is useful insofar as should there be tumour expansion, medical therapy with the HIF-2$\alpha$ antagonist belzutifan might be considered as treatment (20).

Limitations: Genetic data on possible germline mutations could have provided additional insights and enhanced the diagnostic accuracy. However, this was not performed due to patient preference.

In conclusion, this case contributed to the growing body of evidence linking EPAS1 PV/PPV and PPGLs in SCD by identifying a novel somatic pathogenic variant (p.Tyr532Asp). It expands the known spectrum of PVs associated with this clinically relevant context. Currently, routine molecular evaluation in PPGLs primarily includes germline testing. However, this case underscores the importance of incorporating somatic sequencing, particularly in patients with SCD and PPGL, to guide personalised management and improve diagnostic precision.

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**Data availability:** the data sets analysed in this study are included in the manuscript.

**Table 1.** Published studies on somatic mutations in *EPAS1* gene in patients with PPGL

| Year, country | Type of study | Number of patients with EPAS1 somatic variants and the disease associated with it | Description |
|---|---|---|---|
| USA, Brazil, Spain, 2018 (16) | RS | 4 out of 5 patients with PPGL and CCHD had somatic mutation in *EPAS1* gene | • 5 patients (age: 13 – 54 years) had synchronous PPGL and CCHD<br>• 4 patients (80%) out of 5 had somatic *EPAS1* mutation |
| Japan, 2022 (17) | RS | 7 patients with PPGL complicated by CCHD. A total of 16 PPGL samples were analysed for somatic and germline mutations for *EPAS1* | • *EPAS1* somatic mutation was found in 15 out of 16 CCHD-PPGL samples (94%).<br>• Median age of 26 years (18-46 years)<br>• Median cumulative duration of cyanosis of 26 years (range, 12-46 years) |
| UK, 2023 (8) | RS | 4 patients out of 128 patients with PPGL had simultaneous diagnosis of sickle cell anaemia and PPGL. They were included in the study for somatic *EPAS1* mutation | • *EPAS1* somatic mutations were found in all four patients.<br>• 3 out of 4 patients were female (75%)<br>• All were homozygous for HbSS<br>• 2-4 unplanned admissions to hospital for SCD related complications |
| France, 2024 (18) | RS | 40 patients with *EPAS1* related PPGL | • 4 patients had cyanotic congenital heart disease<br>• 13 out of 22 patients (59%) with haemoglobin electrophoresis results had haemoglobinopathies (6 SCD, 5 SCT, 2 heterozygous haemoglobin C disease) |

EPAS-1: endothelial PAS domain contain protein-1; Endothelial RS: retrospective; PPGL: pheochromocytoma and paraganglioma; CCHD: cyanotic congenital heart disease.

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**Table 2.** Published cases of pheochromocytoma or paraganglioma with haemoglobinopathies

| Year, country | Age at diagnosis, sex | Diagnosis | Somatic mutation status *EPAS1* | Description |
|---|---|---|---|---|
| 1990, United Kingdom (21) | 32 Female | PCC and HbSC disease | n/d | • History of intermittent headache, palpitations during pregnancy<br>• Right sided 4 cm PCC<br>• Patient underwent caesarean section at 35 weeks of pregnancy followed by right adrenalectomy |
| 2002, United Kingdom (22) | 22 Male | PCC and HbSS | n/d | • Patient with HbSS, required admission for frequent crisis<br>• Developed resistant hypertension, renal failure<br>• CT left adrenal PCC<br>• Removed successfully |
| 2003, Ireland (23) | 33 Female | PGL and HbSS | n/d | • 36 weeks pregnancy<br>• Presented with headache, malaise and hypertension<br>• Surgical removal of PGL post-partum<br>• No lymph node involvement |
| 2003, USA (24) | 13 | PCC and HbSS | n/d | • n/d |
| 2005, Germany (25) | 27 Female | PCC and HbS-beta thalassaemia | n/d | • Incidental right adrenal mass during pregnancy detected on US scan<br>• Asymptomatic pregnancy with uneventful delivery<br>• Right adrenalectomy post-partum |
| 2014, Sweden (26) | 43 Female | PCC | Somatic *EPAS1* variant c.1589C>A. Not present in germline DNA | • n/d |
| 2016, USA (27) | n/d | Extra-adrenal paraganglioma | n/d | • Metastatic PGL |
| 2019, Nigeria (28) | 34 Female | PGL and HbSS | n/d | • Patient presented with generalised abdominal pain and distension<br>• Left retroperitoneal mass with extensive mesenteric and omental LN involvement<br>• Laparotomy and resection<br>• Died of multi-organ failure |
| 2019, USA (29) | 46 Male | PGL | n/d | • Abdominal pain, refractory hypertension<br>• Surgical resection |
| Our case, UK | 33 Female | PGL with sickle cell nephropathy | Somatic *EPAS1* mutation variant c.1594T>G, p. Tyr532Asp | • Flank pain otherwise asymptomatic<br>• FDG avid and DOTATATE non-avid right infra-renal mass, no metastatic disease elsewhere<br>• Histology confirmed paraganglioma with Ki67 < 1% |

PCC: pheochromocytoma; HbSC: haemoglobin SC disease; HbSS; sickle cell anaemia; PGL: paraganglioma.

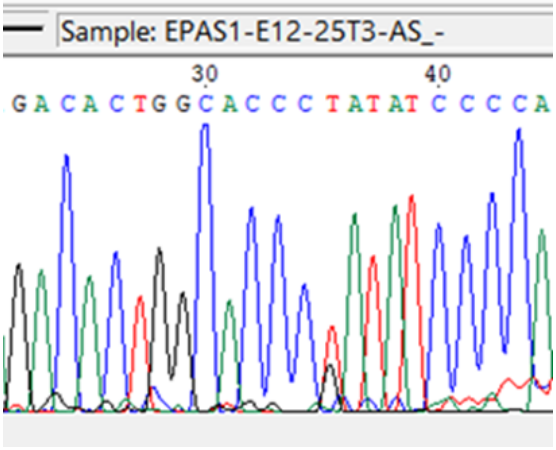
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## SUPPLEMENTARY MATERIAL




**Supplementary Figure 1.** According to ClinGen/CGC/VICC guidelines, the variant was classified as likely oncogenic. Furthermore, previous functional studies have demonstrated that mutations affecting residues 530–532 impair prolyl hydroxylation of the HIF-2α protein, thereby disrupting its oxygen-dependent degradation. This evidence supports the oncogenic character of the variant.

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case report

# Tirzepatide-induced ketoacidosis with hyperglycemia in a patient without diabetes

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## ABSTRACT

Tirzepatide, a dual GLP-1 and GIP receptor agonist, is increasingly used for weight management in both patients with diabetes and patients without diabetes. While gastrointestinal side effects such as nausea are common, severe metabolic complications like ketoacidosis are rare and often overlooked. We report the case of a 38-year-old woman with congenital heart disease who developed acute ketoacidosis with hyperglycemia following five months of tirzepatide therapy. Laboratory findings confirmed high anion gap metabolic acidosis, elevated ketones, and significant hyperglycemia. With supportive care, including intravenous fluids, insulin infusion, and electrolyte replacement, she recovered fully. This represents a rare case of tirzepatide-induced hyperglycemic ketoacidosis in a patient without diabetes. In contrast, the two previously reported cases by Singh and cols. and Iqbal and cols. involved patients without diabetes who developed euglycemic ketoacidosis with normal glucose levels while on tirzepatide. This case underscores the importance of vigilant monitoring for metabolic complications, including hyperglycemia and ketoacidosis, in patients without diabetes, particularly those with comorbidities.

**Keywords:** Tirzepatide, ketoacidosis; GLP-1 agonist; GIP; obesity pharmacotherapy; weight loss; adverse drug reaction

## INTRODUCTION

With obesity rates rising globally, new medications like tirzepatide are transforming weight management. Tirzepatide is increasingly utilized in both patients with diabetes and patients without diabetes for weight control (1,2). This once-weekly injectable targets GLP-1 and GIP receptors to reduce appetite, delay gastric emptying, and enhance glucose-dependent insulin release. It is effective for weight loss and glycemic control (3), though most adverse effects are gastrointestinal, including nausea, vomiting, and diarrhea (4).

Rare metabolic complications such as euglycemic ketoacidosis (EKA), characterized by acidosis and ketone accumulation despite normal blood glucose, have been reported. Two recent cases described EKA in women without diabetes using tirzepatide (5,6). We present a distinct case of a patient without diabetes with congenital heart disease who developed *hyperglycemic* diabetic ketoacidosis (DKA) while on tirzepatide. To our knowledge, this represents the first reported case of hyperglycemic DKA in a patient without diabetes associated with tirzepatide use.

## CASE PRESENTATION

A 38-year-old woman with a history of bicuspid aortic valve and repaired aortic coarctation arrived at the emergency department with two days of nausea, vomiting, abdominal pain, and constipation. She had no fever, diarrhea, or unusual dietary changes and had eaten very little in the prior 48 hours. She had been on Zepbound (tirzepatide) for five months, gradually increasing to 7.5 mg weekly, for weight management.

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Over the five-month span on tirzepatide, her weight dropped from 86 kg to 72 kg. She did not have a history of diabetes, glucose metabolism disorders, alcohol abuse, or eating disorders, and had never experienced acidosis before. She reported a family history of Hashimoto's thyroiditis in a maternal aunt and a first cousin. Her only allergy was to amoxicillin. On arrival, her vital signs were: temperature 97.4°F, heart rate 135 bpm, BP 177/102, respiratory rate 17 breaths per minute, $spO_2$ of 100 on room air, and BMI 26.7. She was dehydrated but alert and oriented. Her abdomen was moderately tender in the left lower quadrant, with no signs of peritonitis. A CT scan of the abdomen and pelvis showed no obstruction, perforation, or other acute pathology (**Table 1**).

Given her high anion-gap metabolic acidosis, elevated ketones, and hyperglycemia, we considered various differentials, including lactic acidosis, ketoacidosis (diabetic, alcoholic, or starvation), and toxin ingestion (methanol, ethylene glycol, salicylates), as well as renal failure. Lactic acidosis was present but insufficient alone to explain the degree of acidosis (lactate 4.7 mmol/L); renal function was near normal; toxic alcohol and salicylate levels were not clinically suspected based on history and presentation; and there was no evidence of sepsis or shock. Alcoholic ketoacidosis was excluded by her negative alcohol history, and starvation ketosis alone seemed unlikely to account for the severity of metabolic derangement, though her recent poor intake may have contributed or precipitated the event in conjunction with tirzepatide's appetite-suppressing effects. After these considerations, our leading diagnosis was tirzepatide-associated ketoacidosis with hyperglycemia. She also had a Naranjo Adverse Drug Reaction Probability scale of 3, indicating a possible ADR.

**Table 1.** Initial laboratory findings on admission

| Parameter | Value | Reference range |
|---|---|---|
| WBC | 27,000/μL | 4,000–11,000 |
| Hemoglobin | 16.9 g/dL | 12.0–15.5 g/dL |
| Hematocrit | 51.6% | 35–45% |
| Platelets | 381,000/μL | 150,000–400,000/μL |
| Neutrophils | 92% | 40–75% |
| Chloride | 116 mmol/L | 98–106 mmol/L |
| Phosphorus | 2.0 mg/dL | 2.5–4.5 mg/dL |
| Potassium | 5.4 mmol/L | 3.5–5.0 mmol/L |
| Bicarbonate ($CO_2$) | 10.6 mmol/L | 22–29 mmol/L |
| Glucose | 285 mg/dL | 70–110 mg/dL |
| Anion gap | 26.4 mmol/L | 8–16 mmol/L |
| Lactate | 4.7 mmol/L | 0.5–2.2 mmol/L |
| Beta-hydroxybutyrate | 62 mmol/L | <0.6 mmol/L |
| Total protein | 9.9 g/dL | 6.0–8.3 g/dL |
| HbA1c | 5.1% | ≤ 5.6% |
| Urine Glucose | > 1000 mg/dL | Negative |
| Creatinine | 1.2 mg/dL | 0.55-1.02 mg/dL |
| BUN | 9 mg/dL | 7-18 mg/dL |
| AST/ALT | 0.66 | 0.7-1.2 |
| ABG ($PCO_2$) | < 18 mmHg | 35-45 mmHg |
| ABG (pH) | 7.13 | 7.35-7.45 |
| Urine Ketones | > 150 | Negative |

She was admitted to the Intensive Care Unit and management followed the current standard diabetic ketoacidosis (DKA) protocol, including aggressive intravenous fluid resuscitation to correct dehydration, continuous insulin infusion to reduce blood glucose levels and suppress ketogenesis, and careful electrolyte monitoring, particularly potassium replacement to prevent hypokalemia. Frequent laboratory assessments guided adjustments in therapy until metabolic parameters normalized, including blood glucose levels consistently below 200 mg/dL, closure of the anion gap (less than 12 mEq/L), serum bicarbonate levels rising above 18 mEq/L, and absence of ketones in serum. These criteria guided the safe transition from intravenous insulin to subcutaneous therapy and ultimately marked clinical and biochemical recovery. She was discharged on day 5 with instructions to stop tirzepatide and follow up with endocrinology.

After discharge, the patient underwent additional outpatient evaluation to assess for an underlying predisposition to ketosis and to exclude LADA-type diabetes. IA-2, glutamic acid decarboxylase 65, and ZnT8 antibodies were all within normal limits, and a repeat hemoglobin A1c was 5.3%, indicating preserved long-term glycemic control. While no Oral Glucose Tolerance Test was performed, and serum insulin and C-peptide were not measured, the completed tests argue against autoimmune-mediated beta-cell dysfunction or chronic insulin deficiency, making an underlying insulinopenic state unlikely in this patient.

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## DISCUSSION

Euglycemic ketoacidosis involves high anion gap acidosis and elevated ketones without significant hyperglycemia (7). While often linked to SGLT2 inhibitors (7-11), it has now been seen with GLP-1 and dual GLP-1/GIP agonists like tirzepatide (5,6). In this case, the patient had two days of severe nausea, vomiting, and abdominal pain, during which she had little food intake. This reduced food intake, likely due to tirzepatide's appetite suppression as well as known side effects, triggered fat breakdown and ketone production, similar to starvation states (12). Unlike diabetic ketoacidosis, which results from severe insulin deficiency, this ketoacidosis can occur with relative insulin sufficiency, allowing partial ketone suppression (8). This case differs from those by Singh and cols. (5) and Iqbal and cols. (6), who reported EKA with normal glucose levels (5.6 mmol/L and 4.9 mmol/L, respectively). Singh and cols. described a 48-year-old woman with EKA after titrating to 5 mg weekly (5). Iqbal and cols. reported a 21-year-old with no comorbidities who presented with EKA (6). Our patient, however, had a glucose of 285 mg/dL, despite a normal HbA1c of 5.1% and no diabetes history.

To our knowledge, this is the first reported case of hyperglycemic ketoacidosis in a patient without diabetes following tirzepatide use. The explanation for hyperglycemia in this patient may include increased stress hormones like cortisol. In fact, Weiss and cols. reported rare cases of extreme stress hyperglycemia in the setting of acute illness (13). Dungan and cols. also described hyperglycemia due to stress-induced counter-regulatory hormones, such as cortisol and epinephrine, which promote hepatic glucose production and insulin resistance in acute illness (14). This interplay of stress-induced hyperglycemia and tirzepatide-driven ketogenesis gives a possible explanation to the novel presentation of hyperglycemic ketoacidosis in this patient without diabetes.

In addition to reduced oral intake and stress-related hyperglycemia, another theoretical explanation is the presence of polymorphisms in the GIP receptor, which could lead to excessive stimulation of glucagon secretion and relative suppression of insulin secretion. In this scenario, the observed hyperglycemia might be driven primarily by increased hepatic glucose production rather than by peripheral insulin resistance or caloric restriction alone. Although this mechanism is speculative and was not directly assessed in this patient, including it provides a biologically plausible pathophysiologic explanation and strengthens the overall clinical interpretation.

However, the definitive cause of hyperglycemia in our patient is uncertain. This case expands the known risks of tirzepatide, showing ketoacidosis can occur later in treatment, in complex patients, and with varying glucose levels. Her high white blood cell count and lactate initially suggested sepsis, but no fever, infection, or abnormal imaging supported this. Antibiotics were discontinued once a non-infectious cause was clear.

The patient was successfully treated for tirzepatide-induced hyperglycemia using the standard diabetic ketoacidosis (DKA) protocol, which included intravenous fluids, insulin infusion, and electrolyte management, leading to normalization of glucose levels, anion gap closure, and resolution of ketosis. However, this case underscores the emerging challenge of managing DKA presentations linked to GLP-1 receptor agonists like tirzepatide, which may manifest with atypical features such as euglycemia or variable glycemic patterns.

These observations highlight the potential need for developing updated or adjunctive treatment protocols tailored specifically for GLP-1–associated DKA to optimize patient outcomes and guide clinicians in managing these increasingly recognized presentations. Potential changes include earlier and more frequent monitoring of ketones regardless of glucose levels, more cautious insulin dosing to avoid hypoglycemia, and a greater emphasis on identifying and correcting precipitating factors unique to GLP-1 therapies, such as dehydration from gastrointestinal side effects. Additionally, protocols might incorporate extended monitoring after initial resolution due to the prolonged pharmacodynamic effects of these agents. These adaptations aim to improve detection, treatment safety, and outcomes in this evolving clinical context.

While the patient's poor oral intake undoubtedly contributed to her ketosis, the direct pharmacological

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effects of tirzepatide on metabolic pathways, independent of starvation, must be considered, especially given the patient's hyperglycemia. The drug's dual agonism of GLP-1 and GIP receptors alters insulin and glucagon secretion, potentially disrupting the balance that prevents excessive ketogenesis. Given the limited number of reported cases on this topic, the exact mechanism by which tirzepatide directly induces ketoacidosis is not yet fully understood. Further research is warranted to understand the direct pharmacological effects of this class of drugs on ketone body metabolism, especially in patients without diabetes.

## CONCLUSION

Tirzepatide is highly effective for weight loss and glycemic control, but its appetite-suppressing effects can lead to serious metabolic complications. Clinicians should consider ketoacidosis in patients on GLP-1 or GIP therapies presenting with nausea, vomiting, or acidosis, regardless of diabetes status or glucose levels. This case highlights the importance of vigilant monitoring, especially in patients with complex medical histories, to distinguish from euglycemic cases and ensure timely treatment.

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**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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brief report

# Cushing's syndrome: a systematic review of psychiatric and cognitive symptoms in case studies

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## ABSTRACT

**Objective:** Cushing's syndrome, characterized by chronic hypercortisolism, is associated with various health risks, including psychiatric symptoms and cognitive impairments. This systematic review of case studies aimed to map and categorize these symptoms. **Materials and methods:** We hypothesized that a broader range of psychiatric and cognitive manifestations would be observed beyond anxiety, depression, and memory impairment. The review followed PRISMA guidelines and was preregistered in PROSPERO (CRD42024433186). We conducted searches in PsycINFO, Embase, PubMed, and Scopus, identifying 273 potentially relevant studies. After screening, 66 studies were included, comprising 74 cases (81% female; mean age 35.7 years, range 13–81). **Results:** Results revealed that 93% of cases presented psychiatric complaints, including depression (39.2%), psychosis/schizophrenic symptoms (35.1%), suicidal ideation/attempts (20.3%), anxiety (17.5%), panic attacks (2.7%), and post-traumatic stress disorder (1.4%). Cognitive complaints were reported in 32% of cases, primarily as general cognitive complaints (18.9%), memory impairment (9.5%), and attentional deficits (5.4%). An overlap of psychiatric and cognitive symptoms was observed in 26% of cases. **Conclusion:** This review underscores the clinical relevance of symptoms such as mania, psychosis, and suicidal behavior in Cushing's syndrome, which are often underreported. Individualized clinical assessment informed by these case studies is crucial for comprehensive management that extends beyond the typical focus on depression and memory. Moreover, greater awareness of the full spectrum of neuropsychiatric manifestations in hypercortisolism is needed.

**Keywords:** Cushing's syndrome; psychiatric disorders; cognitive impairment; case studies; systematic review

## INTRODUCTION

Cushing's syndrome (CS) is a rare neuroendocrine disorder, with an estimated prevalence of 39.1 per million (1), characterized by prolonged exposure to elevated cortisol levels (2). It may cause multiple serious health risks, including cardiovascular, metabolic, musculoskeletal, and skin disorders (3). Furthermore, CS presents a wide range of symptoms, with some studies reporting psychiatric manifestations, primarily anxiety and depression (4), and cognitive impairments, most commonly affecting memory (5).

Case studies can provide a broader scope of highly relevant data for studying rare diseases, as they allow exploration of less common but equally or more severe and clinically significant symptoms (6). A systematic review of case studies is particularly justified for rare diseases due to their low prevalence, clinical and diagnostic heterogeneity, and the scarcity of randomized controlled trials and observational studies with large enough samples to generate robust evidence. Moreover, earlier systematic reviews examining larger samples of CS have provided limited information on less common psychiatric symptoms such as mania and psychosis. Therefore, this study aimed to map psychiatric and cognitive symptoms reported in case studies of patients with CS.

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## MATERIALS AND METHODS

This systematic review included case studies and series published in Portuguese, English, or Spanish that reported psychiatric and/or cognitive symptoms in patients with CS of all ages and sexes, regardless of disease stage. Studies were excluded if they were unavailable online, incomplete, or did not meet the first seven objective items of the Critical Appraisal Checklist for Case Reports (7). Item 8, 'take away lessons', was excluded due to its subjective nature and reduced relevance to methodological quality and risk of bias assessment.

The methodology adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (8) and was preregistered under the International Prospective Register of Systematic Reviews (PROSPERO) registration no. CRD42024433186, covering studies published up to April 2024. Database searches were conducted in April 2024 using the terms "cogn*" OR "psyc*" AND "Cushing" in PsycINFO, Embase, PubMed, and Scopus. Data selection and analysis were conducted by two blinded researchers, with conflicts resolved by a third reviewer in Rayyan (9). Data from the selected studies were systematically tabulated in Excel. Detailed information regarding the article selection process is provided in the PRISMA Flow Diagram (see **Supplementary Figure 1**).

## RESULTS

A comprehensive search yielded 273 potentially relevant case studies. After screening based on predefined inclusion criteria, 66 case studies were included in this systematic review. Most included studies originated from the United States (n = 26), with the remainder primarily from Europe and none from Latin America. One study included a case series of nine patients, resulting in 74 individual cases analyzed. Of these, the method of cortisol measurement was explicitly detailed in 71 cases (97.2%). Regarding diagnostic imaging, 34 cases (46%) utilized magnetic resonance imaging, 31 (41.9%) used computed tomography, and 16 (21.6%) used both imaging methods.

The participants were diagnosed by an endocrinologist and exhibited psychiatric or cognitive symptoms identified during hospital evaluation. Of the 74 cases, 60 were female (81%). Ages ranged from 13 to 81 years (mean = 35.7 years; standard deviation = 15.9). Analysis of reported symptomatology revealed that a substantial majority (n = 69, 93%) presented psychiatric complaints. Cognitive complaints were reported in 24 cases (32%). Notably, 19 cases (26%) presented both psychiatric and cognitive symptoms (**Table 1**).

The distribution of psychiatric symptoms (**Figure 1**) indicated that the most frequently reported were depression (n = 29, 39.2%), followed by psychosis/schizophrenic symptoms (n = 26, 35.1%), suicidal ideation and/or attempts (n = 15, 20.3%), and anxiety (n = 13, 17.5%). Less frequently reported psychiatric symptoms included seasonal bipolar disorder/mania (n = 7, 9.5%), fatigue (n = 6, 8.1%), panic attacks (n = 2, 2.7%), and post-traumatic stress disorder (n = 1, 1.4%). Regarding cognitive functioning, the most commonly reported symptoms were general cognitive complaints (n = 14, 18.9%), memory impairment (n = 7, 9.5%), and attentional deficits (n = 4, 5.4%).

**Table 1.** Distribution of psychiatric, cognitive, and overlapping symptoms (psychiatric and cognitive reported in the same patient) by etiology

| Etiology | Case studies (n) | Symptoms (n) | | |
|---|---|---|---|---|
| | | Psychiatric | Cognitive | Overlap |
| Disease | 35 | 32 | 17 | 14 |
| Adrenal | 25 | 23 | 3 | 2 |
| Ectopic | 10 | 10 | 3 | 3 |
| Lung | 8 | 8 | 3 | 3 |
| Pancreas | 1 | 1 | 0 | 0 |
| Undetectable source | 1 | 1 | 0 | 0 |
| Exogenous | 4 | 4 | 0 | 0 |
| All etiologies | 74 | 69 | 23 | 19 |

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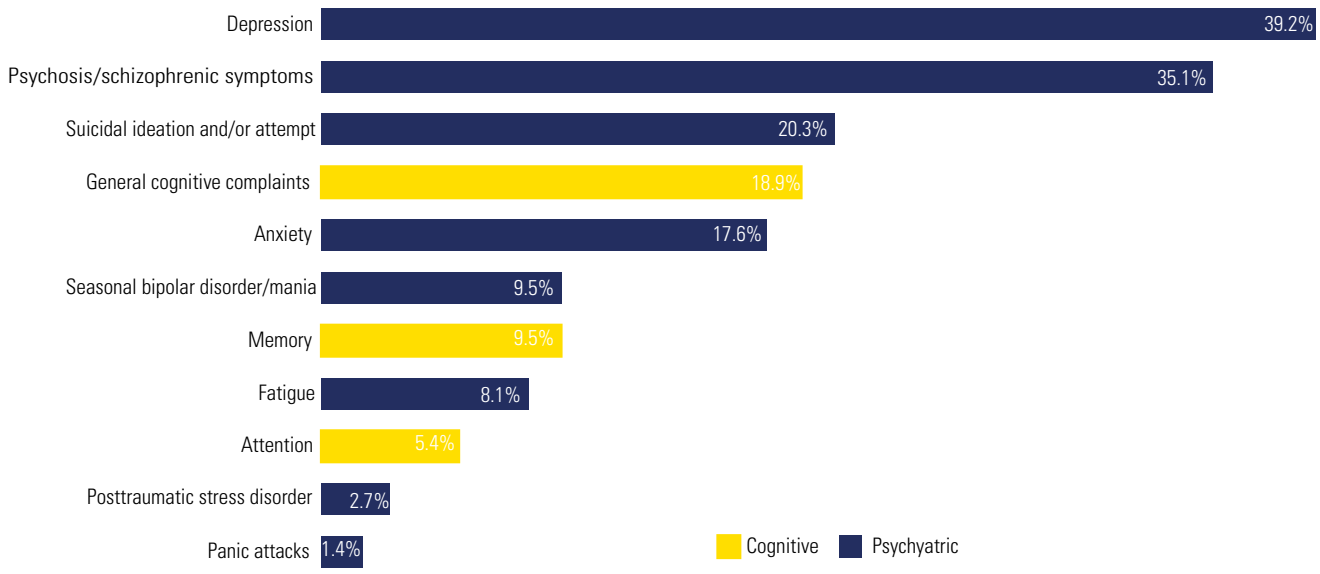



**Figure 1.** Frequency of specific psychiatric or cognitive symptoms reported in published case studies of Cushing's syndrome.

## DISCUSSION

This review aimed to map and categorize the cognitive and psychiatric dysfunctions in CS through a systematic review of case studies. In addition to the high rates of anxiety and depression widely documented in the literature, we identified an increased occurrence of psychosis and suicide attempts, as well as various other less common symptoms. Most articles reporting cognitive complaints did not specify them, limiting interpretation as to whether difficulties pertained exclusively to memory or encompassed other cognitive domains. Over one-quarter of participants experienced both cognitive and psychiatric symptoms, highlighting the complex interplay between these domains. Analysis of cognitive functioning revealed a heterogeneous presentation, with varying degrees of impairment across different domains. Cognitive complaints were reported in 18.9% of cases, encompassing a range of difficulties identified through physician observation or self-report instruments. Notably, the majority of included studies relied on the Mini-Mental State Examination rather than comprehensive or objective neuropsychological assessments, with only one study employing the Trail Making Test (10). This lack of specificity is attributable to the absence of formal neuropsychological testing in most case reports. Consequently, the true nature of cognitive deficits in CS may be inadequately characterized by this literature. Difficulty concentrating was the most frequently reported cognitive complaint (11–14). Although less common, memory and attention deficits were also present, consistent with previous studies associating these impairments with neurocognitive dysfunction in CS (14,15).

This study underscores the need for greater awareness of the full spectrum of psychiatric manifestations in hypercortisolism. Nearly 40% of cases exhibited depressive symptoms, albeit our findings also indicate the presence of other clinically relevant symptoms such as mania, hypomania, or seasonal bipolar disorder in nearly 10% of cases. The lower frequency of manic or hypomanic episodes is consistent with reports suggesting that these symptoms are less common in hypercortisolism and often underreported (16). When documented, these typically appear in case studies demonstrating the emergence of mania or hypomania related to CS (17–19) or the exacerbation of existing symptoms due to hypercortisolism (20). In contrast, psychosis was reported in 35% of cases, even though it is less commonly associated with CS. This discrepancy is likely due to the bias inherent in case studies, in which such conditions are more frequently documented because of their rarity and severity (21–24).

Panic attacks are less frequently reported in the literature (25), despite some authors indicating them

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as the third most prevalent psychiatric disorder in hypercortisolism (26). In this review, both identified cases of panic attacks also involved psychosis, suggesting a possible correlation between these symptoms in CS (25,27). Fatigue also appears to be common in CS, with studies citing rates of up to 42% (28), while our findings up to 8.1%, suggesting underreporting in case studies, perhaps due to lower risk of mortality. Only one case had post-traumatic stress disorder symptoms, comorbid with psychosis (11); previous sample studies have suggested a correlation between CS and persistent post-traumatic stress disorder lasting over one year (29). Finally, the presence of suicidal ideation and/or attempts in 20.3% of cases shows the substantial clinical impact of hypercortisolism on mental health. Even after remission, patients may continue to experience significant psychiatric outcomes and risk of suicide (30). Given these findings, the scarcity of observational studies specifically investigating suicidal ideation in CS is concerning.

This study should be interpreted in light of several limitations. First, the results should be interpreted cautiously as they derive from highly heterogeneous case studies. A key limitation is the scope of the search strategy: we focused on major international databases and did not include other relevant databases (e.g., Psicodoc, Biblioteca Virtual em Saúde, and Web of Science), the grey literature, and studies published in other languages. Furthermore, most studies represented WEIRD (Western, Educated, Industrialized, Rich, and Democratic) populations, thereby not adequately represent diverse populations worldwide. Despite these limitations, the results are consistent with the literature that provides robust evidence for the relationship between cortisol and major depressive and anxiety disorders, panic, post-traumatic stress, and cognitive complaints. Future research would benefit from standardized neuropsychological batteries to go beyond general complaints, enabling a more precise characterization of cognitive impact in CS.

## CONCLUSION

The findings of this systematic review of CS case studies highlight the importance of closely monitoring symptoms such as depression, anxiety, and cognitive complaints, including memory problems. Importantly, our study emphasized the presence of additional symptoms, including mania, psychosis, panic attacks, post-traumatic stress disorder, and suicide attempts, which are often linked to CS but frequently underreported in observational studies. Comprehensive psychological and psychiatric evaluation is crucial for accurate mental health diagnosis and appropriate, individualized intervention. This approach is essential to providing comprehensive, personalized care that may enhance these patients' quality of life.

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**Disclosure:** no potential conflict of interest relevant to this article was reported.

**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

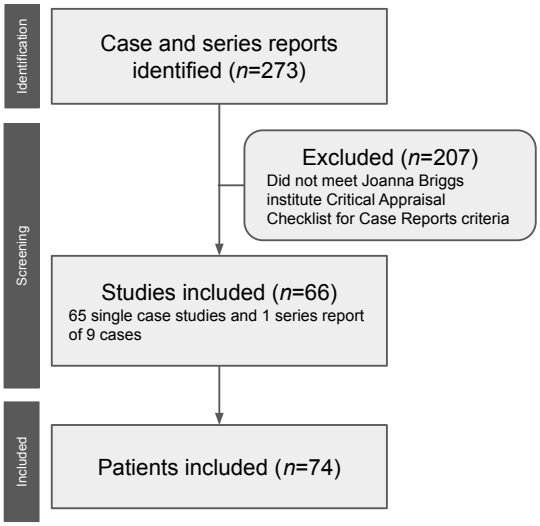
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## SUPPLEMENTARY MATERIAL




**Supplementary Figure 1.** PRISMA Flow Diagram.

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letter to the editor

# Comment on: Metabolic comorbidities and post-transplant outcomes in Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD): a cohort study from a Brazilian tertiary center

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Dear Editor,

We read the paper by Caprini and cols., entitled "Metabolic comorbidities and post-transplant outcomes in Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD): a cohort study from a Brazilian tertiary center" with great interest (1). The article is commendable, addressing the rising issue of MASLD, its association with increased liver transplants, and post-transplant outcomes. However, there are some methodological weaknesses which deserve a mention.

Initially, a notable methodological limitation of the study is immortal time bias in MASLD classification. MASLD was treated as a fixed exposure, but its diagnosis through imaging requires long enough survival. Early mortalities occurring in patients who could have been diagnosed with MASLD over time were automatically grouped with non-MASLD, leading to underestimated MASLD post-transplant hazards and biased comparison between MASLD and non-MASLD post-transplant survival analysis. Recent studies illustrate that observational studies are highly vulnerable to immortal time bias (2). Categorizing MASLD as a time-dependent exposure and controlling for immortal time bias would have strengthened the survival analysis.

Secondly, a notable limitation is failure to stratify MASLD group based on fibrosis stage. Evidence suggests that fibrosis stage, rather than steatosis alone, is a key determinant of long-term outcomes, primarily mortality, in MASLD. A recent meta-analysis demonstrates that mortality risk in MASLD rises in parallel with fibrosis stage, highlighting fibrosis stage as an important prognostic determinant (3). The lack of stratification by fibrosis stage may have obscured important prognostic differences among MASLD recipients and potentially affected the observed survival analysis.

Thirdly, the study is limited by failure to report pre-transplant diagnostic methods for MASLD, introducing misclassification bias in categorization of post-transplant recurrent and de novo MASLD. As Sharma and Arora emphasize, pre-transplant histological assessment is crucial to ensure mild or subclinical steatosis does not remain unnoticed. No histologic feature can accurately distinguish between post-transplant de novo and recurrent MASLD (4). This limitation may have caused overestimation of post-transplant de novo MASLD cases and mitigated important prognostic differences between the two groups.

Lastly, a key limitation of the article is the risk of residual confounding due to the immunosuppressive therapy administered after transplantation. Medications used for immunosuppression, such as corticosteroids, tacrolimus, and sirolimus, can lead

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to metabolic issues such as weight gain, dyslipidemia, insulin resistance and diabetes, all of which increase risk of MASLD. The study failed to adjust for specific type, dosage, or modifications of immunosuppressive regimens over time. This suggests that some of the outcomes may reflect the metabolic effects of these medications rather than MASLD itself. Kang and cols., highlight that different immunosuppressive regimens are associated with distinct risks of post-transplant MASLD, indicating that immunosuppressant exposure is an important confounding factor that should be considered in analyses (5).

In conclusion, the study is commendable. Further research with controlling for immortal time bias, fibrosis staging, standardized reporting of pre-transplant diagnostic methods and immunosuppressive therapy would strengthen survival analysis and clinical reliability of study.

**Contributions:** Zainab Mumtaz: conceptualization and design of the work, drafting manuscript and critical review, final approval, Accountable for accuracy and integrity. Sumiaya: conceptualization and design of the work, drafting manuscript and critical review, final approval, Accountable for accuracy and integrity. Hoorain Talpur: conceptualization and design of the work, drafting manuscript and critical review, final approval, Accountable for accuracy and integrity.

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**Disclosure:** no potential conflict of interest relevant to this article was reported.

**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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letter to the editor

# Response to the letter to the editor: Metabolic comorbidities and post-transplant outcomes in Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD): a cohort study from a Brazilian tertiary center

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Dear Editor,

We thank the authors for their thoughtful Letter to the Editor (1) regarding our article on metabolic comorbidities and post-transplant outcomes in MASLD (2). We appreciate the constructive methodological considerations and address them below:

**1. Regarding immortal time bias in MASLD classification:** We recognize the potential impact of immortal time bias, a known challenge in studies that define exposures or outcomes dependent on a survival period. It is important to note that, given the retrospective nature of our study and the reliance on medical record data collected between 2005 and 2015, a rigorous reclassification of MASLD as a time-dependent exposure with the granularity necessary to mitigate this bias entirely was not feasible. At our institution, post-transplant clinical follow-up to investigate possible injuries to the transplanted liver is performed using liver biopsy or imaging studies, depending on the clinical indication and availability. In real-world practice, patients do not always attend on the scheduled date or can undergo the requested tests at the desired frequency. Therefore, it was not possible to obtain a continuous, standardized MASLD diagnostic status for patients throughout post-transplant follow-up, a prerequisite for such a time-dependent exposure analysis.

**2. Stratification by fibrosis stage:** In this retrospective study, systematic data on post-transplant fibrosis stratification were not available in medical records. We used available data in accordance with routine clinical practice. Additionally, the five-year follow-up period would be insufficient to document significant fibrosis progression in grafts, particularly given the natural history of MASLD. We acknowledge, however, that systematic fibrosis assessment would be valuable in future prospective studies with extended follow-up and standardized evaluation protocols.

**3. Pre-transplant diagnostic methods for MASLD:** We fully agree that histological assessment represents the gold standard for MASLD diagnosis. In our retrospective study, we used data from medical records, which, although potentially resulting in misclassification bias, reflect routine clinical practice in many liver transplant centres. Future prospective studies with standardized diagnostic protocols, including systematic biopsies, would provide more robust data on this issue.

**4. Regarding adjustment for immunosuppressive therapy:** The influence of immunosuppressive therapy on post-transplant metabolic outcomes is undeniable

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and was acknowledged in our Discussion. However, obtaining detailed, time-varying data on immunosuppressive regimens (type, dosage, adjustments, adherence) across 15 years of follow-up poses significant challenges regarding data completeness and accuracy. Importantly, our observational, retrospective study aimed to characterize the clinical-epidemiological profile of MASLD patients undergoing transplantation and describe associations with outcomes, rather than establish causal relationships. We acknowledge that correlation does not imply causality, and findings should be interpreted as descriptive associations. Future prospective studies should meticulously capture and adjust for these confounding effects.

In conclusion, we express our sincere gratitude for the thoughtful and constructive methodological considerations that will undoubtedly improve future research in this field. We appreciate these observations and recognize our study's limitations. Future prospective research with standardized methods will enhance the understanding of MASLD transplant outcomes.

**Disclosure:** no potential conflict of interest relevant to this article was reported.

**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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letter to the editor

# Comment on: Oxytocin: a neglected hormone in pituitary disease – From function to the diagnosis of a deficiency, resulting clinical relevance, and potential treatment options in endocrinology

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The authors of the article in question were invited by the Editorial Office to respond to these comments; however, no response has been received to date.

Dear Editor,

I read with great interest the recent article by Sevenja Leibnitz and cols., entitled "Oxytocin: a neglected hormone in pituitary disease – From function to the diagnosis of a deficiency, resulting clinical relevance, and potential treatment options in endocrinology" (1).

The authors provide an extensive and perceptive analysis of oxytocin, emphasizing its frequently overlooked functions beyond reproduction. The study highlights the potential significance of oxytocin deficiency in pituitary dysfunction by effectively integrating molecular, physiological, and clinical perspectives. However, I would like to highlight a few points to strengthen the research.

First, while the study relies extensively on animal research to understand the role of oxytocin, these models provide important foundational insights. Future human research could expand on this strong preclinical work to validate and translate these findings, thereby improving our understanding of oxytocin's role in pituitary dysfunction (2).

Although the research provides a thorough overview of oxytocin physiology, future research could strengthen the topic by accounting for sex differences. Since oxytocin biology and receptor expression fluctuate between males and females, as well as across different life stages, integrating these factors may help elucidate discrepancies in clinical presentation and treatment response (3).

Moreover, the study emphasizes the possible involvement of oxytocin insufficiency in pituitary disorders; yet, subsequent research could enhance these conclusions by accounting for prevalent co-morbidities in hypopituitary patients. A more comprehensive approach could help illuminate the independent role of oxytocin in clinical symptoms, as many people have numerous hormone deficits and metabolic problems (4).

Lastly, the authors meticulously inspect analyses the therapeutic potential of intranasal oxytocin. However, future research could further strengthen this domain by concentrating on long-term safety, appropriate dose, and probable interactions with other hormone replacement therapies. Well-designed clinical trials will be desired to show that oxytocin-based treatments are both effective and safe before widespread clinical use (5).

In conclusion, the authors' perceptive analysis emphasizes the significant but frequently disregarded function that oxytocin plays in pituitary disorders. Future research will advance our knowledge of oxytocin and the potential clinical uses by addressing areas concerning human validation, sex variations, co-morbidities, and long-term therapeutic safety.

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**Data availability:** the datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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# erratum

In the article **"Supplements for bone health"**, published in Archives of Endocrinology and Metabolism, DOI: 10.20945/2359-4292-2025-0374:

Page 4, Table 2
Where it reads:
Fruits – Banana – 221.99 mg/100 g

It should read:
**Fruits – Banana – 4.29 mg/100 g**

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