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✦ Original research

A Novel Approach to the Levothyroxine Absorption Test Using Only Two Free T4 Measurements

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Abstract

Background and aims: Levothyroxine (LT4) pseudomalabsorption is a factitious disorder. The LT4 absorption test is a non-invasive method for distinguishing true LT4 malabsorption from LT4 pseudo-malabsorption in people with hypothyroidism. This study aimed to evaluate a simplified method by estimating the optimal cutoff point for free T4 (FT4) increment at four hours to predict levothyroxine absorption.

Methods: Data was retrieved from the medical records of patients with hypothyroidism and persistent elevated thyroid stimulant hormone (TSH) who underwent levothyroxine absorption. Next, we estimated the two and four-hour FT4 increment. Finally, we calculated an optimal threshold value for the four-hour FT4 delta that maximizes the absolute sensitivity and specificity values.

Results: Data from 76 patients was analyzed; 90% were women. The median age at the time of the test was 39 (IQR 16.5) years. The median FT4 increment at two hours was 0.8 ng/dL (95%CI 0.75

to 0.95); after four hours, it was 1.0 ng/dL (95%CI 0.92 to 1.07). To predict a qualitative absorption greater than 60% using the FT4 four-hour increment, we found an optimal cutoff point of 0.62 ng/dL with 94% sensitivity, 100% specificity, and a 0.98 area under the curve (AUC).

Conclusions: Using 60% qualitative absorption of FT4 as the threshold, a 0.62 ng/dL increase after four hours of initial LT4 dose could be used to differentiate pseudomalabsorption from true malabsorption. This simple approach simplifies the levothyroxine absorption test.

Introduction

Hypothyroidism is a common endocrine disorder treated since 1949 with a synthetic thyroxine called levothyroxine (LT4).[1] Patients with minimal endogenous thyroid function usually require a dose of LT4 of about 1.6 to 1.8 µg/kg daily to normalize their thyroid stimulating hormone (TSH) concentrations.[2]

The primary site of LT4 absorption is the small intestine, particularly the duodenum and the jejunum. Approximately 62–82% of levothyroxine taken orally after an overnight fast is absorbed within the first three hours of intake.[2] The absorption peak of levothyroxine occurs at approximately two hours in healthy people and three hours in patients with primary hypothyroidism, and blood levels of free T4 (FT4) increase linearly during the first 60–90 minutes before reaching a plateau.[3]

In clinical practice, clinical and biochemical hypothyroidism persists even after an adequately prescribed dose of LT4. Despite repeated counseling, non-adherence or noncompliance is the most common explanation for this elevated TSH level.[4] This lack of adherence to LT4 is known as pseudomalabsorption. Pseudomalabsorption is a factitious disorder that can mimic the decreased gastrointestinal absorption of drugs.[5] Ain et al. first used this term in 1991 after evaluating four cases of persistent hypothyroidism despite high oral doses of thyroid replacement therapy, which was eventually confirmed to be related to non-adherence.[6] Pseudomalabsortion requires ruling out important organic causes and concomitant medications such as ferrous sulfate, calcium carbonate, and antacids; intrinsic gastrointestinal disorders like celiac disease, atrophic gastritis, tropical sprue, resection of the small intestine, and diabetic diarrhea; and pharmacokinetic and pharmacodynamic modifying drugs such as orlistat, rifampicin, carbamazepine, phenytoin, and amiodarone, or the presence of antiT4 antibodies.[7, 8]

Subjects with pseudomalabsorption usually require doses greater than 225 mcg/day of LT4.[5] Therefore, the LT4 absorption test is recommended to distinguish levothyroxine malabsorption from pseudomalabsorption.[4] However, there is no single protocol, and the interpretation of the results is difficult, even when using total T4 (TT4) or FT4 measurements.[9, 10]

Previous studies have reported good absorption of levothyroxine in patients administered a bolus dose of 1,000 mcg.[11] Most of the evidence comes from studies with small sample sizes that utilized multiple sampling points and complicated methods, such as computing qualitative T4 absorption from several samples to rule out pseudomalabsorption.[12, 13, 14] The primary aim of this study was to estimate whether there is an optimal cutoff point for FT4 delta—defined as the difference between the basal and four-hour measurement of FT4 in ng/dL—to idetermine LT4 pseudomalabsorption.

Materials and Methods

Study design and patients

In this single-center, cross-sectional study, we extracted data from the patient's electronic medical records (EMR) between 2017 and 2021. First, we identified for eligibility the records of adults (aged ≥ 18 years) with primary hypothyroidism who underwent an LT4 absorption test (LT4AT). LT4AT is usually performed in individuals with persistently high TSH levels despite LT4 doses of ≥ 1.9 mcg/kg. The records of pregnant women, patients with central hypothyroidism, and records with incomplete data were excluded.

LT4 absorption test protocol

LT4AT was conducted under the supervision of laboratory staff. Patients were asked to come after an overnight fast in the morning. Ingestion of medications that could interfere with LT4 absorption was inquired about before the test. All patients received a 1,000 mcg dose of LT4 (Eutirox®, Merck Healthcare KGaA) supplied as ten 100 mcg pills with two full glasses of water (400 mL) in the morning and were asked to remain seated for at least one hour. Swallowing of the tablets was supervised.

Variables

The following data were extracted from the EMR: demographic variables (age and sex), weight, height, body mass index (BMI), FT4 concentration at the beginning of the test and two and four hours after the LT4 dose, and medical prescription of medications before the test that could interfere with levothyroxine absorption, such as calcium and iron supplements, proton pump inhibitors, tyrosine kinase inhibitors, antiseizure medications, antidepressants, cholestyramine, and orlistat.[15] In addition, relevant medical conditions were extracted, including a history of gastric bypass surgery, inflammatory bowel disease, atrophic gastritis, depression, anxiety disorder, lactose intolerance, and Helicobacter pylori infection.

From the FT4 concentrations, the two and four-hour increases were computed. The qualitative absorption of levothyroxine was calculated using the following formula, adapted from Ghosh et al.:[13]

$$\%\ Levothyroxine\ absorption = \frac{FT4\Delta \times 10 \times VD}{LT4\ dose\ in\ mg}$$

The FT4Δ is the difference between the basal and four-hour measurement of the FT4 in ng/dL. The original formula in Ghosh et al. used the total serum T4; however, a strong correlation has been reported between the total serum T4 and the FT4 increment.[9] VD is the volume of distribution, which is calculated in dL using the following formula:

$$VD = BMI \times 0.442$$

Hormone Assays

Serum TSH and FT4 were estimated using the chemiluminescence technique with commercially available kits from Abbot (Alinity I Free T and Alinity I TSH) (Abbott Laboratories, North Chicago, IL).[8] Analytical sensitivity and total precision for TSH are 0.0083 mIU/L and 2.0%, respectively, and for FT4, are 0.42 ng/dL and 2.6%, respectively. The laboratory reference range for TSH is 0.35 mIU/L to 4.94 mIU/L, for FT4, it is 0.70 ng/dL to 1.48 ng/dL, and the inter-assay coefficient of variation are 1.5% and 2.2%, respectively.

Definitions

Levothyroxine malabsorption was defined as an absorption percentage of less than 60%[9] using the lower limit (0.70 ng/dL) of the expected LT4 absorption as the threshold.

Hashimoto's thyroiditis was confirmed by measuring antithyroid autoantibodies or typical ultrasonographic features. Atrophic gastritis was diagnosed using a gastric biopsy and an anti-parietal cell antibody test. Helicobacter pylori infection was confirmed by gastric biopsy or breath tests. A high levothyroxine dose was defined as a daily LT4 dose beyond 1.9 mcg/kg.[16]

Statistics methods

Continuous variables are summarized using median and interquartile range (IQR), and categorical variables using absolute and relative frequencies. We used the Mann-Whitney test to compare basal FT4 with the two and four-hour measurements and the two and four-hour delta FT4. Comparison between samples was made using the Wilcoxon signed ranks test, and a 95% confidence interval (95%CI) was computed for the differences. Finally, we calculated an optimal threshold value for the four-hour FT4 delta that maximizes the absolute values of sensitivity and specificity after locally weighted scatterplot smoothing (LOEES), as it produces less bias estimation in small-sample studies and tests the out-of-sample performance of the cutoff point by bootstrapping using 500 samples.[17] Finally, given that our definition of malabsorption is based on the qualitative LT4 absorption, we planned a sensitivity analysis to test the found cutoff point in two scenarios where the absorption percentage to define malabsorption was changed from less than 60% (original definition) to less than 80%[9] and less than 98%.[13] All studies were conducted using the R statistical package.[18] As the levothyroxine absorption between the two-hour and four-hour marks was similar, both measurement points were compared.

Ethics approval

This study complied with the amendment made in 2013 during the general assembly in Fortaleza, Brazil, of the World Medical Association to the Declaration of Helsinki. This study was approved by the Ethics and Research from the sponsor institution. The committee did not require informed consent due to the study design. Only one of the researchers had access to patient identification data, whereas the remaining authors could only access a de-identified database.

Results

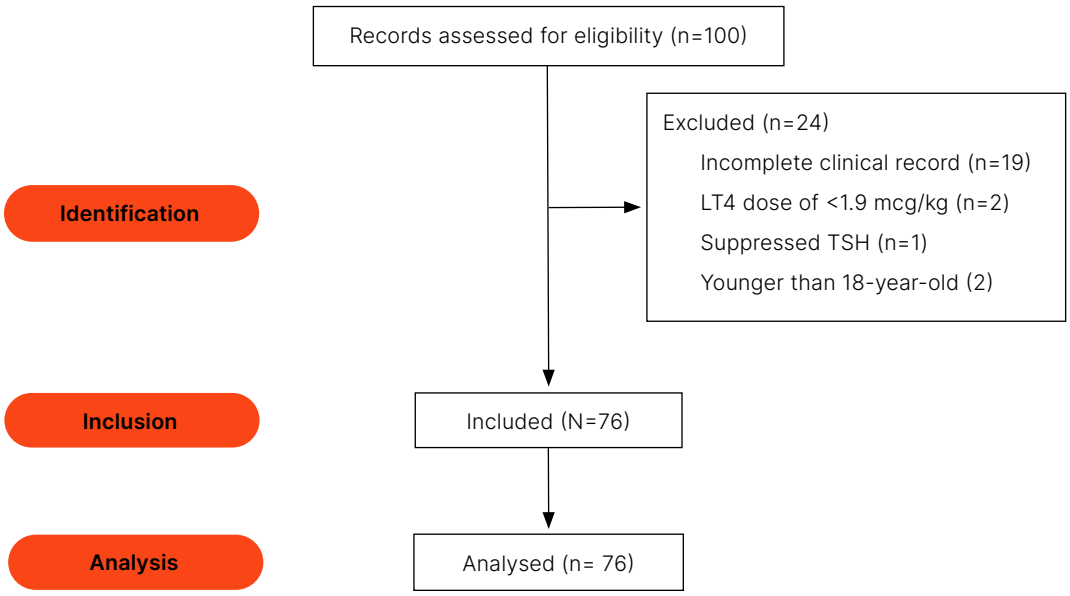
Data were retrieved from the electronic medical records of 76 patients who underwent an FT4AT, (Figure 1). Most of the patients were female (N= 69), with a median age of 39 (IQR 16.5) years. Most patients (N=43) had autoimmune thyroiditis as the cause of hypothyroidism. Several patients were also treated with medications that could alter levothyroxine absorption or clearance. Patients' characteristics are presented on Table 1.

Table 1. Population characteristics

	Overall (N=76)
Age (in years)	39.0 [16.5]
Sex, female	69 (90.8%)
Weight (in kilograms)	71.5 [28.0]
Height (in meters)	1.60 [0.09]
Body mass index (Kg/m²)	28.2 [8.56]
Hypothyroidism etiology	
Autoimmune thyroiditis	43 (56.6%)
Postoperative cancer	24 (31.6%)
Postoperative non-cancer	6 (7.9%)
Radioactive iodine therapy	3 (3.9%)
Medications records in EMR	
Calcium supplements	10 (13.2%)
Proton pump inhibitors	9 (11.8%)
Iron supplements	4 (5.3%)
Antidepressants	4 (5.3%)
Orlistat	3 (3.9%)
Antiseizure medications	1 (1.3%)
Comorbidities	
Depression or anxiety disorder	13 (17.1%)
Atrophic gastritis	6 (7.9%)
Helicobacter pylori infection	4 (5.3%)
Gastric bypass	1 (1.3%)
Basal TSH (in mUI/L)[IQR]	41.4 [90.3]
Levothyroxine dose per Kg of body weight in µg	3.49 [2.70]

Data presented as median with interquartile range or proportions. EMR: Electronic medical records, IQR: interquartile range.

Figure 1. Patients flow diagram



FT4 changes during the test

The FT4 concentration increased significantly following LT4 loading. The median increase at two hours was 0.8 ng/dL (95%CI 0.75 to 0.95) and 1.0 ng/dL (95%CI 0.92 to 1.07) after four hours (Figure 2). The difference between the two and four-hour delta was 0.12 ng/dL (95%CI 0.05 to 0.19) (Table 2).

Figure 2. Changes in free T4 during the test. Presented as median with interquartile range. FT4: Free T4. The red line represents the lower reference limit of the FT4 test, 0.7 ng/dL.

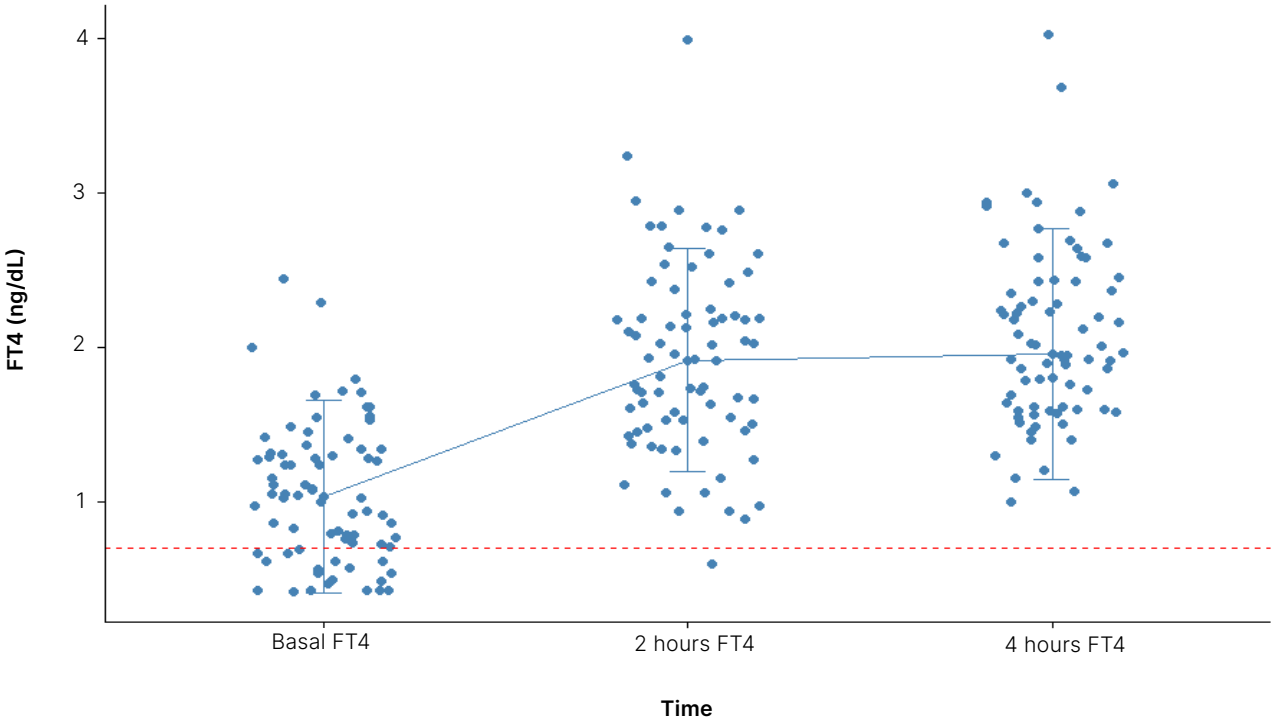


Table 2. Free T4 changes during the test. Data presented as median with interquartile range or proportions.

	Overall (N=76)
Basal free T4 (in ng/dL)	1.03 [0.628]
Free T4 two hours after the load	1.92 [0.725]
Free T4 four hours after the load	1.96 [0.815]
Free T4 two-hour delta	0.835 [0.503]
Free T4 four-hour delta	0.965 [0.350]
Free T4 two-hour rise	1.78 [0.838]
Free T4 four-hour rise	2.02 [0.767]
T4 two-hour absorption (in %)	98.2 [59.8]
T4 four-hour absorption (in %)	117 [52.6]
Two-to-four-hour T4 delta	16.7 [50.7]

FT4 qualitative absorption

Using the four-hour increment of the FT4, the median LT4 qualitative absorption was 117% (IQR 52.6) (Table 2). Three subjects had a 60% or less qualitative absorption, labeled as true malabsorption. In the malabsorption group, the median qualitative absorption was 40.6 % (IQR 4.92), while in the pseudomalabsorption group, it was 118% (IQR 51.2). Only 1 patient diagnosed with true malabsorption was on treatment with a medication that potentially could interfere with levothyroxine absorption. Table 3 summarises the medications reported on the EMR.

Table 3. Medications reported on the EMR of the patients

	True malabsortion (N=3)	Pseudomalabsortion (N=73)
Calcium supplements	0 (0%)	10 (13.7%)
Orlistat	0 (0%)	3 (4.1%)
Proton pump inhibitors	0 (0%)	9 (12.3%)
Iron supplements	1 (33.3%)	3 (4.1%)
Antiseizure medications	0 (0%)	1 (1.4%)
Antidepressants	0 (0%)	4 (5.5%)

FT4 four-hour delta cutoff point

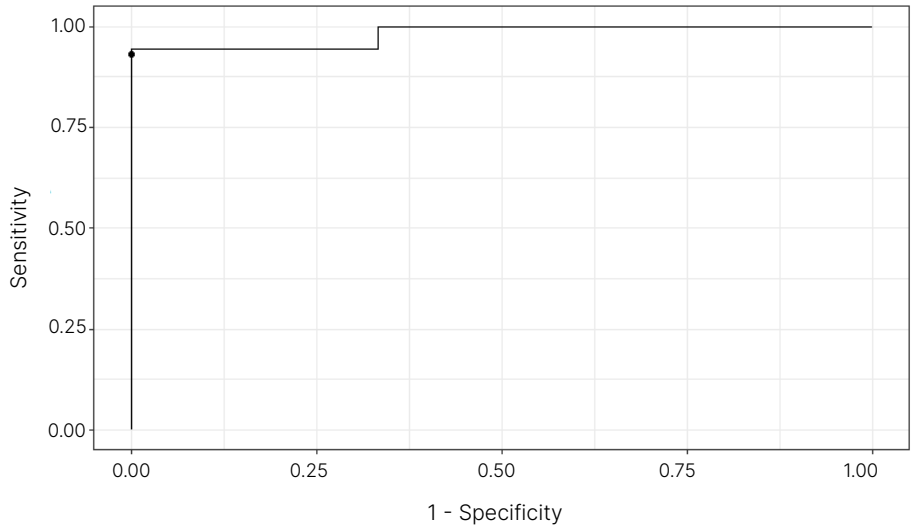
We found that 0.62 ng/dL increment of the FT4 after four hours of receiving 1,000 mcg of LT4 has 93.1% sensitivity and 100% specificity to differentiate pseudo-malabsorption from true malabsorption with an area under the curve of 0.98 (Table 4, Figure 3). Furthermore, the sensitivity analysis revealed that changing the malabsorption definition does not affect the cutoff point sensitivity and the AUC but reduces its specificity (Table 4).

Table 4. Optimal cutoff point

	Cutoff point	AUC	Sensitivity	Specificity
Originally planned analysis	0.62	0.98	93.1%	100%
Sensitivity analysis 1	0.62	0.93	97%	66%
Sensitivity analysis 2	0.62	0.86	100%	44%

AUC: area under the curve, PPV: Positive predictive value, NPV: Negative predictive value. Originally planned analysis, malabsorption was defined as less than 60% of levothyroxine absorption. Sensitivity analysis 1, malabsorption was defined as less than 80% of levothyroxine absorption. Sensitivity analysis 2, malabsorption was defined as less than 98% of levothyroxine absorption.

Figure 3. ROC Curve for FT4 four-hour delta cutoff point



Two and four-hour absorption comparison

The two-hour absorption was 16% lower (95%CI -26.03 to -8.00) compared to the four-hour absorption. The optimal increment for diagnosis at the two-hour mark was a 0.58 ng/dL increase of the FT4, after four hours of receiving 1,000 mcg of LT4 has 92% sensitivity and 92% specificity to differentiate pseudo-malabsorption from true malabsorption with an area under the curve of 0.98.

Discussion

We presented an alternative to traditional LT4AT. In a group of patients with high LT4 requirements, our results indicate that the duration of the LT4AT could be reasonably reduced to four hours, and a delta greater than ≥ 0.62 ng/dL from baseline suggests pseudomalabsorption.

In our study, the thyroid hormone delta measurement and absorption percentage calculation were performed using FT4 values. Furthermore, although the original LT4 absorption studies were validated using total thyroxine TT4 measurements, the use of FT4 in LT4AT was validated by Sun et al. in 2014. The correlation between FT4 and TT4 was 0.88 (95% confidence interval, 0.56 to 0.97;

P<0.001). Therefore, the authors concluded that FT4 may be used interchangeably with TT4 for quantitative assessment of suspected malabsorption using oral LT4AT.[6,9]

LT4AT is an essential tool that can distinguish between non-adherence and true intestinal malabsorption of LT4. There is no gold standard method for LT4AT with various protocols advocated in the literature.[12, 19, 20] The time commitment for implementation ranged from two to five days. Marques et al., in a study published in 2016, indicated that a 2.5 increase in FT4 from the baseline is suggestive of pseudomalabsorption. However, in contrast to our findings, a previous study did not determine the sensitivity and specificity of the FT4 cutoff point using ROC curve analysis, which prevented the determination of a good cutoff point. In addition, the sample size was small.[21]

Similar to our results, Gonzales et al., in a study performed with 16 patients diagnosed with refractory hypothyroidism who had completed the LT4AT, showed that most patients who completed the LT4AT had normal intestinal absorption at four hours. Nevertheless, this study characterized normal absorption by calculated absorptions of ≥60%, corresponding to an increase in TT4 toward peak levels.[10]

Previous studies used the computed percentage of LT4 absorption to define normal absorption.[22, 23] However, the use of this formula is controversial, and its calculation is complex for routine use by clinicians, thus limiting its use. Our findings support a simplified interpretation of FT4AT, using only the delta of FT4 at four hours.

Most subjects in our study had basal FT4 levels within the normal range. Similarly, Van Wilder et al.[24] described cases in which a subject presented FT4 levels close to the reference range, which could indicate intentional ingestion before testing. However, during LT4AT with supervised intake, an increase in FT4 within the first 120 min, with the maximum level observed at four hours, strongly contradicts the true malabsorption.[25] The possibility of true malabsorption should be evaluated when the absorption percentage is less than 60% or when there is no increase in FT4.[20, 26, 27] Also, although there is only a minor difference in levothyroxine absorption between the two-hour and four-hour marks, the two-hour delta has lower specificity compared to the four-hour.

However, the expected time interval for the absorption of LT4 by the small intestine is within the first 120 min.[28] Nevertheless, we also described the FT4 four-hour delta cutoff point because our cohort reached maximum levels at four hours. Therefore, adopting this simplification may facilitate and increase the use of LT4AT and reduce costs by measuring FT4 only at four hours.

Following the diagnosis of pseudomalabsortion, a mental health evaluation should be performed. Psychiatric disorders such as depression and anxiety are associated with autoimmune hypothyroidism.[29, 30] In addition, some patients exhibit neuropsychiatric symptoms even after successful treatment of hypothyroidism.[31] In contrast, others persist in low compliance with levothyroxine and are at risk of serious complications that lead to unnecessary medical and surgical procedures.[32] Therefore, supervised weekly doses of LT4 have been proposed to improve treatment adherence in this subgroup of patients.[33, 34, 35]

Strengths, limitations, and final remarks

Our study constitutes the largest cohort of patients published on this topic. Furthermore, we established an FT4 four-hour delta cutoff point. This could make it easier to perform the LT4AT and facilitate its use in routine care.

The limitations of our study include its retrospective nature, the absence of a comparison with well-controlled hypothyroid individuals, and the small number of patients with true malabsorption, which restricted our findings. In addition, the lack of follow-up data made it difficult to establish the optimal dose for achieving euthyroidism and to determine whether patients should undergo psychiatric evaluation after being diagnosed with pseudomalabsorption. We had also planned to conduct several subgroup analyses for more prevalent conditions and medications that affect levothyroxine absorption, but the sample size was not large enough for this. Therefore, our results need to be confirmed in further research with a larger sample size. Ultimately, although the results may not be generalizable, they could serve as a starting point for future larger prospective cohorts.

Conclusion

Here, we present a simplified protocol for diagnosing pseudomalabsorption. The increase of FT4 to 0.62 ng/dL at four hours in people with a baseline FT4 below the reference range suggests pseudomalabsorption without needing to calculate the absorption percentage. We emphasize the importance of early LT4AT application in cases of refractory hypothyroidism, given the benefits of the test in distinguishing between pseudomalabsorption and malabsorption and, as a result, guiding clinical decisions in this context.

Conflicts of interest

The authors certify that they have no affiliation or are involved in any organization or entity with any financial interest (such as fees, financial aid for education, shares, employment contracts, work as consultants, or any other type of interest) or non-financial interest (such as personal or professional relationships, affiliations, or beliefs) on the topic of interest or any material discussed in this manuscript. Carlos A. Builes-Barrera received consulting and speaker fees from Merck, Abbott, Sanofi, Novo Nordisk, Novartis, Bayer, Merck Sharp and Dhome, Lilly, Amgen, Boehringer Ingelheim, Siegfried and Lafrancol. Carlos E. Builes-Montaño received consulting and speaker fees from Sanofi, Novo Nordisk, Novartis, and Boehringer Ingelheim. María Carolina Fragozo-Ramos received financial support from Novo Nordisk and Ipsen for the medical events.

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Data availability statement

The datasets produced or analyzed during the current study are available from the sponsor institution upon reasonable request.

Authors' contributions

CAB conceived and designed the study. CAB, MCF, and CEBM further developed the study design. CAB, MCF, and CEBM managed the study and evaluated outcomes. CEBM developed the statistical analysis plan for outcomes; CAB, MCF, and CEBM analyzed and interpreted the data and wrote the first draft of the manuscript. CAB, MCF, and CEBM provided an intellectual contribution to the final version of the manuscript. All authors reviewed and approved the final version of the manuscript.

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