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CASE REPORT

Interference of High Dose Biotin Supplementation with Thyroid Parameters in Immunoassays Utilizing the Interaction between Streptavidin and Biotin: a Case Report and Review of Current Literature

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SUMMARY

Background: **Automated immunoassays utilizing the interaction between streptavidin and biotin are widely used. Nonetheless, biotin remains an often overlooked confounder.**
Methods: **We report the case of a 54-year-old female patient with progressive multiple sclerosis and Hashimoto's thyroiditis who presented herself for a follow-up. Measurements on Roche's cobas® 8000 modular analyzer series suggested severe hyperthyroidism. Initially, no relevant confounders could be identified.**
Results: **All requested thyroid parameters were measured with alternative methods, yielding plausible results.**
Conclusions: **Biotin is a significant confounder in many immunoassays. Alternative measurement methods or methods of biotin neutralization need to be implemented for certain situations.**
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KEY WORDS

biotin, thyroid, Roche, cobas, interference, multiple sclerosis

INTRODUCTION

About half of all current automated immunoassays for the determination of thyroid parameters utilize the interaction between streptavidin and biotin, which offers advantages including signal amplification and increased sensitivity [1,2]. However, the design remains vulnerable to interference from high biotin concentrations causing false low results in sandwich immunoassays and false high results in competitive immunoassays, which may mimic hyperthyroidism, thyrotoxicosis or autoimmune disorders like Graves' disease [1-4].
Since the recommended daily biotin intake of 30 - 75 µg causes no relevant disturbance in current assays, biotin interference used to be an uncommon phenomenon that was mostly limited to biotin-substituting patients with rare inherited metabolic disorders such as

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biotinidase deficiency, holocarboxylase synthetase deficiency, and biotin-thiamine-responsive basal ganglia disease [1,2,5].
However, long-term biotin supplementation has been adopted as a promising therapeutic strategy in progressive multiple sclerosis (MS), which is not a rare disease and has a global prevalence of 22/100,000 [1,2,6-9]. Additionally, biotin is included in many commercially available poly-vitamin complex preparations, and it is very popular as a self-medication to reduce hair loss or improve the condition of skin and nails in supra-physiological doses of up to 30 mg daily [2,5].
Patients tend not to report these supplementations in their medical history, which may lead to inappropriate medical procedures due to apparently pathologic test results [2].

CASE PRESENTATION

A 54-year-old female patient with known progressive MS and Hashimoto's thyroiditis presented herself for a routine follow-up examination which included thyroid function tests (TFTs) and thyroid antibody tests ordered by her treating physicians. Our clinical colleagues reported a daily substitution of 75 µg L-thyroxine and 300 ng biotin and verbally reconfirmed this medication in response to further inquiry by us, which was triggered by the unfamiliar dosage of biotin. The patient showed no clinical signs of hypo- or hyperthyroidism.
Serum levels of TSH, FT3, FT4, anti-TPO and TRAb were measured via electro-chemiluminescence immunoassays (ECLIAs) on Roche's cobas® 8000 modular analyzer series (Table 1). The results did not reflect the patient's clinical presentation and we could identify no relevant confounder at the time. It was agreed to take a new blood sample during her next appointment 2 weeks later.
Again, the parameters mentioned above were measured on Roche's cobas® 8000 modular analyzer series with practically identical results (Table 1). At that time, the patient's medication was reported as: 75 µg L-thyroxine and 300 mg biotin, i.e., 10^6 times the initially reported biotin dosage.
In light of the new information, we decided to determine the serum concentration of biotin by manual ligand binding assay, which showed extremely elevated biotin levels > 50,000 ng/L (Reference interval: > 250 ng/L = sufficient biotin levels; > 400 ng/L = optimal biotin levels).
According to Roche's method sheets, biotin levels > 10,000 - 70,000 ng/L can interfere with their thyroid-associated ECLIAs [11]. TSH and FT4 are among the more susceptible assays with interference occurring at biotin levels > 25,000 ng/L and > 20,000 ng/L, respecttively. For their FT3 assay, Roche warns of possible interference, if the sample contains > 70,000 ng/L of biotin [11].
We concluded that biotin would be the most likely confounder and decided to measure TSH, FT3, and FT4 with methods that should not be influenced by high levels of biotin. The chemiluminescence assays on Abbott's ARCHITECT I2000SR and Siemens' ADVIA Centaur XP yielded results which reflected the patient's clinical presentation (Table 2).
Since the results on both machines were almost identical, we decided to rely on Abbott's ARCHITECT I20000SR for the determination of anti-TPO and TgAb levels. TRAb levels were measured via radioimmunoassay (RIA). The results were consistent with the patient's history of Hashimoto's disease (Table 2). Fortunately, the patient suffered no negative consequences due to the diagnostic delay.

DISCUSSION

Immunoassays are widely available, relatively cheap, time-efficient, and automatable. Often they are sufficient for the determination of thyroid parameters despite the fact that they tend to overestimate FT3 and FT4 at low concentrations [15].
However, medications like furosemide, salicylate, antiepileptics, and heparin as well as mutations in transthyretin or albumin and physiologic states like pregnancy (with increased thyroxine-binding globulin levels) can confound free hormone measurements. There is limited comparability between the results of free thyroid hormone immunoassays by different manufacturers [15]. Additionally, there have been reports of interference in Roche's ECLIAs in patients who substitute high doses of biotin [1-5].
In TFTs, this interference produces a plausible scenario from the laboratory's point of view due to the fact that TSH is a sandwich immunoassay and FT3/FT4 are competitive immunoassays. The obtained results mimic a severe state of hyperthyroidism as in the presented case [2,5].
Additionally, the sandwich immunoassays for ACTH, AFP, CA125, CA15-3, CA19-9, CEA, C-peptide, FSH, hCG, insulin, LH, prolactin, total/free PSA, NT-proBNP, PTH intact, SHBG as well as troponin T hs and competitive immunoassays for cortisol, DHEA-S, digoxin, estradiol, progesterone, folate, testosterone, vitamin B12 and HIV are potentially affected by biotin interference [1,12].
This may lead to potentially fatal situations including failure to diagnose a myocardial infarction, overestimation of a tumor therapy response, a missed reoccurrence of malignancy or a false diagnosis of drug intoxication. We found reports of patients who were subjected to unnecessary diagnostic procedures and treatments because of biotin interference [1,5,12].
Due to the reasons stated above, we expect to see biotin interference with increasing frequency. Therefore, it appears highly advisable to make clinicians more aware of the problem of biotin interference in certain immunoas-

Table 1. Laboratory results Roche's cobas® 8000 modular analyzer series.

Parameter	Initial concentration	Concentration after 2 weeks	Reference interval	Units
TSH	< 0.01	< 0.01	0.40 - 4.00	µU/mL
FT3	7.07	6.00	2.00 - 4.40	pg/mL
FT4	> 7.77	7.69	0.93 - 1.70	ng/dL
anti-TPO	> 600	> 600	< 34	IU/mL
TRAb	> 40	> 40	< 1.75	IU/L

Table 2. Laboratory results using alternative methods.

Machine/Test	Parameter	Concentration	Reference interval	Units
Abbott ARCHITECT I2000SR	TSH	0.33	0.35 - 4.94	µU/mL
	FT3	2.58	1.71 - 3.71	pg/mL
	FT4	1.37	0.70 - 1.48	ng/dL
	anti-TPO	595	< 5.61	IU/mL
	TgAb	63.2	< 4.11	IU/mL
Thermo-Scientific RIA	TRAb	< 1.0	< 1.0	IU/L
Siemens ADVIA Centaur XP	TSH	0.34	0.35 - 3.50	µU/mL
	FT3	2.6	2.30 - 4.20	pg/mL
	FT4	1.7	0.89 - 1.76	ng/dL

says and the importance of reporting high dose biotin substitution to the laboratory. Additionally, counter measures need to be established in order to obtain valid results in samples of patients who substitute high-dose biotin.

So far, the predominant strategy was to discontinue the biotin substitution for several days in order to eliminate the interference [1,5]. The American Thyroid Association Guidelines recommend a cessation of high-dose biotin for at least 2 days prior to TFTs [13]. However, high-dose biotin substitution has led to reported peak serum concentrations of up to 10^6 ng/L [1]. The half-life of biotin in 100 - 300 mg substitutions was found to be 7.8 - 18.8 hours [14].

With peak serum biotin concentrations of 10^6 ng/L and a half-life of 7.8 - 18.8 hours, it would take 54.6 to 131.6 hours (i.e., 2.3 to 5.5 days) to reach Biotin levels of 7,812.6 ng/L, which can safely be assumed not to interfere with Roche's assays.

Therefore, a cessation of 2.5 upper half-lives (i.e., 48 hours) seems insufficient to assure biotin levels that would not interfere with the ECLIAs mentioned above [1].

Since biotin is predominantly cleared via the kidneys, a significantly increased half-life must be assumed in patients with chronic kidney disease as well as in the elderly, neonates, and many diabetic patients who have decreased renal function. Kummer et al. reported that withdrawal of biotin therapy for up to 7 days was necessary to return TRAb levels to normal ranges in some patients with chronic kidney disease on high-dose biotin therapy [1,4,15].

So, the above-mentioned approach seems unfeasible, especially in medical emergencies and on intensive care units. Additionally, it is unknown what effects the discontinuation of biotin supplementation might have on the clinical course of affected MS patients.

In this light, we decided to determine all requested thyroid parameters with methods that have not been associated with interference by significantly increased biotin levels.

However, our approach has its own drawbacks which include divergent reference intervals, limited comparability to the patient's results prior to the onset of high-dose biotin substitution (and the switch to alternative measurement methods) and higher financial costs due to multiple testing.

This led us to screen the literature for an alternative, which would allow us to continue using our standard machine, Roche's cobas 8000 modular analyzer series, in future cases. The method reported by Piketty et al. might be this alternative:

They treated lithium heparin plasma samples with magnetic microparticles coated with streptavidin to adsorb

biotin and verified that the interpretation of the hormonal profile was unchanged by the neutralization method in patients without biotin-supplementation.
In samples of patients receiving high-dose biotin supplementation of up to 300 mg daily, the neutralization protocol successfully eliminated the interference by biotin. Highly abnormal initial results returned to the reference intervals and corresponded to the clinical presentation of the patients [12].

CONCLUSION

Since increasing numbers of cases of immunoassay interference by high-dose biotin supplementation are to be expected in the future, it appears prudent for laboratories to establish counter measures in order to deal with biotin interference. The neutralization method of Piketty et al. might prove very useful in this regard.

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Declaration of Interest:
Nothing to declare. There are no conflicts of interest.

References:

1. Trambas C, Zhong L, Yen T, Sikaris K. Characterization of the scope and magnitude of biotin interference in susceptible Roche Elecsys competitive and sandwich immunoassays. Ann Clin Biochem 2018;55:205-15 (PMID: 28875734).

2. Piketty ML, Polak M, Flechtner I, Gonzales-Briceño L, Souberbielle JC. False biochemical diagnosis of hyperthyroidism in streptavidin-biotin-based immunoassays: the problem of biotin intake and related interferences. Clin Chem Lab Med 2017;55: 780-8 (PMID: 27732554).

3. Barbesino G. Misdiagnosis of Graves' disease with apparent severe hyperthyroidism in a patient taking biotin megadoses. Thyroid 2016;26:860-3 (PMID: 27043844).

4. Kummer S, Hermsen D, Distelmaier F. Biotin treatment mimicking Graves' disease. N Engl J Med 2016;375:704-6 (PMID: 275 32849).

5. Minkovsky A, Lee MN, Dowlatshahi M, et al. High-dose biotin treatment for secondary progressive multiple sclerosis may interfere with thyroid assays. AACE Clin Case Rep_2016;2:370-3 (PMID: 27917400).

6. Tourbah A, Lebrun-Frenay C, Edan G, et al. MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: a randomized, double-blind, placebo-controlled study. Mult Scler 2016;22:1719-31 (PMID: 27589059).

7. Sedel F, Papeix C, Bellanger A, et al. High doses of biotin in chronic progressive multiple sclerosis: A pilot study. Mult Scler Relat Disord 2015;4:129-69 (PMID: 25787192).

8. McCarty MF, DiNicolantonio JJ. Neuroprotective potential of high-dose biotin. Med Hypotheses 2017;109:145-9 (PMID: 2915 0274).

9. Feigin VL, Abajobir AA, Abate KH, et al. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol 2017;16:877-97 (PMID: 28931491).

10. Li D, Radulescu A, Shrestha RT, et al. Association of biotin ingestion with performance of hormone and nonhormone assays in healthy adults. JAMA 2017;318:1150-60 (PMID: 28973622).

11. Roche Diagnostics GmbH. Roche Elecsys method sheets. Mannheim: Roche Diagnostics GmbH, 2016 (https://pim-eservices.roche.com/eLD_SF/gb/en/Documents/GetDocument?documentId=2fae2c32-e3c0-e711-b48d-00215a9b3428).

12. Piketty ML, Prie D, Sedel F, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. Clin Chem Lab Med 2017;55:817-25 (PMID: 28222020).

13. Ross DS, Burch HB, Cooper DS, et al. 2016 Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid 2016;26:1342-421 (PMID: 27521067).

14. Peyro Saint Paul L, Debruyne D, Bernard D, Mock DM, Defer GL. Pharmacokinetics and pharmacodynamics of MD1003 (high-dose biotin) in the treatment of progressive multiple sclerosis. Expert Opin Drug Metab Toxicol 2016;12:327-44 (PMID: 26699 811).

15. Welsh KJ, Soldin SJ. Diagnosis of endocrine disease: how reliable are free thyroid and total T_3 hormone assays? Eur J Endocrinol 2016;175(6):R255-63 (PMID: 27737898).