Review Article

BIOTIN INTERFERENCE WITH ROUTINE CLINICAL IMMUNOASSAYS: UNDERSTAND THE CAUSES AND MITIGATE THE RISKS

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ABSTRACT

Objective: The objectives of this report are to review the mechanisms of biotin interference with streptavidin/biotin-based immunoassays, identify automated immunoassay systems vulnerable to biotin interference, describe how to estimate and minimize the risk of biotin interference in vulnerable assays, and review the literature pertaining to biotin interference in endocrine function tests.

Methods: The data in the manufacturer's "Instructions for Use" for each of the methods utilized by seven immunoassay system were evaluated. We also conducted a systematic search of PubMed/MEDLINE for articles containing terms associated with biotin interference. Available original reports and case series were reviewed. Abstracts from recent scientific meetings were also identified and reviewed.

Results: The recent, marked, increase in the use of over-the-counter, high-dose biotin supplements has been accompanied by a steady increase in the number of reports of analytical interference by exogenous biotin in the immunoassays used to evaluate endocrine function. Since immunoassay methods of similar design are also used for the diagnosis and management of anemia, malignancies, autoimmune and infectious diseases, cardiac damage, etc., biotin-related analytical interference is a problem that touches every area of internal medicine.

Conclusion: It is important for healthcare personnel to become more aware of immunoassay methods that are vulnerable to biotin interference and to consider biotin supplements as potential sources of falsely increased or decreased test results, especially in cases where a lab result does not correlate with the clinical scenario. **(Endocr Pract. 2017;23:989-998)**

Abbreviations:
FDA = U.S. Food & Drug Administration; **FT3** = free tri-iodothyronine; **FT4** = free thyroxine; **IFUs** = instructions for use; **LH** = luteinizing hormone; **PTH** = parathyroid hormone; **SA/B** = streptavidin/biotin; **TFT** = thyroid function test; **TSH** = thyroid-stimulating hormone

INTRODUCTION AND RELEVANCE OF ISSUE

Biotin is a water-soluble B-complex vitamin with multiple roles in a variety of metabolic pathways (1,2). It is a small molecule that can be covalently coupled to proteins, polypeptides, and low-molecular weight antigens including thyroid and steroid hormones with minimal effects on the biological and antigenic activities of the products, thus enabling the use of biotin conjugates as ligands in competitive and immunometric assay formats (3). Consequently, avidin/biotin chemistries have played a significant role in the development of the field of clinical immunoassay over the past 25 years and have been incorporated into many of the immunoassay methods that are currently used for patient care (4).

Supraphysiologic biotin supplementation is increasingly marketed in the United States as an over-the-counter

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remedy for common hair and skin problems, as well as beneficial for weight loss, enhancing glucose metabolism, and boosting energy. Pharmacologic use of biotin includes inherited metabolic diseases such as genetic biotin deficiency and biotin-thiamine responsive basal ganglia disease. It is also used as supportive treatment in patients with disorders of mitochondrial energy metabolism (5). High-dose biotin has also been recently studied in progressive multiple sclerosis (6), for use in alleviating muscle cramps in hemodialysis patients (7), and in patients with malabsorption syndromes or in total parenteral nutrition (8).

Biotin is available over the counter in doses up to 100 mg, mega doses that greatly exceed the requirements of 30 mcg per day. In fact, 15 to 20% of individuals in the U.S. report consuming biotin-containing dietary supplements (9,10). There are favorable tolerability and safety profiles in individuals that received pharmacological doses of up to 300 mg a day. Many may not consider it a medication and worth reporting to their physician. Unfortunately, this marked increase in the use of biotin has led to an increase in the risk of clinically significant analytical errors in subjects that use high-dose biotin supplements. This can result in misdiagnosis and potential inappropriate treatment (5,11). The purpose of this review is to provide an awareness of potential biotin interference that can falsely increase or decrease results for a variety of routine immunoassays. This includes thyroid, steroid and polypeptide hormones, tumor markers, vitamins, and infectious disease serologies (5). This review will detail the mechanisms of analytic interference utilizing streptavidin/biotin (SA/B) methodology. We also examine the product labeling for popular immunoassay systems in use in the United States in order to assess the risk of biotin interference in vulnerable immunoassays. Finally, a summary of the clinical literature is presented with an emphasis on hormonal immunoassays.

BIOTIN PHARMACOKINETICS

Biotin, also termed vitamin B7 and vitamin H, is a component of a normal diet and is present in trace amounts in natural foods such as egg yolk, pork, liver, whole cereals, soybean and leafy vegetables (12). It is an essential cofactor in enzymatic carboxylation reactions. As such, it plays a vital role in fatty acid and branched-chain amino acid metabolism, gluconeogenesis and in the Krebs cycle (13). It has also been found to have a role in glucose metabolism, islet cell gene expression, and insulin secretion (14).

The adequate daily intake of biotin in adults is 30 μg (15). The dietary intake of biotin in Western populations is approximately 35 to 70 μg/day (16). Oral biotin is completely absorbed and is 100% bioavailable, even in doses that exceed the normal dietary intake by 600 times (9,16). Toxicity with high dose biotin has not been reported(15). The amount of usual dietary intake is not believed to be high enough to affect the SA/B-based immunoassay, however supraphysiologic doses greater than 3 times the adequate daily intake are a concern (13). Fortunately, biotin absorption and excretion are rapid. The pharmacokinetics of oral biotin supplementation has been investigated in healthy subjects following single oral doses of 1 mg (17), and 100 to 300 mg (18). The results of these studies indicate that peak serum biotin levels occur approximately 1 to 3 hours after an oral dose and decrease with a half-life in the range of 8 to 16 hours. The peak serum biotin levels (mean [SEM]) after single doses of 1 mg or 100 mg were 35 [8] nmol/L and 2,024 [658] nmol/L, respectively. The major route of clearance is urinary excretion, and the half-life of biotin can potentially be impacted by altered renal function and should be considered.

In vivo data from Wijeratne et al in which 1 of the authors ingested 30 mg of biotin in order to examine the kinetics of SA/B assay interference, demonstrated a peak interference around 2 hours after intake for all analytes measured (19). A time-dependent decline in interference over 24 hours was seen for free thyroxine (FT4), free triiodothyronine (FT3), and thyroglobulin on the platform under investigation (19). The effect lasted approximately 5 hours for dehydroepiandrosterone sulfate (DHEAS), estradiol, testosterone, and ferritin utilizing a different analyzer (19). The magnitude of change and duration of biotin interference varies significantly according to the particular analyte, biotin concentration, assay design, and assay manufacturer as detailed below (11,20).

MECHANISMS OF BIOTIN INTERFERENCE

SA/B-based immunoassays are vulnerable to interference when they are used to analyze a sample that contains biotin. Exogenous biotin in the sample competes with biotinylated reagents for the binding sites on streptavidin reagents. Examples of 2 common types of immunoassay formats used by clinical laboratories are shown in Figure 1. In the immunometric (sandwich) assay format (Fig. 1 *A*), the concentration of the analyte of interest (e.g., thyroid stimulating hormone [TSH]) is directly proportional to the signal intensity of the washed solid phase. Exogenous biotin competes with the binding of labeled complexes to the solid phase, reduces the signal intensity of the bound fraction, and produces falsely decreased test results.

In competitive immunoassay formats (Fig. 1 *B*), the concentration of an analyte (e.g., estradiol) is inversely proportional to the signal intensity of the washed solid phase. Again, exogenous biotin reduces the signal intensity of the washed solid phase, this time producing a falsely increased test result. In general, biotin interference falsely decreases test results in immunometric assays and falsely increases test results in competitive immunoassays that use SA/B-based methods.

A third immunoassay format that is vulnerable to biotin interference utilizes biotinylated antigens and/or

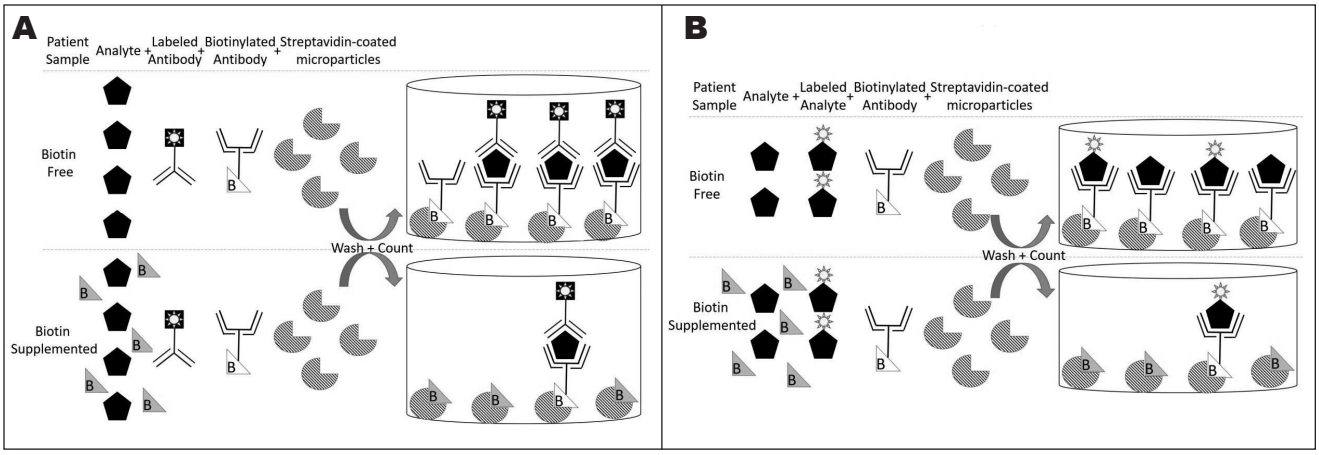

Fig. 1. Mechanisms of biotin assay interference. *A*, Sandwich immunoassay. In these formats, 2 antibodies (1 labeled, and 1 biotinylated) bind to different epitopes on the analyte, and the sandwich is bound to a streptavidin-coated solid phase. The signal intensity of the washed solid phase is proportional to the analyte concentration. Supplemental biotin interferes with the binding of the sandwich to the solid phase, decreases the signal intensity, and causes a falsely low result. *B*, Competitive immunoassay. In these formats, the analyte in the sample competes with a labeled analyte reagent for binding to a biotinylated antibody. The biotinylated antigen/antibody complex binds to a streptavidin-coated solid phase. The signal intensity of the washed solid phase is inversely proportional to the analyte concentration. Supplemental biotin interferes with the binding of antigen/antibody complexes to the solid phase, decreases the signal intensity, and causes a falsely elevated result.

labels and an anti-biotin antibody as the binding protein (instead of streptavidin). Exogenous biotin in the sample competes with the binding of biotinylated complexes to anti-biotin coated solid phases, alters the signal intensity of the bound fraction, and produces falsely decreased or increased test results in noncompetitive and competitive assays, respectively.

The mere presence of a biotin/streptavidin binding event does not necessarily imply that an assay will be vulnerable to biotin interference. In some methods, the streptavidin or the anti-biotin antibody and the biotin-labeled reagents are combined during the manufacturing process. Assay methods that use these "prebound" reagents (rather than individual reagents that are combined in the presence of the patient's sample during the analysis) are expected to be resistant to, or unaffected by biotin interference—provided that these expectations are supported by the results of interference studies that were performed by the manufacturer when the method was initially cleared by the U.S. Food & Drug Administration (FDA).

ESTIMATING THE RISK OF BIOTIN INTERFERENCE IN VULNERABLE IMMUNOASSAYS

All clinical immunoassay methods that use biotinylated reagents and biotin binding proteins should be considered to be potentially vulnerable to interference by the exogenous biotin until proven otherwise. Immunoassay manufacturers are expected to identify and test substances that might interfere with their methods, and the FDA is authorized to require that potential interfering substances be disclosed and listed as limitations to a particular method. However, the instructions for use (IFUs) that the manufacturers provide to the clinical laboratories that perform an SA/B-based method may not list biotin interference as an analytical limitation or present evidence that the vulnerability of the method to biotin interference was formally evaluated. To address the vulnerability issue, we reviewed the product labeling, as presented in the current IFUs for all of the methods available on 7 of the most popular immunoassay systems used in the United States (Table 1). Our review showed that SA/B- and anti-biotin/biotin-based methods are being used to measure a wide variety of analytes including thyroid, steroid and polypeptide hormones, infectious disease serologies, cardiac markers, tumor markers, biomarkers of anemia and autoimmune diseases, and immunosuppressive drugs. The information that was presented regarding the potential for biotin interference, whether or not exogenous biotin is identified as an analytical limitation of the method, the concentration at which biotin causes significant analytical interference, and recommendations for mitigating interference by withdrawing biotin therapy prior to specimen collection varies considerably from system to system and from method to method within a particular immunoassay system.

The next step in predicting the risk of biotin interference in a particular method is to determine the threshold at which exogenous biotin in the sample causes a significant increase or decrease in a test result. Clinical diagnostics manufacturers typically evaluate analytical interferences by spiking human serum or plasma specimens with increasing concentrations of a putative interfering substance and identifying the concentration at which the interfering substance (e.g., biotin) causes a ±10% change in the test result. The thresholds at which significant inter-

Table 1
Biotin Interference in Tests Performed by 7 Automated Clinical Assay Systems[a]

Multitest assay system	All immunoassays			Endocrine immunoassays		
	Total	Vulnerable to biotin interference	Biotin interference threshold (range, nmol/L)	Total	Vulnerable to biotin interference	Interference thresholds of potentially vulnerable endocrine tests (test [threshold in nmol/L], direction of IF)[b]
Roche Elecsys®	81	81	21-491	46	46	http://www.roche-diagnostics.ch/content/dam/corporate/roche-dia_ch/documents/serumindices/Interferences_Immunologie.pdf[30]
Ortho Vitros®	43	29	10-82	17	11	TSH[21]↓, iPTH[20]↓, FSH[41]↓, LH[21]↓, hCG[40]↓, PRL[41]↓, E2[21]↑, PRG[82]↑, TSTN[41]↑, CORT[40]↑, 25OHD[62]↑
Siemens Dimension®	26	21	205-8,200 (3 n/a)	10	9	TSH[2,050]↓, FT4[205]↑, FT3[205]↑, FSH[409]↓, LH[409]↓, hCG[n/a], PRL[8,200]↓, PROG[406]↑, TSTN[406]↑
Siemens Centaur®	67	18	41-4,090 (6 n/a)	20	4	FT4[n/a], iPTH[4,090]↔, DHEAS[409]↑, SHBG[409]↓
Beckman Coulter Access®/DXI®	48	14	41-1,000 (9 n/a)	26	6	FT4[n/a], FT3[41]↑, TT3[n/a], TG[n/a], TGAb[n/a], TPOAb[n/a]
Abbott Architect i2000®	46	2	120 (1 n/a)	18	2	TSTN[120]↔, 25OHD[n/a]
Diasorin Liaison XL®	42	0	------	20	0	------

Abbreviations: CORT = cortisol; DHEAS = dehydroepiandrosterone-sulfate; E2 = estradiol; FSH = follicle stimulating hormone; FT3 = free triiodothyronine; FT4 = free thyroxine; hCG = human chorionic gonadotropin; iPTH = intact parathyroid hormone; LH = luteinizing hormone; 25OHD = 25 hydroxyvitamin D; PRG = progesterone; PRL = prolactin; TSTN = testosterone; SHBG = sex hormone-binding globulin; TG = thyroglobulin; TGAb = anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TSH = thyroid stimulating hormone; TT3 = total triiodothyronine.

[a] The potential for biotin interference was evaluated by reviewing the manufacturer's published data regarding interference thresholds of the immunoassays performed on the Roche Elecsys® system, or by reviewing the current instructions for use (IFUs) for each of the immunoassays performed by the other six immunoassay systems. A method was considered to be potentially vulnerable to biotin interference when it utilized a streptavidin/biotin reaction, an anti-biotin/biotin reaction, or a prebound avidin/streptavidin or biotin/anti-biotin reagent in the analysis.

[b] The interference thresholds are the concentrations (nmol/L) at which exogenous biotin in a sample was reported to cause a significant analytical interference (more than a ±10 % change) in the test result. The expected directions of the interference observed in the presence of exogenous biotin at levels exceeding the threshold based on the assay format are as follows: ↓, a falsely decreased result in non-competitive (i.e., "sandwich" or immunometric) formats; ↑, a falsely increased result in competitive formats; or ↔, no effect up to the limit at which the method was shown to be free from interference during premarket testing; n/a, a biotin interference threshold was not presented in the manufacturer's IFU.

ference occurs may vary from system to system depending on assay design. A method that has been shown by the manufacturer (or the end user) to have a low threshold for interference is more likely to generate an erroneous result in the presence of exogenous biotin than a method that has a high interference threshold. The use of the published interference thresholds for risk prediction can be limited by the accuracy of the interference studies performed by the manufacturer. In the case of the Roche Elecsys® system, the accuracy of the published thresholds were verified by users of the method (21). In contrast, users of the Ortho Vitros® methods found significant analytical interference below the thresholds that were reported by the manufacturer (22). Despite the importance of the interference threshold in risk assessment, the IFUs for some FDA-cleared methods that utilize biotinylated reagents and biotin binding proteins do not present interference thresholds or other information to alert the user that the method may be vulnerable.

Once a method's vulnerability to biotin interference and the threshold for interference have been ascertained, the risk that it will give an inaccurate test result in a particular subject will depend on the amount of biotin that was taken, the pharmacokinetic parameters associated with an oral dose of that size, and when the specimen was collected relative to the subject's last dose. Based on the pharmacokinetics reviewed above, a single oral dose of biotin <1 mg/day is unlikely to interfere in an immunoassay whose manufacturer-determined interference threshold is >40 nmol/L. On the other hand, a single oral dose of 100 mg per day could result in peak serum biotin concentrations of >3,000 nmol/L. This concentration far exceeds the manufacturer-determined interference thresholds of most of the vulnerable assays listed in Table 1. In fact, the risk of an erroneous test result could persist for up to 8 days depending on the interference threshold of the method such that it might be necessary to avoid the use of vulnerable methods altogether when testing subjects who use the 100-mg biotin supplements that are now available. A recent evaluation of biotin interference in a multiple sclerosis patient who was treated with a single dose of 300 mg biotin showed falsely increased or decreased test results in 12 different endocrine assays (23). Unfortunately, comprehensive single-

dose studies of biotin pharmacokinetics in healthy subjects taking oral doses in the range of 1 to 100 mg per day have yet to be published. Once this data is available, it will be possible to estimate the likelihood of inaccurate test results after supplementation with the 5- and 10-mg dosage forms that are most commonly used by the general public.

The short-term solution for reducing the risk of erroneous test results owing to biotin interference, and the one recommended by many of the manufacturers of the vulnerable assays, is to refrain from collecting a specimen until the plasma biotin level drops below the interference threshold of the assay. The current convention is to attach a biotin interference warning at the point of order entry or to a reported test result to advise the clinical staff that the test may be subject to biotin interference and that specimens should be collected at least 8, 24, 48, etc. hours after the patient's last oral dose, depending on the interference threshold for the assay method. However, this short term solution is both impractical and error prone (24) because of the difficulty in obtaining an accurate and complete history of biotin use from the patient. The most appropriate intervention would call for the manufacturers of vulnerable methods to redesign or reformulate their assays so as to eliminate the possibility of biotin interference. Indeed, in 2011, the Siemens Centaur® intact parathyroid hormone (PTH) method, which was shown to generate falsely low test results in renal dialysis patients treated with multivitamin supplements containing (only) 300 mcg of biotin (25), was withdrawn from the market and replaced with a modified method that used prebound SA/B reagents (26). This particular incident served both as an early warning of the biotin interference problem and as a model of a long-term solution to the problem.

Other approaches that clinical laboratories could use to minimize the risk of interference with their vulnerable methods would be to move those tests to interference-free immunoassays on another analyzer. Labs that perform liquid chromatography-tandem mass spectrometry could utilize this technique for the analysis of some the endocrine tests that are affected by biotin interference (particularly thyroid and steroid hormones and thyroglobulin). However, it is a complex method that requires technical expertise and is not adapted to high-throughput assay systems. Laboratories could also develop and validate pre-analytical sample pretreatment procedures to eliminate exogenous biotin from a sample prior to analysis by a vulnerable method. For example, Piketty et al removed biotin via adsorption with magnetic microparticles coated with streptavidin (27). This technique reduced the biotin concentrations below the interference thresholds of 10 immunoassays in all but 2 of 23 plasma samples from biotin-treated patients (27). Most of the erroneous hormone results normalized after the adsorption procedure. Though effective, this approach would be expensive and time-consuming and would require the laboratory to assume full responsibility for the analytical (and possibly the clinical) performance of the modified method.

CLINICAL BIOTIN INTERFERENCE WITH COMMON IMMUNOASSAYS

Thyroid Assays

Pharmacologic biotin's most striking and prominent interference has been reported with thyroid function testing. Various case reports have been published that exhibit both positive and negative effects on thyroid function test (TFT) results depending on whether a competitive or sandwich immunoassay platform was employed. Table 2 summarizes 17 cases of biotin interference with susceptible thyroid assays described to date in the literature.

The first case reported in 1996 described a newborn female with a positive screen for congenital hypothyroidism (20). Cord blood TSH was markedly elevated and FT4 was low as measured by the Boehringer Mannheim ES700 analyzer. However, subsequent TFTs on day 2 and day 3 of life showed downtrending TSH and elevated FT4, inconsistent with the newborn screen. Further investigation revealed that the baby was started on a vitamin cocktail (including 10 mg of biotin daily) immediately after birth as the baby's sibling had died of organic acidosis 2 years prior. When TFTs were remeasured employing a solid phase chemiluminescence assay independent of SA/B interaction, the discrepancy between cord blood and day 3 samples was not observed. Two days after discontinuation of biotin therapy, TSH and FT4 levels on the ES700 analyzer resembled the cord blood samples indicating hypothyroidism, and treatment with thyroxine was initiated. The authors concluded that the variance in TFTs resulted from interference with high dose biotin supplementation.

In 2012, Kwok et al described a case of a 3-year-old Chinese girl with propionic acidemia on treatment with 10 mg of biotin 4 times a day (13). Given mild developmental delay and poor weight gain, TFTs were obtained and displayed repeatedly suppressed TSH with normal FT4 and FT3 by the Roche Cobas e601 sandwich and competitive immunoassays, respectively. The patient showed no signs of thyrotoxicosis or goiter. Given the discrepancy between the TFTs and clinical picture, TSH was repeated with serial dilution, resulting in normalization of the TSH concentration, suggesting interference. The Cobas e601 TSH assay utilized SA/B in a sandwich platform. Simultaneous samples were re-analyzed with the Beckman Coulter assay exhibiting normal TSH, but now significantly elevated FT4 and FT3. The Beckman TSH assay did not use SA/B interaction, while the Beckman FT4 and FT3 were competitive binding assays using monoclonal anti-T4 antibody coupled to biotin and biotinylated T3 analogue, respectively. The authors consequently confirmed that addition of exogenous biotin to a concentration of ≥20 μg/L to a serum sample of known TSH and free hormone concentrations lowered

TSH values in the Cobas e601 assay, but increased FT4/FT3 as measured by the Beckman Coulter analyzer.

Subsequently, several more cases in children (5,19) and adults (11,19,23,28-32) have been reported ranging in age from newborn to 74 years. All cases have demonstrated that ingestion of biotin can produce erroneous biochemical diagnoses dependent on the manufacturer and biotin sensitivity of SA/B immunoassays utilized. Since TSH is a sandwich assay while FT4/FT3 are competitive assays, the expected derangement with biotin interference is false depression of TSH along with a false elevation of FT4/FT3. Children taking biotin in supraphysiologic doses (2 mg/kg/day to 30 mg/day) for inborn metabolic disorders displayed a pattern of low or normal TSH levels and elevated total or free T4 and T3 dependent on the assay. Similar patterns were evident in adult patients receiving biotin at doses up to 300 mg/day based on recent data suggesting benefit in multiple sclerosis (6). Barbesino recently described a case of a 55-year-old man with multiple sclerosis presenting with labs suggestive of severe hyperthyroidism (28). Importantly, data also showed a positive TSH binding inhibiting antibody at a high concentration, supporting a diagnosis of Graves disease. However, the patient lacked any signs or symptoms consistent with Graves and imaging demonstrated a normal thyroid radioiodine uptake/scan and thyroid ultrasound. A subsequent bioassay for thyroid-simulating immunoglobulin was undetectable, a finding inconsistent with Graves disease. Further questioning revealed the recent initiation of biotin 100 mg 3 times a day for multiple sclerosis. Two weeks after discontinuation of biotin, TFTs normalized.

It is noteworthy to recognize that interference of biotin can also impact thyroglobulin assays leading to falsely low results in sandwich assay formats (16,28). In vivo data from Wijeratne et al in which one of the authors ingested 30 mg of biotin displayed an 11-fold decline in thyroglobulin levels. The interference effect was persistent for at least 24 hours after biotin ingestion (19). This interaction poses a challenge in patients with thyroid cancer in whom thyroglobulin levels may aid in determining persistent or recurrent disease. A biotin-related underestimation of thyroglobulin levels may produce false reassurance (28).

Parathyroid Assays

Biotin supplementation can lead to falsely low PTH as revealed in several case reports (Table 2). The first generation PTH assay is a radioimmunoassay binding the C-terminus (PTH 39-84). Second- and third-generation PTH assays are a 2-site immunometric sandwich assay that utilize a capture antibody in addition to a biotinylated detection antibody that noncovalently binds to streptavidin-coated particles.

The first case describes a postmenopausal female with laboratory evidence of hypercalcemic primary hyperparathyroidism with a femoral fracture and renal insufficiency (8). She then started biotin for hair growth and lab data obtained during pre-operative evaluation revealed undetectable PTH (8). Repeat labs 1 month after biotin discontinuation once again revealed an elevated PTH. In a subsequent case, a postmenopausal female with a history of nephrolithiasis and primary hyperparathyroidism underwent subtotal parathyroidectomy (8). Postoperative calcium levels normalized; however, follow-up PTH levels were inappropriately undetectable after the initiation of biotin for neuropathy. Repeat PTH normalized after biotin discontinuation, but PTH was again undetectable after the resumption of biotin supplementation. A third case depicts a postmenopausal female with end-stage renal disease secondary to failed renal transplant and severe osteoporosis. Biochemistries revealed an elevation of serum calcium and alkaline phosphatase in conjunction with a discordantly low PTH. The discovery of elevated PTH when repeated by an immunoassay from a different manufacturer re-affirmed assay interference and the patient revealed biotin intake for restless leg syndrome (33).

Other Immunoassays

Biotin interference with SA/B immunoassays have also been anecdotally described in patient samples for estradiol, testosterone, progesterone, DHEAS, vitamin B12, prostate-specific antigen, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (19,23,30). In a recent abstract, graded concentrations of biotin were added to pooled plasma to determine interference with a variety of analytes (21). The analytes studied of relevance to this review were FSH, LH, prolactin, beta-human chorionic gonadotropin, and progesterone. In each case, there was substantial interference. Negative interference was seen, as expected, in the sandwich assays, whereas the competitive assays showed positive interference (22). There are also hormonal assays for which there is theoretical biotin interference, but for which we could find no full-length published reports in patients. These include prolactin, growth hormone, insulin-like growth factor 1, adrenocorticotropic hormone, cortisol, and aldosterone. Other nonhormonal areas for vigilance given our review of biotin-based methodology include infectious disease serologies, cardiac and tumor markers, biomarkers of anemia and autoimmune diseases, and concentrations of immunosuppressive drugs.

CONCLUSION

The prevalence of high-dose biotin usage in the United States has dramatically increased over the past 2 years and is likely to continue, placing more individuals at risk for erroneous test results. Until a permanent solution to the problem of biotin interference is offered by the clinical diagnostics industry or mandated by the FDA, healthcare providers should be aware that biotin supplements can cause falsely increased or decreased results for a variety

Table 2
Summary of Case Reports Describing Biotin Interference on Thyroid and Parathyroid Immunoassays[a,b]

Serial no.	Paper (year)	Age/ sex	Diagnosis	Biotin dose	Magnitude of change in TSH on biotin	Magnitude of change in FT4 on biotin	Magnitude of change in T3 (free or total) on biotin	Effect on other thyroid parameters	Laboratory re-assessment after biotin discontinuation (days)	Assay manufacturer
1.	Henry et al (1996)	New-born	Routine screening for congenital hypothyroidism Family history of organic academia in deceased sibling	10 mg QD	↓	↑	-	-	2	Boehringer Mannheim ES700
2.	Wijeratne et al (2012)	1-week old baby	Liver failure, lactic acidosis	10 mg TID	↔	↑	↑↑	-	-	Beckman Coulter DxI
3.	Kummer et al (2016)	1 mo M	Neonatal mitochondrial disease	7 mg/kg/day	↓	↑↑	↑	TRAb +	1-7	Roche Diagnositics GmbH
4.	Kummer et al (2016)	1 mo M	Neonatal mitochondrial disease	8 mg/kg/day	↓	↑↑	-	TRAb +	1-7	Roche Diagnositics GmbH
5.	Kummer et al (2016)	5 mo M	Infantile mitochondrial disease	2 mg/kg/day	↓	↑↑	↑	TRAb +	1-7	Roche Diagnositics GmbH
6.	Kummer et al (2016)	2 yr F	Biotin-thiamine-response basal ganglia disease	14 mg/kg/day	↓	↑↑	-	TRAb +	1-7	Roche Diagnositics GmbH
7.	Kummer et al (2016)	2 yr M	Biotin-thiamine-response basal ganglia disease	15 mg/kg/day	↓	↑↑	↑	TRAb +	1-7	Roche Diagnositics GmbH
8.	Kwok et al (2012)	3 yr F	Propionic acidemia	10 mg QID	↓↓ ↔	↔ ↑	↔ ↑	-	-	Roche Cobas e601 Beckman Coulter DxI
9.	Kummer et al (2016)	9 F	Biotin-thiamine-response basal ganglia disease	10 mg/kg/day	↓	↑↑	↑	TRAb +	1-7	Roche Diagnositics GmbH
10.	Wijeratne et al (2012)	M author	None	30 mg single dose	-	↑↑	↑	Tg ↓↓↓	1	Beckman Coulter Dxl
11.	Trambas et al (2016)	-	Multiple sclerosis	300 mg QD	↓	↑↑	↑	-	-	-
12.	Willeman et al (2017)	39 M	Multiple sclerosis	50 mg QD	↓↓	↔	↔		30	Siemens Dimension

Continued on next page

Table 2 ***Continued***

13.	Batista et al (2016)[c]	52 F	Hair loss	10 mg QD	↓	↔	↔	TRAb +	2	Vitros 5600 (Ortho-Clinical) E170/Cobas 602 Modular (Roche)
14.	Lee and Brennan (2016)	52 F	Multiple sclerosis	300 mg QD	↓	↑↑	↑↑	-	5	LabCorp No assay manufacturer reported
15.	Barbesino (2016)	55 M	Multiple sclerosis	100 mg TID	↓	↑↑	↑	TSH binding inhibiting Ab + TSI Ab - Tg ↓ Tg Ab	14	Elecsys Roche Thyretain Quidel Access Tg Beckman Coulter Access Tg Ab II Beckman Coulter
16.	Elston et al (2016)	63 F	Multiple sclerosis	100 mg TID	↓	↑↑↑	↑	TRAb +	1	Roche Elecsys 2010 Beckman Coulter
17.	Minkovsky et al (2016)	74 F	Multiple sclerosis, chronic autoimmune thyroiditis	100 mg TID	↓ /↓↓	↑↑	↑	-	3	Roche Diagnostic
18.	Waghray et al (2013)	60 F	Hypercalcemia, suspicion for primary hyperparathyroidism on prior labs	1.5 mg QD	↓↓				30	Siemens Advia Centaur Roche Cobas E411
19.	Waghray et al (2013)	62 F	Primary hyper-para-thyroidism s/p subtotal para-thyroidectomy	5 mg QD	↓↓				180 270	Siemens Advia Centaur Roche Cobas E411
20.	Meany et al (2009)	64 F	Renal osteodystrophy and elevated alkaline phosphatase	10 mg QD	↓					Roche Elecsys 2010
21.	Trambas et al (2016)	-	Multiple sclerosis	300 mg	↓				-	-

Abbreviations: F = female; FT4 = free thyroxine; FT3 = free tri-iodothyronine; M = male; QD = once daily; QID = 4 times daily; Tg = thyroglobulin; Tg Ab = thyroglobulin antibodies; TID = 3 times daily; TRAb = anti-thyrotropin receptor antibodies; TT3 = total tri-iodothyronine; TSH = thyroid-stimulating hormone; TSI = thyroid stimulating immunoglobulin.

[a] TSH/Tg/Tg Ab/TRAb: Normal ↔ ; Low ↓; Undetectable ↓↓ ; Positive +

[b] Magnitude of Change in Serum FT4, FT3, and PTH Concentrations:
0-3×↑ / ↓
3.1-6× ↑↑ / ↓↓
>6× ↑↑↑ / ↓↓↓

[c] Index case; additional data from (30) demonstrating biotin interference mimicking hyperestrogenism not shown.

of immunoassays. It would be of benefit to not only review prescribed medications, but also the supplements patients are taking and to always correlate laboratory and clinical findings. Healthcare providers can contact the clinical laboratory that performs their testing for up-to-date information on which lab's test methods are vulnerable to biotin interference and for advice on how to prepare the patient and time the blood draw to the minimize the risk of inaccurate test results. Laboratories can attach a biotin interference warning to test results that were measured with a potentially vulnerable assay and to advise the clinical staff that specimens for these tests should be collected at least 8, 24, 48, etc. hours after the patient's last biotin dose, depending on the interference threshold for the assay method. In general, patients taking over-the-counter biotin should be advised to withhold the supplement for at least 48 hours preceding their blood tests if clinically possible. This should be sufficient time to allow for biotin clearance and removal of the majority of assay interference independent of manufacturer, with the exception of patients taking biotin doses over 100 mg/day who may require further withdrawal.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

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