A 45-year-old woman presented to our clinic in July 2019 for evaluation of intermittent fever, rigors, diaphoresis, malaise, and urinary symptoms of frequency and urgency of 5 days’ duration. Her history was notable for Graves disease after total thyroidectomy 8 months previously after failed medical management, with no neck swelling or pain at present; an incidentally discovered and resected papillary thyroid microcarcinoma; and hypertension. A reconciled medication list included levothyroxine (125 μg/d), sertraline (100 mg/d), amphetamine-dextroamphetamine (20 mg/d), propranolol (40 mg twice daily), selenium (200 μg/d), and biotin (10 mg/d). She reported no relevant family history or sick contacts, but she may have had tick exposure. Physical examination revealed a temperature of 39.4°C, heart rate of 64 beats/min, blood pressure of 98/56 mm Hg, weight of 85 kg, exophthalmos and lagophthalmos, and healed Kocher incision with no neck masses or tenderness. Abdominal examination revealed mild suprapubic tenderness.

The differential diagnosis included infection (urinary tract infection [UTI], viral syndrome, tick-borne illness) and hyperthyroidism in the setting of ongoing levothyroxine therapy and her history of Graves disease. Subsequent laboratory studies revealed the following (reference ranges provided parenthetically): hemoglobin, 10.7 g/dL (11.6 to 15.0 g/dL); white blood cell count, 16.7 × 10⁹/L (3.4 to 9.6 × 10⁹/L); creatinine, 0.91 mg/dL (0.59 to 1.04 mg/dL); 2 sets of blood cultures with negative results; urine culture growing *Escherichia coli*, greater than 100,000 colony-forming units per milliliter; and negative results on a tick-borne pathogen panel including Lyme disease serology. Chest radiography revealed no abnormalities. Thyroid function testing (TFT) revealed the following: thyrotropin, 0.01 mIU/L (0.3 to 4.2 mIU/L); free thyroxine (fT4), 1.3 ng/dL (0.9 to 1.7 ng/dL); and free triiodothyronine (fT3) 2.4 pg/mL (2.8 to 4.4 pg/mL).

Antimicrobial therapy was initiated for a UTI.

1. Which one of the following is the most likely explanation for this patient’s abnormal TFT results?
   a. Excessive levothyroxine dose
   b. Assay interference by biotin
   c. Recurrence of Graves disease
   d. Subacute thyroiditis
   e. Euthyroid sick syndrome

Patients without endogenous thyroid hormone production require levothyroxine at approximately 1.6 to 1.7 μg/kg per day, translating to 136 to 145 μg/d. At our patient’s dosage of 125 μg/d, excessive dosing of levothyroxine is unlikely to be the cause of her suppressed thyrotrpin concentration, particularly with her normal fT4 and mildly reduced fT3 levels. It is further premature to assume excessive thyroid hormone replacement, as suggested by the abnormal thyroid test results, because the patient endorsed daily biotin use.

Biotin (vitamin B7) is known to interfere with TFT results. Biotin’s strong affinity for streptavidin is employed in many commonly used immunoassays, including those for thyrotropin, fT4 and fT3. Excess serum biotin interferes with biotin-streptavidin binding, leading to falsely low or high results depending on the type of assay. Thyrotrpin is measured using a sandwich assay. In a
sandwich assay, the concentration of the substance being measured is directly proportional to the signal; extra biotin lowers the signal, causing a falsely low value, which could explain this patient’s abnormal TFT results.2

Thyrotoxicosis secondary to Graves disease can rarely present with fever; however, this patient underwent a total thyroidectomy, and concurrent UTI better explains her constitutional symptoms. Although cases of Graves disease secondary to ectopic thyroid tissue, usually along the thyroglossal duct, have been reported, they are very rare.3

Subacute thyroiditis in remnant or ectopic thyroid tissue could present with fever and malaise along with biochemical evidence of hyperthyroidism in initial stages. However, neck pain and tenderness are the predominant clinical features, and both were absent, making subacute thyroiditis unlikely.

Euthyroid sick syndrome (also called nonthyroidal illness syndrome) refers to abnormal TFT results sometimes seen in euthyroid individuals during acute illness or stress. Usually, the thyrotrpin level is low-normal (0.05 to 0.3 mIU/L), and fT3 and fT4 values are also reduced.4 Suppressed thyrotrpin (<0.01 mIU/L) is rare and typically reflects thyrotoxicosis. Euthyroid sick syndrome is also a diagnosis of exclusion, and potential confounding by biotin interference must be addressed.

2. In view of the findings thus far, which one of the following is the best next step in the evaluation of this patient’s condition?

a. Assume interference from biotin and do nothing further
b. Advise patient to switch to a multivitamin with a lower (5-mg) dose of biotin
c. Withhold the morning dose of biotin and retest
d. Retest after withholding biotin for 1 week
e. Request alternate TFT assay

Although biotin is a potential source of aberrant TFT results in our patient, this hypothesis should be established definitively, and appropriate levothyroxine dosing should be confirmed. Switching to a lower biotin dose of 5 mg will not alleviate concerns for assay interference. A 5-mg daily supplement approximates a 30-ng/mL serum biotin concentration. Interference has been noted at serum biotin concentrations as low as 10 ng/mL.2

The half-life of biotin is approximately 15 hours in a healthy adult, although the duration of interference after ingestion depends on the dose ingested, duration of use, and the patient’s kidney function.2 Several studies have found elevated serum biotin levels up to 24 hours after ingestion. Therefore, retesting the following day would not definitively eliminate the risk of interference.5

Retesting 1 week after withholding biotin would be appropriate to ensure adequate elimination of excess serum biotin in our patient with normal renal function who was taking 10 mg of biotin daily. Even patients taking massive doses of biotin (30 to 300 mg) generally clear biotin to levels below the threshold for interference within 1 week.5

Radioimmunoassays and liquid chromatography–mass spectrometry are good alternative assays that are not susceptible to interference from biotin. However, cost and local availability limit their use.5

Our patient was advised to return for retesting in 1 week. For unrelated personal reasons, this appointment was delayed and she was retested in 6 weeks. Her presenting symptoms had resolved with antibiotic therapy for UTI. Repeated TFT while off biotin for 6 weeks revealed a thyrotrpin level of less than 0.01 mIU/L, fT4 value of 2.1 ng/dL, and fT3 level of 3.3 pg/mL.

3. Which one of the following is the best next step to address the patient’s persistently low thyrotrpin level?

a. Check serum biotin levels to rule out interference
b. Reduce the levothyroxine dose
c. Nothing, as this is likely persistent euthyroid sick syndrome
d. Start methimazole
e. Check for thyrotrpin receptor antibodies
This patient’s thyrotropin concentration is now suppressed with newly elevated fT4. Although this pattern could be consistent with biotin interference, the patient reported biotin discontinuation 6 weeks before retesting. Serum biotin can be measured using various assays including liquid chromatography and spectrophotometric and biological assays. However, these tests are used primarily in the evaluation of congenital or acquired disorders of biotin metabolism. This step was unnecessary in our case.

Suppressed thyrotropin and elevated fT4 are consistent with excessive thyroid hormone levels. In the setting of thyroid hormone replacement, this problem is due to excessive dosing. The patient’s dose of levothyroxine should be reduced with laboratory values rechecked in 6 to 8 weeks.

Euthyroid sick syndrome manifests during an acute illness, with normalization of TFT results within 1 to 2 weeks of recovery. It is a diagnosis of exclusion, and persistence of abnormal results 6 weeks after recovery indicates an alternate etiology.4

Methimazole is an antithyroid medication used in the management of hyperthyroid states like Graves disease. Recurrence of Graves disease after total thyroidectomy is highly unlikely, and methimazole therapy is not indicated. Thyrotropin receptor antibody levels are elevated in Graves disease and may persist after thyroidectomy, irrespective of TFT levels. They are not useful in this case.

The patient’s levothyroxine dose was reduced to 100 μg/d. Follow-up testing after 6 weeks yielded a thyrotropin level of 0.05 mIU/L, an fT4 value of 1.3 ng/dl, and an fT3 level of 2.6 pg/mL. The levothyroxine dose was further reduced to 88 μg/d with ultimate normalization of thyrotropin at 1.2 mIU/L. She continued to have persistent Graves orbitopathy and was referred to the ophthalmology department for further management.

4. To provide accurate information to the patient about the health effects of biotin supplementation, which one of the following statements is correct?
   a. Biotin can affect other assays as well
   b. Biotin interferes with laboratory assays inconsistently, and the effect seen in this patient was driven by concurrent UTI
   c. Taking biotin as part of a multivitamin pill reduces the chance of interference
   d. High biotin doses can be dangerous
   e. There is no medical indication for biotin

Although not all immunoassays utilize biotin-streptavidin binding in their assay formats, a majority do, as evidenced by a recent review of 374 immunoassays that found that 59% used biotin.5 There is growing concern over the potential for falsely low troponin results due to biotin interference in patients evaluated for myocardial infarction. In 2019, the US Food and Drug Administration issued a warning specifying that several laboratory assays are susceptible to biotin interference.6

The likelihood of interference depends on the serum biotin concentration and the assay’s threshold for interference. Although reduced creatinine clearance can raise serum biotin concentrations, a UTI will not. Serum biotin concentration and subsequent potential for interference depend on the ingested dose regardless of the pill composition. Biotin is a water-soluble vitamin that does not accumulate and is renally cleared. There are no known adverse effects or toxicity from high doses of biotin.2,5

A healthy individual consuming a balanced diet is able to obtain the daily adequate intake of biotin (30 μg), making additional supplementation unnecessary.8 However, there are several conditions in which high-dose biotin is beneficial, including biotinidase deficiency, biotin-thiamine-responsive basal ganglia disease, and malabsorption syndromes and also the use of total parenteral nutrition.5 Recent studies have documented benefit in progressive multiple sclerosis, disorders of mitochondrial energy metabolism, and in patients undergoing dialysis who experience muscle cramps.5

5. What advice should you give the patient regarding further biotin supplementation?
   a. Stop taking biotin because of the risk for hyperthyroidism
   b. Continue taking biotin for healthy hair, skin, and nails
c. Consume raw eggs for a good dietary source of biotin

d. Continue taking biotin at an increased dose of 300 μg/d to prevent neuropathy

e. Resume biotin supplement if desired because there is no known physiologic harm, but do not take it prior to undergoing laboratory testing

Biotin ingestion does not lead to hyperthyroidism but rather may cause TFT results to mimic hyperthyroidism. Although biotin is claimed to improve the health of nails, skin, and hair, there is no definitive evidence of benefit and it should not be recommended. Raw egg white contains the glycoprotein avidin, which tightly binds biotin and thus prevents intestinal absorption. Consuming large quantities of egg white can lead to biotin deficiency. Cooking denatures avidin, preventing this interaction. Dietary biotin sources include sweet potatoes, nuts, fish, and beef liver. Megadoses of biotin (300 μg/d) have shown benefit in individuals with progressive multiple sclerosis. However, there is no evidence that high doses can prevent neuropathy.

The patient can continue taking biotin if she wishes, provided she does not take it for at least 2 days prior to any laboratory studies and ensures documentation in her medication list in the event that laboratory tests are needed urgently. There are no known toxicities that would necessitate stopping the supplement.

DISCUSSION
An estimated 12% of the US population will have a thyroid disorder in their lifetime. Thyroid hormone levels are checked in a variety of clinical settings because of the heterogeneity of presentations from thyroid hormone excess and insufficiency. The most common means of evaluating thyroid function is by measuring the thyrotropin level. In 2008, approximately 59 million thyrotropin tests and 18 million fT4 tests were performed in the United States. Although thyrotropin is generally sufficient to evaluate the hypothalamic-pituitary-thyroid axis, there is some physiologic diurnal variation in thyrotropin and levels can be affected by acute illness and non-thyroidal medications, most notably glucocorticoids. Levels of fT4 should be measured in patients with known or suspected central hypothyroidism and in patients with abnormal thyrotropin levels, unless the reason for the abnormality is known/expected. Measuring triiodothyronine (total or free) levels is not routinely recommended.

Thyroid function tests evaluating the hypothalamic-pituitary-thyroid axis are conducted using immunoassays that take advantage of streptavidin’s strong affinity for biotin. At our institution, thyrotropin and fT4 are measured with the Roche Cobas immunoassay analyzer, while fT3 is measured with the Beckman Coulter Dxl 800 analyzer. In the thyrotropin immunoassay, a sandwich complex is formed between a biotinylated monoclonal thyrotropin-specific antibody, a ruthenium-labeled monoclonal thyrotropin-specific antibody, and serum thyrotropin. Streptavidin-coated microparticles are added, binding to the biotin-labeled sandwich, and the measured thyrotropin concentration is directly proportional to the emitted signal intensity. In contrast, fT4 is measured using a competitive immunoassay, in which ruthenium-labeled antithyroxine antibodies bind fT4 in the sample. Biotinylated thyroxine and streptavidin-coated microparticles are added and bind the leftover ruthenium-labeled antibodies. The serum fT4 concentration is therefore inversely proportional to the signal intensity. Free triiodothyronine is similarly measured using a competitive immunoenzymatic assay, but using an antitriiodothyronine monoclonal antibody conjugated to alkaline phosphatase, streptavidin-coated particles, and a biotinylated triiodothyronine analogue.

Ingestion of biotin supplements and the subsequent increase in serum biotin concentrations lead to interference resulting in artificially lower thyrotropin and elevated fT4 and...
fT3 levels. In the thyrotropin sandwich immunoassay, circulating biotin competes with the biotinylated thyrotropin immunocomplex for streptavidin-coated microparticles, such that some thyrotropin remains unbound and uncaptured by the electrode for chemiluminescent quantification. In the fT4 and fT3 competitive immunoassays, circulating biotin competes with biotinylated thyroxine and triiodothyronine, respectively, for streptavidin binding, which results in more biotinylated thyroxine and triiodothyronine left unbound and quantified along with endogenous fT4 and fT3 in the patient's serum. It is interesting to note that biotin interference mimics the pattern expected with Graves disease, reinforcing the importance of rechecking TFT results without biotin interference.

Biotin (vitamin B7) is a small water-soluble vitamin that participates in several carboxylation reactions in the body. A balanced diet provides the daily adequate intake of 30 μg. Over the past few decades, biotin has been purported to have cosmetic benefits in hair, nails, and skin growth, prompting increased intake in over-the-counter biotin supplements despite the lack of evidence for benefit.

Biotin interference with TFT results is the most frequently reported in the literature, but other immunoassays may be affected as well. For example, corticotropin, luteinizing hormone, follicle-stimulating hormone, and parathyroid hormone levels are falsely low in the presence of biotin, while assays testing for cortisol, testosterone, and estrogen result in falsely high levels. Levels of tumor markers like carcinoembryonic antigen, CA-125, CA 19-9, α-fetoprotein, and prostate-specific antigen are falsely lowered, hindering monitoring for cancer recurrence. Infectious disease serologies for HIV and hepatitis B and C are can be falsely lowered, with potential for missed diagnoses. Finally, cardiac markers like troponin may be spuriously low, leading to potential misdiagnosis of acute coronary syndrome in an emergency setting. A recent study found that 7.4% of 1442 samples collected in the Mayo Clinic emergency department had biotin concentrations at or above the lowest known threshold for interference in Roche immunoassays (10 ng/mL) and 2% of patient samples had biotin concentrations greater than 20 ng/mL, which is the threshold for interference with the troponin T immunoassay used in the emergency department.

It is evident that biotin, although physiologically “harmless,” can result in several potentially hazardous clinical situations through interference with immunoassays. The susceptibility for interference of a specific immunoassay depends on the test threshold for interference and the serum biotin concentration. The normal serum biotin concentration is 0.05 to 0.83 ng/mL. The threshold for interference can range from 10 ng/mL to 200 ng/mL. Although the exact dose-response relationship of oral to serum biotin is unavailable, most over-the-counter supplements contain between 5 and 100 mg of biotin, with higher doses posing increased risk of interference.

Improving awareness is the first step toward mitigating this issue. A thorough medication review including the intake of over-the-counter supplements is imperative. Abstaining from biotin is the best strategy to avoid interference. The half-life of biotin is about 15 hours in a healthy individual; however, levels depend on the dose ingested and kidney function. A general recommendation of withholding biotin for 2 to 3 days prior to testing is reasonable given evidence of clearance in prior studies. Removing excess biotin from a sample is viable but expensive, as are alternative testing modalities such as radioimmunoassays and liquid chromatography–mass spectrometry. Ultimately, it is important to remember that the full clinical picture needs to be considered when encountering an unexpected test result.

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REFERENCES


CORRECT ANSWERS: 1. b. 2. d. 3. b. 4. a. 5. e