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When vitamin supplementation leads to harm: The growing popularity of biotin and its impact on laboratory testing

High biotin concentrations in blood samples for immunoassays that employ biotin-streptavidin interactions can interfere with investigations for cardiac disease, endocrine disorders, malignancies, anemias, and infectious diseases and lead to falsely low or falsely high results.

ABSTRACT: Biotin, also known as vitamin B7 or vitamin H, has seen a surge in popularity in recent years based on limited evidence that it enhances hair, skin, and nail growth. Due to its water-soluble properties, biotin is excreted through the urinary system and is considered nontoxic even at large doses. However, a high concentration of biotin in blood can interfere with laboratory tests that use technology dependent on biotin-streptavidin interactions. These tests include immunoassays used to investigate or monitor cardiac disease, endocrine disorders, malignancies, anemias, and infectious diseases. Increasingly, cases of erroneous laboratory results due to biotin use have been reported in the medical literature. The results can be falsely low or falsely high, and in either case can lead to patient misdiagnosis and mismanagement. Mitigation is possible when biotin interference is identified. Patients can be advised to discontinue the supplement before follow-up testing or physicians can order an alternative testing method. While laboratory professionals have been aware of biotin interference for many years, greater awareness among health care providers in general is needed to ensure that biotin supplementation is identified and mitigation strategies are considered.

A 54-year-old female was being assessed for thyroid dysfunction following self-reported symptoms of weight gain and lethargy. The patient had no neck tenderness and exhibited no symptoms of goiter. Past medical history was significant for relapsing-remitting multiple sclerosis and anxiety. Laboratory investigations were ordered and the results were consistent with thyrotoxicosis: thyroid-stimulating hormone (TSH) of 0.08 mU/L (reference range 0.34–4.82 mU/L), free thyroxine (FT4) of 33.2 pmol/L (10.0–20.0 pmol/L), and free triiodothyronine (FT3) of 46.1 pmol/L (3.5–6.5 pmol/L). Given that these laboratory values were not supported by the clini-

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cal presentation, new blood work was ordered. When a similar pattern of values was found (TSH 0.01 mU/L, FT4 > 103.0 pmol/L, FT3 > 46.1 pmol/L), a referral was made to endocrinology. Upon being questioned by the endocrinologist, the patient described taking high-dose biotin for her multiple sclerosis. The patient was advised to stop the biotin for 1 week before repeat testing. Follow-up blood work revealed the patient to be euthyroid, with a TSH level of 1.78 mU/L, FT4 of 12.6 pmol/L, and FT3 of 3.7 pmol/L, along with an undetectable TSH receptor antibody titre.

Clinical and commercial use of biotin

Biotin, also known as vitamin B7 or vitamin H, is a water-soluble vitamin that serves as a cofactor for a number of carboxylase reactions, making it essential to the functioning of various metabolic pathways.[1-4] It is involved in fatty acid synthesis, catabolism of branched-chain amino acids, gluconeogenesis, islet cell gene expression, insulin secretion, and the Krebs cycle.[4] The recommended dietary reference intake for biotin is 30 μg/day. The vitamin is found in various foods, including egg yolk, pork, liver, whole cereals, soybeans, avocado, cauliflower, and leafy greens.[1-4] Because of its abundance in a typical North American diet, biotin deficiency is uncommon, and supplementation is rarely indicated. Clinically, biotin may be prescribed in patients with malabsorptive disorders or in those on total parenteral nutrition. It may also be useful in relieving muscle cramps in hemodialysis patients.[5] High doses of biotin (5 to 30 mg/day) are recommended in certain inborn errors of metabolism, such as in biotinidase deficiency, propionic acidemia, or in holocarboxylase synthetase disorders.[6] More recently, mega-doses of biotin (up to 300 mg/day) have shown promise in secondary progressive multiple sclerosis.[7]

In the past decade, biotin supplementation in doses up to 20 mg a day has been marketed to promote hair, skin, and nail growth. Although there is limited evidence to support this claim, marketing led to biotin sales of up to 49.6 million units in the US alone between July 2016 and July 2017.[1] Biotin is currently the top-selling multivitamin supplement on Amazon.ca. Locally, a survey of 660 patients visiting the Diamond Health Care Centre Outpatient Laboratory at Vancouver General Hospital found that 50 respondents (7.6%) were taking biotin. The majority of users were female (92%) and biotin users tended to be younger than non-users: age 49 (19 to 85) years versus age 54 (19 to 98) years. The prevalence of biotin supplementation in our survey population was similar to that reported by the Mayo Clinic, which found 7.7% of outpatients to be ingesting biotin.[1]

Biotin interference with laboratory tests

As biotin is readily available over the counter and is perceived to be harmless, patients may not disclose their biotin use to physicians unless specifically asked. Further, health care providers may also view biotin supplementation as innocuous. However, when found in high concentrations in blood, biotin can interfere with laboratory tests that employ biotin-streptavidin interactions as part of the assay technology. Biotin-streptavidin binding is commonly used in immunoassays due to its avidity and stability, which facilitate immunoassay sensitivity and specificity. A 2017 study by Holmes and colleagues found that out of the 374 methods operated by 8 of the most popular immunoassay analyzers in the US, 221 (59%) were biotin-based.[4] Depending on the laboratory and the analytical platforms used, a broad range of tests may be affected by biotin supplementation, including those used in the diagnosis

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or monitoring of cardiac disease, endocrine disorders, malignancies, anemias, and infectious diseases (**Table 1**).[6]

Biotin-containing blood samples can affect laboratory tests by competing with biotinylated reagents for binding to streptavidin. Depending on whether the assay is noncompetitive or competitive, the result may be falsely low or falsely high (**Figure**). In noncompetitive or sandwich immunoassays, supplemental biotin competes with reagent biotin for binding to streptavidin-coated beads. With a smaller volume of reagent biotin-streptavidin complexes formed, the assay signal is decreased and a factitiously low result is reported. This is in contrast to competitive immunoassays, which are generally used to measure small molecules such as FT4 or FT3, where the assay signal is inversely proportional to the analyte concentration. In these immunoassays, biotin competes with labelled biotinylated analyte for binding to streptavidin and results can be spuriously high.

Table 1. Immunoassays at high risk for analytic interference from biotin supplementation.

Conditions	Immunoassays
Cardiac disease	• Troponin I, troponin T • Brain natriuretic peptide (BNP), N-terminal pro brain natriuretic peptide (NT-pro BNP)
Endocrine disorders	• Thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroid peroxidase (TPO) antibodies, anti-TSH receptor antibodies, thyroglobulin • Estradiol, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) • Human chorionic gonadotropin (hCG), prolactin, growth hormone • 25-hydroxyvitamin D, parathyroid hormone (PTH) • Cortisol
Malignancies	• Gastrin, alpha fetoprotein (AFP), carcinoembryonic antigen (CEA) • Cancer antigen (CA) 19-9, CA 125, CA 15-3 • Calcitonin • Prostate-specific antigen (PSA)
Anemias	• Ferritin • Folate, vitamin B12
Infectious diseases	• Hepatitis A virus (HAV) serologies: HAV total, anti-HAV total, anti-HAV IgM • Hepatitis B virus (HBV) serologies: HBsAg, anti-HBs, anti-HBc total, anti-HBc IgM, HBeAg, anti-HBe • Hepatitis C virus (HCV) serologies: anti-HCV • Herpes simplex virus (HSV) serologies: HSV-1 IgG, HSV-2 IgG • Rubella serologies: rubella IgG, rubella IgM
Others	• C-reactive protein (CRP) • Procalcitonin • Anti-CCP (cyclic citrullinated peptide) antibodies • IgE • Digoxin, cyclosporine, sirolimus

Adapted from Holmes E, Samarasinghe S, Emanuele A, Meah F.[4]

In the Holmes study referred to above, 37% of the biotin-based immunoassays evaluated were affected by serum biotin levels of less than 51 ng/mL.[4] Mean peak serum biotin concentrations of 8.6 ng/mL have been reported following consumption of a single dose of 1 mg, while concentrations of 495 ng/mL have been reported following consumption of 100 mg.[4] In a study by Grimsey and colleagues, ingestion of biotin for 5 consecutive days was found to produce peak median serum biotin levels of 46 ng/mL (5 mg dose daily), 103 ng/mL (10 mg dose daily), and 184 ng/mL (20 mg dose daily).[8] The higher the dose of biotin ingested, the more likely that biotin-based laboratory assays will be impacted.

Increasingly, biotin consumption is being found to result in inaccurate laboratory findings that lead to misdiagnosis and mismanagement. Misleading thyroid function results are often described in the medical literature,[2,3,9-13] as biotin causes falsely high FT4 and FT3 values and falsely low TSH results that mimic hyperthyroidism. In one reported case, a newborn female with a positive screening test for congenital hypothyroidism was found to have decreasing TSH levels and elevated FT4 levels on subsequent testing.[12] Further investigations revealed that the neonate had been started on a vitamin cocktail containing 10 mg of biotin daily because the baby's sibling had died of organic acidosis a few years prior. Discontinuation of biotin supplementation and repeat thyroid function testing using an alternative methodology confirmed the initial diagnosis of hypothyroidism, and thyroxine therapy was initiated.

In another case, a 64-year-old female with end-stage renal disease was thought to have adynamic bone disease due to a low normal parathyroid hormone (PTH) level, intermittently

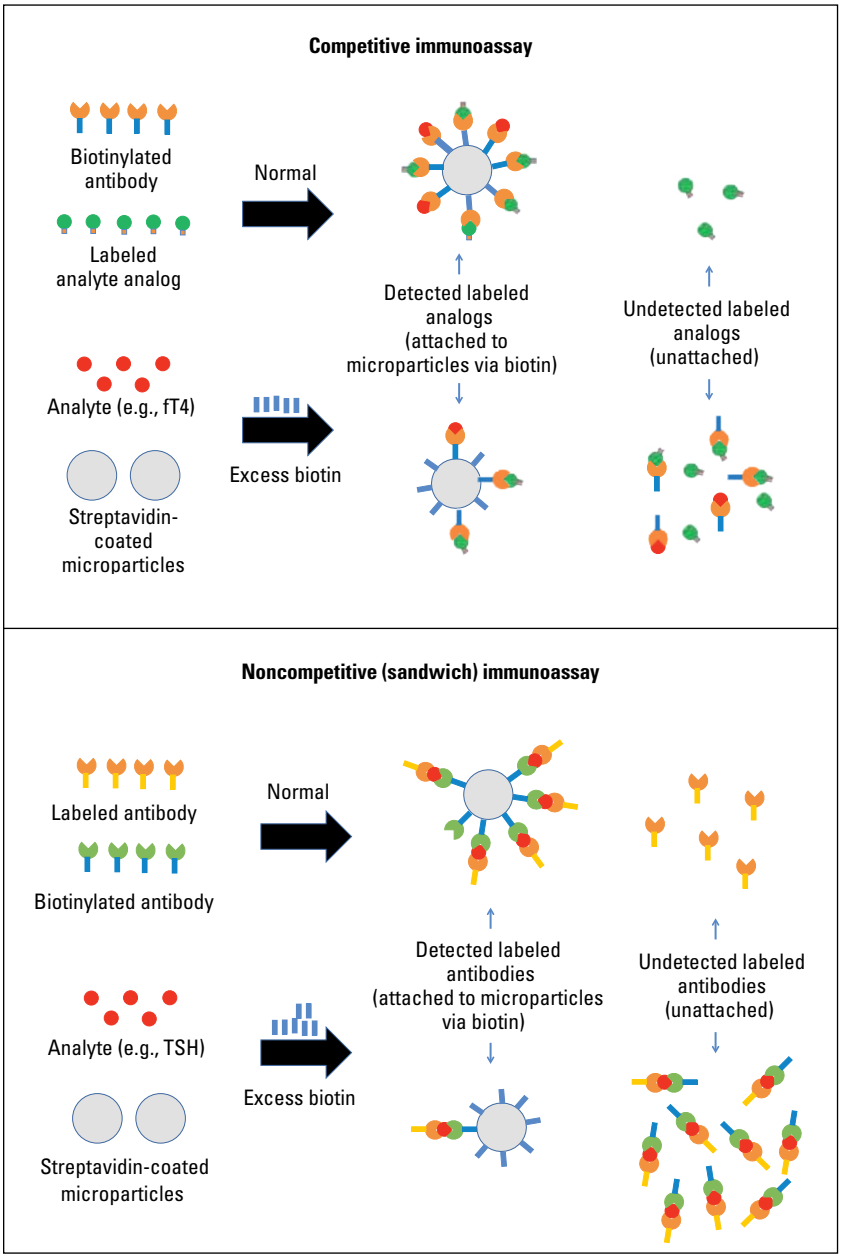

Figure. Mechanism of biotin interference with competitive and noncompetitive immunoassays.

increased serum calcium level, and severe osteoporosis.[14] However, her increased alkaline phosphatase result was inconsistent with the low bone turnover observed in adynamic bone disease. It was later discovered that the patient had been ingesting 10 mg of biotin per day for restless leg syndrome. Repeat testing on a different analytical perform that did not use biotin-streptavidin technology found the patient's PTH concentration to be markedly elevated, consistent with secondary hyperparathyroidism.

Mitigating biotin interference

While laboratory professionals have been aware of biotin interference for many years, the problem has been amplified recently with increasing use of the supplement. In November 2017 a growing number of adverse events associated with biotin, including one patient death following an incorrect cardiac troponin assessment secondary to biotin interference, led the US Food and Drug Administration (FDA) to warn the public, health care providers, and laboratory personnel that biotin may interfere with certain laboratory tests and cause misleading test results.[15]

It is paramount that health care providers and patients be informed of the potential errors in laboratory results with biotin use. Physicians should always ask their patients about biotin supplementation during history taking. The question should be thoughtfully posed (e.g., "Are you taking any supplement for hair, skin, or nail benefits?") since patients may not be aware of the ingredients in a supplement, as these are not always listed clearly on the label.

Patients taking biotin should be advised to discontinue the vitamin for at least 1 day before a blood test, or up to a week before testing if high doses have been consumed. The half-life of biotin is dependent on various factors, including the patient's kidney function and the dose and duration of biotin use. In individuals with normal renal parameters, the half-life of biotin after a single dose of 0.6 mg has been reported as less than 2 hours. In contrast, the half-life following a single biotin dose of 100 mg to 300 mg is between 8 and 19 hours.[4,16]

When patients on biotin require urgent blood work, such as when they present to the emergency department, the ordering physician should consult

local laboratory staff. Laboratory physicians can provide information on which particular assays on the test menu may be affected by biotin, the magnitude and direction of interference, and whether some laboratory tests on the same analytical platform are more vulnerable to biotin interference than others. Further, the laboratory physician can arrange for alternative testing using a different methodology not affected by biotin or may be able to request removal of biotin from the sample via pretreatment with streptavidin-coated beads.[6,17]

Fortunately, the results of a recent survey completed by 18 endocrinologists in BC indicate a good understanding of biotin interference (**Table 2**). However, endocrinology is not the only specialty affected, and further work is needed to ensure information about the impact of biotin on laboratory testing is disseminated to all physicians.

Summary

The case of a 54-year-old female being assessed for thyroid dysfunction demonstrates the harm vitamin supplementation can cause. In this case, laboratory findings erroneously indicated thyrotoxicosis—findings that were eventually determined to be the result of high-dose biotin supplementation when the patient received follow-up testing, and she was ultimately diagnosed as euthryoid.

When found in high concentrations in blood, biotin can interfere with laboratory tests that employ biotin-streptavidin interactions as part of the assay technology. Depending on whether the assay is noncompetitive or competitive, the result may be falsely low or falsely high. Increasingly, biotin consumption is being found to result in inaccurate laboratory findings that can lead to misdiagnosis and mismanagement.

While responses from a recent survey completed by endocrinologists in BC indicate a good level of understanding where biotin interference is concerned, more must be done to ensure that physicians working within and outside the laboratory become familiar with the potential of biotin to interfere with test results. BCMJ

Competing interests

None declared.

References

1. Katzman BM, Lueke AJ, Donato LJ, et al. Prevalence of biotin supplement usage in outpatients and plasma biotin concentrations in patients presenting to the emergency department. Clin Biochem 2018; 60:11-16.
2. Chun K. Biotin interference in diagnostics tests. Clin Chem 2017;63:619-620.
3. Samarasinghe S, Meah F, Singh V, et al. Biotin interference with routine clinical immunoassays: Understand the causes and mitigate the risks. Endocr Pract 2017; 23:989-998.
4. Holmes E, Samarasinghe S, Emanuele A, Meah F. Biotin interference in clinical immunoassays: A cause for concern [letter to the editor]. Arch Pathol Lab Med 2017; 141:1459-1460.
5. Oguma S, Ando I, Hirose T, et al. Biotin ameliorates muscle cramps of hemodialysis patients: A prospective trial. Tohoku J Exp Med 2012;227:217-223.
6. Clinical Microbiology Proficiency Testing website. Wong S, Pudek M. Biotin supplementation and laboratory interference: An unintended consequence in the pursuit of beauty. Posted 27 April 2018. Accessed 14 January 2019. http://cmpt.ca/biotin-supplementation-and-laboratory-interference/.
7. Tourbah A, Lebrun-Frenay C, Edan G, et al. MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: A randomised, double-blind, placebo-controlled study. Mult Scler 2016;22: 1719-1731.
8. Grimsey P, Frey N, Bendig G, et al. Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference. Int J Pharmacokinet 2017; 2:247-256.
9. Arya VB, Ajzensztejn M, Appleby G, et al. High-dose biotin in infants mimics biochemical hyperthyroidism with some commercial assays. Clin Endocrinol (Oxford) 2018;88:507-510.
10. Koehler V, Ulrike M, Nassour A, Mann WA. Fake news? Biotin interference in thyroid immunoassays. Clin Chim Acta 2018;484:320-322.
11. De Roeck Y, Philipse E, Twickler T, Van Gaal L. Misdiagnosis of Graves' hyperthyroidism due to therapeutic biotin intervention. Acta Clin Belg 2018;73:372-376.
12. Henry JG, Sobki S, Arafat N. Interference by biotin therapy on measurement of TSH and FT4 by enzyme immunoassay on Boehringer Mannheim ES700 analyser. Ann Clin Biochem 1996;33:162-163.
13. Kwok JS, Chan IH, Chan MH. Biotin interference on TSH and free thyroid hormone measurement. Pathology 2012;44: 278-280.
14. Meany DL, Jan de Beur SM, Bill MJ, Sokoll LJ. A case of renal osteodystrophy with unexpected serum intact parathyroid hormone concentrations. Clin Chem 2009;55:1737-1739.
15. US Food and Drug Administration. The FDA warns that biotin may interfere with lab tests: FDA safety communication. Issued 28 November 2017. Accessed 23 October 2018. www.fda.gov/medicaldevices/safety/alertsandnotices/ucm586505.htm.
16. Li D, Radulescu A, Shrestha RT, et al. Association of biotin ingestion with performance of hormone and non-hormone assays in healthy adults. JAMA 2017; 318:1150-1160.
17. Trambas C, Lu Z, Yen T, Sikaris K. Depletion of biotin using streptavidin-coated microparticles: A validated solution to the problem of biotin interference in streptavidin-biotin immunoassays. Ann Clin Biochem 2018;55:216-226.

Table 2. Responses from June 2018 survey about biotin supplementation completed by 18 BC endocrinologists.

Question	Possible answers	Responses	Percentage
1. Biotin supplements are known to affect certain laboratory tests when taken in high doses. Which tests are most likely to be affected?	a. Immunoassays	14	78%
	b. Electrolytes	0	0%
	c. Liver and/or muscle related enzymes	0	0%
	d. Creatinine and albumin	0	0%
	e. Mass spectrometry tests	0	0%
	f. I don't know	4	22%
Answer: a. Immunoassays Immunoassays are the most common methodology used in endocrinology testing. Many immunoassay methods employ streptavidin-biotin technology and are known to be affected by biotin supplementation.			
2. What percentage of patients are taking enough biotin to significantly bias one or more laboratory test results?	a. < 0.1%	3	17%
	b. 1%	3	17%
	c. 5%	4	22%
	d. > 10%	3	17%
	e. I don't know	5	28%
Answer: c. 5% Estimates are that approximately 5% of the population is taking enough biotin to bias one or more laboratory test results.			
3. Does biotin cause positive biases (e.g., falsely elevated) or negative biases (e.g., falsely depressed) on test results?	a. Falsely elevated	3	18%
	b. Falsely depressed	0	0%
	c. Neither	0	0%
	d. Both	13	76%
	e. I don't know	1	6%
Answer: d. Both Depending on the assay, laboratory results may be falsely high or falsely low.			
4. If a patient were taking biotin, how long should they stop the supplement before going to the laboratory for blood tests?	a. Overnight (8–12 hours)	0	0%
	b. 24 hours	0	0%
	c. 2 days	10	59%
	d. 7 days	6	35%
	e. I don't know	1	6%
Answer: There is no single biotin washout period that will guarantee interference-free test results. Interference thresholds differ widely among assays. Also, high biotin doses take more time to clear than low doses, and clearance takes longer in patients with impaired renal function. As a general rule: • If a patient has been taking 10 mg of biotin per day, we recommend laboratory testing 24 hours following discontinuation of biotin for tests listed in Table 1. • If a patient has been taking 300 mg of biotin per day, we recommend laboratory testing 7 days following discontinuation of biotin for tests listed in Table 1.			
5. If the patient takes a high dose of biotin prior to the blood sample collection, will the laboratory be able to detect interference before issuing a report?	a. Yes	0	0%
	b. No	16	94%
	c. Not sure	1	6%
Answer: b. No The laboratory will not be able to detect the interference. We recommend health care providers always ask about biotin supplementation . If the patient is taking biotin, be sure to communicate this information to the laboratory. If you encounter a laboratory result that is inconsistent with the patient's clinical presentation, contact the laboratory to perform additional investigations or arrange for alternate testing, as needed.			