A 55-year-old man's laboratory results didn’t make sense. Biochemically he had a clear case of severe Graves’ disease, an immune disease that causes overproduction of thyroid hormones, but he had no clinical symptoms. On further questioning, the man revealed he had taken very high doses of biotin—about 5,000 to 10,000 times the amount in a typical daily multivitamin—to treat multiple sclerosis. The biotin, a water-soluble B-complex vitamin, had interfered with his laboratory tests in just the right way to suggest the erroneous diagnosis (Thyroid 2016;26:860-3).

Reports of biotin interference have drawn more attention recently as dietary supplement companies have marketed over-the-counter biotin supplements in high doses—more than 5 mg—for a variety of medical and non-medical reasons. The Food and Drug Administration (FDA) released a safety communication in November urging more communication among laboratorians, clinicians, and patients about the importance of reporting biotin use to mitigate the risk of clinically significant incorrect lab test results. The agency said it received an increase in the number of reported adverse events related to biotin interference, including one death.

The risk of biotin interference extends to assays critical to internal medicine, such as tests for anemia, malignancies, autoimmune and infectious diseases, and cardiac damage, said Earle W. Holmes, PhD, DAACC, director of the Loyola University Medical Center special chemistry, immunoserology, and endocrinology laboratories in Hines, Illinois.

In an article in Endocrine Practice, he and his coauthors counted 17 reported cases in the medical literature of biotin interference with thyroid assays, most in the past 5 years (Endocr Pract 2017;23:989-98). The cases included six children who were taking biotin to treat inherited metabolic diseases and were erroneously diagnosed with Graves’ disease. Three of them—
1-month-old boy, a 2-year-old girl, and a 2-year-old boy—started anti-thyroid medication before the error was detected (N Engl J Med 2016;375:704-6).

"I think that there are other perfect storms out there, in which biotin interference with several different tests could provide compelling lab evidence for a diagnosis that might not be correct," Holmes said.

There is also the risk that biotin masks a true diagnosis. For example, the death reported to FDA occurred when a patient taking high levels of biotin had falsely low troponin results. "It emphasizes the importance of taking a thorough history, including use of over-the-counter medications, when evaluating patients," commented James Januzzi, MD, director of the cardiac intensive care unit at Massachusetts General Hospital and professor of medicine at Harvard University in Boston.

Examining Unpredictable Effects

Biotin does not interfere with laboratory tests when taken at levels found naturally in food and multivitamins, or at amounts near the Institute of Medicine’s adequate intake determination of 30 mcg per day. However, biotin supplements are sold in doses that far exceed this amount, from 1 mg to 100 mg. At the same time, not all immunoassay systems are susceptible to biotin interference.

For example, some assay methods such as those currently used in the Abbott Architect i2000, DiaSorin Liaison XL, and others do not use what’s known as the biotin-streptavidin capture method. And of course, biotin is one of many possible causes of interference that affect all laboratory tests.

Biotin supplements became popular for their purported ability to promote hair and nail growth, despite no clinical evidence for those claims. However, recent studies have suggested that high doses of biotin might be a safe and effective therapy for certain diseases, most notably multiple sclerosis, which seems to respond to biotin in 300 mg daily doses, though there currently is no FDA-approved therapy (Mult Scler 2016;22:1719-31).

Patients might not be aware they are taking biotin, as it is not always labeled in supplements, or they might not think to mention it to their doctor prior to a blood draw. A recent survey commissioned by Roche Diagnostics found that 85% of patients believe it is the responsibility of their healthcare provider to properly prepare them for a blood draw, according to the company.

Biotin interference can be unpredictable. A study by Danni Li, PhD, DAACC, an associate professor of laboratory medicine and
pathology at the University of Minnesota Medical Center in Minneapolis, and her colleagues found that daily 10 mg biotin supplements caused interference in several immunoassays, including results that could have caused misdiagnosis of thyrotoxicity and a failure to identify congestive heart failure.

Yet several biotin-based assays that were predicted to experience interference did not (JAMA 2017;318:1150-60). It is a complex problem with no easy solution, Li said. “I think everybody just kind of wishes this never happened.”

As awareness of potential biotin interference has spread, laboratorians have different opinions on how to respond. Some, like Holmes, worry that this could prove to be a problem on the scale of heterophilic antibody interference, which led to misdiagnoses and lawsuits, and ultimately required manufacturers to issue warnings and reconfigure their products.

In June 2016, Holmes’ endocrinologist colleagues at Loyola University Medical Center called his attention to an article called “Beware of Biotin” in Endocrine News. Holmes immediately reviewed the manufacturers’ package inserts for the 62 automated immunoassays performed in his laboratory, and identified three that were at high risk for interference by exogenous biotin. He moved one assay to another platform, but the other two—both tumor marker assays—could not be moved or replaced easily.

The technical specialist in Holmes’ lab conducted studies to estimate the thresholds for biotin interference in the latter two tests, and Holmes estimated how long patients should refrain from taking biotin before having their blood drawn. His lab attached biotin warnings to the reported results for these tests and later began to display warnings at the point of order entry in the institution’s electronic medical record system.

“I am lucky that my lab has multiple instruments with overlapping menus and is able to largely avoid this problem, because I would rather not use methods that have this kind of vulnerability,” Holmes said.

Others, like Nam K. Tran, PhD, an associate professor in the department of pathology and laboratory medicine at the University of California, Davis (UC Davis), see biotin interference as just one of many interferences that laboratories deal with routinely. “The question is, is it really an issue or not, especially in our particular population,” Tran said.

When Tran’s laboratory decided to purchase a Roche automation line about a year ago, UC Davis endocrinologists immediately raised concerns about biotin interference, he noted. “At the time, there was a handful of published papers that detail rare events
where, most likely, a patient took an ultra-high dose of biotin causing erroneous results that may have affected patient care,” Tran said. “Fair enough.”

He decided to explore UC Davis’ electronic medical record data to find out how many patients self-reported biotin use. “We found the numbers to be really small,” he said. “Less than fifteen percent of patients reported taking biotin, and most of them were taking a once-a-day, regular dose—2.5 to 5 mg—that wouldn’t interfere with the Roche assay. That gave us confidence.” However, self-reported biotin users are only one part of the puzzle.

Knowing this, Tran and his laboratory began measuring biotin in blood from patients who self-report biotin or multivitamin use. Their study has since been expanded to monitor biotin in patients being tested for other analytes such as thyroid stimulating hormone and cardiac troponin. Tran and his colleagues are measuring biotin on multiple platforms, including those with and without suspected biotin interference.

“The biotin problem remains limited in our population at UC Davis in Sacramento,” he said. “I’m sure there are people who take massive doses, don’t get me wrong. My argument is that this is one out of the innumerable other interferences that we know or don’t know that we encounter every day in the clinical lab. It just doesn’t make sense to me that people are making such a big deal about biotin when I have plenty of other interferences, known or otherwise, that could cause inaccurate results.”

Tran cautioned against a “kneejerk” reaction, and said that it would be a mistake to switch platforms every time an issue such as biotin interference arises. Not only would it be cost-prohibitive, especially for smaller facilities, but it wouldn’t solve the issue of mitigating all interferences. “Education and leveraging informatics are the solution,” he said, adding that any discussion of interference risk mitigation is an opportunity for labs to take the lead in educating clinicians not only on biotin, but also other interferences in general.

“Clinicians are the eyes and ears of the laboratory, and we must continue to train current and future healthcare providers to look beyond a singular lab result,” Tran said. He also noted that medical schools spend very little time on laboratory testing, and rarely if ever discuss in depth the topic of interfering substances. “We must improve our education of physicians on how a lab result should be evaluated in the context of patients’ clinical presentations, as well as knowing conditions where lab results may become inaccurate,” he said.

Building Communication and Collaboration
Stephen R. Master, MD, PhD, FAACC, chief of clinical chemistry laboratory services and director of the central lab at Weill Cornell Medicine in New York City, also sees biotin in the context of many other interferences that clinical laboratorians must be aware of. “To the extent that the lab becomes aware of it through interactions with clinicians, it’s a standard part of the practice of laboratory medicine, trying to work through those interferences,” he said.

One difference with biotin, though, is that biotin is used in so many lab tests that interference by one external source potentially impacts a whole class of assays, Master noted. His research has focused on another newly discovered interference with biotin-based assays—antibodies against streptavidin. In the past 5 years there have been at least three reports of patients who naturally have anti-streptavidin antibodies in their blood—including one identified by Master and his colleagues—causing interference in their lab results.

Whatever the cause of an interference, it is usually discovered when a clinician notices a discrepancy between test results and clinical presentation and alerts the laboratory, Master said. This makes interference a particularly difficult issue for high-volume and reference laboratories.

“Ideally, you’d love to have some common way of screening and knowing ahead of time what a patient’s taking,” Master said. “The reality is that most of the time we get our samples coming to the lab with very minimal clinical history.”

Collaboration between clinicians and laboratorians is the key to detecting interference and preventing misdiagnosis, Master said. Laboratorians should educate clinicians about the risks of biotin interference, and encourage them not to ignore strange results but rather to contact the laboratory to investigate. “That’s our job,” Master said. “That’s what we do … as long as the clinicians know they can come to us, I think this is a place we can provide a real service. [Biotin] is neither the first nor the last of these interferences that will be important.”

Li, at the University of Minnesota Medical Center, has been involved in two biotin interference cases, both of which began when an endocrinologist asked the lab for help with a puzzling result. Though her study showed that biotin interference may occur when patients take relatively moderate supplement doses, she has not advocated for dramatic changes at her own institution in response.

“I think the most critical information that everybody wants to know, including FDA and diagnostic companies, is how prevalent is this problem?” Li said. “We really don’t have enough data to understand this.”
On the high end, the National Health and Nutritional Examination Survey suggests biotin use is as high as 32%, but this includes doses too low to cause interference, Li said. Roche quotes Nielsen sales data which show that between July 2014 and June 2017, biotin sales in the U.S. were trending slightly upwards with the steadiest sales growth in doses 2.5 mg or less, levels which pose a very low risk of interference. Sales of 5 mg doses have declined.

Holmes agreed that accurate prevalence data is essential, and said national data may not reflect local trends and the risk of biotin interference for any given laboratory. “I think there’s going to be geographical variation, and potentially cultural differences,” he suggested. One option for laboratorians is to make sure all the manufacturers they work with address biotin interference, Holmes said. In the short term, laboratorians should demand that manufacturers provide complete information about which methods are vulnerable and each method’s threshold for interference.

FDA’s safety communication directed all test manufacturers and test developers who use biotin technology to determine their interference thresholds and communicate with their customers. FDA is monitoring reports of biotin interference and has encouraged the voluntary reporting of all adverse events. After contacting the manufacturers of the instruments in his own laboratory, Holmes contacted other companies and reviewed their instructions for use, ultimately reviewing 600 to 700 such documents.

At that time, Roche’s interference thresholds were already available online and the company had biotin warnings and specimen collection guidelines for mitigating interference in the company’s instructions for use, he said. Most of the other manufacturers could not provide him with a list of their vulnerable assays, and in many instances their product labelling did not provide the information that was necessary to evaluate the potential risk of erroneous test results in patients taking biotin supplements. In several cases, customers received this information some 16–19 months after the Endocrine News article was published, he said.

All manufacturers should report their biotin interference thresholds as the concentration of biotin that causes a 10% change in a test result, so that the relative risks of different methods can be readily appreciated, Holmes emphasized.

**BIOTIN BASICS**

**Why is biotin used in immunoassays?**

Biotin is a small molecule that can be attached by covalent bond
to a variety of targets—from large proteins such as antibodies to tiny steroid hormones—with minimal effect on their biological activity. Biotin then makes the target easy to capture because it forms a strong, stable, and specific non-covalent bond with avidin, streptavidin, or NeutrAvidin proteins.

Biotin-based detection systems are at the core of immunoassays on many platforms, including the Roche Elecsys; Ortho Clinical Diagnostics Vitros; Beckman Coulter Access/DXi; and Siemens Centaur, Immulite 2000, and Dimension.

What effect does biotin interference have?

The direction of interference depends on the design of the assay. Some results are falsely elevated, some falsely lowered. Two of the most common immunoassay designs are the sandwich assay and the competitive assay.

Sandwich assay

Direction of biotin interference: Falsely lowered results

Typical use: Measuring large molecules, such as hormones and proteins

Example assays: thyroid stimulating hormone, pituitary glycoprotein hormones, human chorionic gonadotropin, parathyroid hormone, insulin-like growth factor-1, insulin, thyroglobulin, C-peptide, ferritin, N-terminal pro b-type natriuretic peptide, prolactin, prostate-specific antigen

How it works: Two antibodies are the “bread” on either side of a sandwich with analyte as the filling. One antibody is labeled with a signal to be measured, the other is labeled with biotin for capture. The antibody complex is captured when biotin sticks to streptavidin-coated beads. The more analyte present in the sample, the more “sandwich” complexes are formed and captured, and the stronger the signal.

How biotin interferes: Free biotin sticks to the streptavidin-coated beads, leaving fewer places for the antibody complexes to stick. Antibody complexes that have successfully captured the analyte have no place to bind and get washed away undetected. The resulting signal is weaker than it should be.

Competitive assay

Direction of biotin interference: Falsely elevated results

Typical use: Measuring small molecules, such as steroids
Example assays: 25 hydroxyvitamin D, free triiodothyronine (T3), free thyroxine (T4), total T3, total T4, cortisol

How it works: Analyte sticks to antibodies labeled with biotin. However, the analyte must compete for those antibodies with the reagent, a version of itself but with a label for detection. If no analyte is present, the reagent occupies all the antibody binding sites, the complexes are captured, and they emit a strong signal. If analyte is present, unlabeled analyte sticks to some of the antibodies, outcompeting some of the labeled reagent, and the signal weakens.

How biotin interferes: Free biotin sticks to streptavidin, leaving fewer spaces for antibody complexes to be captured. Many antibodies, whether bound to analyte or reagent, get washed away. This weakens the signal, regardless of how much analyte has competed with labeled reagent. This may give the false impression that analyte is present, even if it is not. (See Clin Chem Lab Med 2017;55:780-8 | JAMA 2017;318:1150-60 | Endocrine Practice 2017;23:989-98).

Are all immunoassays vulnerable?

No, some immunoassays, such as the Abbott Architect i2000 and DiaSorin Liaison XL, are not susceptible. Also, some biotin-based assays are designed with biotin pre-bound to streptavidin before the sample is present, so theoretically they are not vulnerable to biotin interference. It is always best to consult the manufacturer’s package insert to check for wait times and thresholds.

How long does it take biotin to clear the body?

There is no single biotin washout period that will guarantee interference-free test results. Interference thresholds differ widely among assays, even on a single platform. Also, higher doses of biotin take more time to clear than low doses, and clearance takes longer in patients who have poor renal function.

Results of a pharmacokinetics study supported by Roche Diagnostics found an 8-hour washout period for healthy people who are taking 10 mg of biotin a day, assuming the assay has a biotin interference threshold of 30 ng/mL or higher. However, some assays have thresholds lower than 30 ng/mL and some people take up to 300 mg biotin a day. In the cases of patients on high dose biotin experimental therapy, who would be closely monitored by a healthcare provider, study authors recommend a washout of up to 73 hours (Int J Pharmacokinetics 2017;2:247-56).

FURTHER READING

Case studies of biotin interference

Analysis of biotin interference in common immunoassays


Biotin supplement pharmacokinetics


Methods for detecting biotin interference


Other interferences with biotin-streptavidin systems

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