

Beauty fad's ugly downside: test interference

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September 2016—It's the kind of health promotion advice one might pick up casually over lunch with friends, in a quick Google search, or during a visit to the hairdresser. Take megadoses of an over-the-counter vitamin called biotin—a common supplement in multivitamin compounds—and watch your skin improve and your hair and nails thicken and gleam. In recent years, online social networks and health-related websites have begun to teem with ads claiming that people have seen a transformation since they jumped on the biotin bandwagon.

The trend has meant that biotin megadoses up to hundreds of times the recommended daily intake of 30 µg per day have become a wellness routine for many. For clinical laboratories, for clinicians, and for patients getting diagnostic tests, however, this phenomenon is happening without regard for an alarming fact: taking unusually large doses of biotin can interfere with a broad range of immunoassay test results. In an unexpected and dismaying coincidence, it turns out, the design of many immunoassays, including thyroid tests, cardiac markers, and others, relies on biotin to capture antibodies.

In fact, “a significant percentage of immunoassays rely on biotin-streptavidin binding as part of the assay architecture,” says Ed Reineks, MD, PhD, staff pathologist at the Cleveland Clinic, which conducts millions of immunoassays per year on automated systems. “It's a common mechanism used to capture antibodies. So this biotin interference could affect virtually every area with any immunoassay testing.”

Potentially hundreds of assays could be affected by high-dose biotin—and not just because of high hopes for skin, hair, and nails. “There are certain areas like rheumatology and multiple sclerosis where high-dose biotin is thought to be helpful by certain clinicians,” says André Valcour, PhD, director of the Esoteric Immunoassay, Allergy, Coagulation, Toxicology, and Biological Monitoring departments at LabCorp's Center for Esoteric Testing, Burlington, NC. The possibility that supplements might interfere with assay results, of course, is familiar. “There are so many over-the-counter formulations that are new and novel, so many different tests we measure. And the regulatory process for over-the-counter supplements is such that we don't know their purity. We don't know their contents or whether other substances are in there,” Dr. Valcour points out. “So it's always possible, and should be considered by clinicians, that a patient taking over-the-counter supplements or drugs might produce anomalous results in a lab test.”

However, the use of high-dose biotin supplements, either over the counter or by prescription, is fairly new. “I believe it's less than a couple of years old,” Dr. Valcour says. And biotin is in a category of its own. “I'm not aware of any other situation where a component of an assay design is being taken as a supplement,” he says.

“Biotin is actually attached to one of the proteins of the assay or to the surface of the substrate. It's part of the



design and it's chemically bound to a component of the assay. I don't know of anything else that's quite like that."

At meetings in which he has participated and asked about biotin use, Dr. Valcour has been surprised at how many of the participants say they take biotin—usually based on the belief that biotin's role as a key contributor to keratin will help improve hair and nails. "This included people on both sides of the table and a large portion of people in the room. So utilization is higher than one might expect. And they didn't know anything about the potential effect of biotin megadoses on lab testing."

Package inserts from Roche Diagnostics, Abbott Diagnostics, and Siemens Healthcare Diagnostics all contain warnings about limitations of their tests due to possible interferences, Dr. Reineks notes. "The diagnostics manufacturers are required, when they seek Food and Drug Administration approval, to examine common interferences for tests, and the most routine are things that can go wrong with the specimen, such as hemolysis," Dr. Reineks says. "But then certain tests have additional interferences they have to consider."

Biotin is one such potential interference. Patients who are taking high doses of biotin should not have their blood drawn for laboratory testing for a certain period after they take their biotin. However, Dr. Valcour says, what typically would happen is a clinician might get unexpected test results for a patient, then call the lab to find out if something was wrong with the test. "If it's determined that the patient was on a high dose of the vitamin, they usually ask the patient to skip one day."

Biotin, Dr. Reineks notes, has a very short half-life in most patients, according to the literature—under two hours—and it is usually 99 percent cleared from the body within four to five half-lives. "That's one mitigating factor in this. The other is that the recommended intake for biotin is something on the scale of 30 µg per day, under expert guidelines for daily supplements." When he learned of biotin interference a few years back, he looked at what kind of doses people are taking. "And the Roche package inserts I reviewed warn about patients taking 5 mg a day, which is more than 150 times the recommended dose." So he considered an interference to be an unlikely problem.

"What I've learned since then, from reading and informal ground level research, is that the manufacturers of these supplements set the pills at 10,000 µg, more than 300 times the recommended dose. The pills are far in excess of what the recommended supplement level is," Dr. Reineks says. And despite almost no one having a biotin deficiency, an extremely rare condition, usually associated with a genetic abnormality, "there are a lot more people taking biotin than I would have guessed."

Dr. Valcour had a similar experience when he investigated. "When the problem first came to my attention in January 2016 with an Endocrine News article, I immediately went to the Web, and saw many, many sources of biotin advertised at megadoses and many claims of its clinical utility as an over-the-counter supplement. So it was clearly something that may have an impact on the results of immunoassays performed in a laboratory."

Dr. Valcour, who oversees testing in LabCorp's special coagulation laboratory, says that there is precedent for medication adversely affecting the accuracy of laboratory testing.

"Clinicians sometimes order clotting-based laboratory tests on patients who are treated with anticoagulants. In these cases, test results do not reflect the patient's underlying condition, but rather the impaired clotting caused by the medication. Like with biotin, an exogenous compound taken by the patient can result in clinically erroneous results being reported." What is new about the biotin situation, he says, is that, in many cases, the



LabCorp is taking steps to make clinicians aware of the potential for biotin interference in immunoassays, says Dr. André Valcour. "We are being proactive to get the issue out in the forefront."



Dr. Reineks

patients are taking the drug without informing their clinicians. “However, the common feature to both situations is the need for clinicians to be aware of their patient’s treatment regimen, both prescribed and unprescribed, and the potential impact of the treatment on testing results.”

With biotin, it’s not always the case that an obvious interference exists, Dr. Valcour notes. “You may have a patient on a very high dose of biotin that can cause results to be significantly abnormal, alerting the doctor there is something funny going on. In these cases, the results can suggest an extreme pathology that is inconsistent with the patient presentation. What we don’t understand is the effect of residual biotin in the patient’s blood several hours after they take their dose. This may produce inaccurate results that are clinically plausible. This is the more insidious situation,” he says.

In Dr. Valcour’s experience, “Many clinicians are unaware of the effect of biotin on laboratory results.” Labs are beginning to address the issue, he says. “I know our lab has been aware for several months, we’ve engaged with the diagnostics manufacturers, and are formulating an approach for dealing with it.” He has experienced three cases in which clinicians contacted him, and he discovered through investigation that biotin was causing anomalous results. “That’s helped me become more aware of it too.”

The diagnoses that could be affected by potential

biotin interference run the gamut, Dr. Reineks says. “We use the biotin-streptavidin technique in many immunoassays. That’s how we measure cardiac markers such as troponin, natriuretic peptides, some iron studies. That’s how we do hormone studies. In addition to thyroid, we have testosterone, estradiol, and hCG—it could affect all of those.”

A New Zealand study published this year (Elston MS, et al. *J Clin Endocrinol Metab*. Epub ahead of print June 30, 2016. doi:10.1210/jc.2016-1971) demonstrated that these possibilities are real. In the study, a patient who had markedly abnormal thyroid function tests that did not match the clinical context was given a factitious Graves’ disease diagnosis due to biotin immunoassay interference. The researchers found that once the patient’s biotin ingestion was halted, her thyroid function tests normalized far more rapidly than possible given the half-life of thyroxine. Significantly, multiple other analytes also tested abnormal in the presence of biotin.

In a letter to the editor published Aug. 18 in the *New England Journal of Medicine* (Kummer S, et al. 2016;375[7]:704–706), physicians from Heinrich Heine University Hospital in Duesseldorf, Germany, report on six children receiving high-dose biotin treatment in the context of inherited metabolic diseases for whom laboratory results were suggestive of Graves’ disease. The children had excessively elevated levels of free thyroxine and total triiodothyronine, low levels of thyrotropin, and elevated levels of anti-thyrotropin receptor antibodies. Only one child had symptoms attributable to hyperthyroidism, and scans of the thyroid were unremarkable in all examined patients. After biotin was discontinued, thyrotropin and thyroid hormone levels were normalized 24 to 28 hours later; levels of anti-thyrotropin receptor antibodies took up to seven days to normalize. “Although manufacturers are aware of this potential problem,” the authors write, “this source of error is usually not referenced to the clinician in laboratory reports.”

At the Cleveland Clinic, Dr. Reineks, who is medical director for automated chemistry at the clinic’s main campus, is not being flooded with calls about laboratory results, and he cautions that the kind of widespread impact that might result from biotin interference does not seem to be happening yet. “I’d be getting a lot more complaints. The number of calls I get about potential interference are pretty consistent—maybe one or two calls a week—but the denominator is very large, because we run so many tests. I would say biotin is on the radar as a possible explanation, but there are a lot of other interfering substances, or causes for inconsistent immunoassay results, that are probably higher on the list of suspects.”

For example, he says, a number of people have autoimmune diseases and are making antibodies that interfere with tests, and a number of therapeutic antibodies are emerging too. “There are basically in-vitro–produced medications that are antibodies and people get those as treatments for various things like arthritis or lymphoma. Some prescribed drugs can also cause false-positives, but they are usually very specific to a particular individual test.”

Biotin is unusual, Dr. Reineks emphasizes. “Something that applies to so many tests that can cause problems—

there are not that many substances that would fall into that category. Biotin would have a very unique position in that regard.”

At least two types of common assays use the biotin-streptavidin formulation: competitive assays, which are typically used for low-molecular-weight targets such as thyroid hormone T4, and sandwich assays, used for bigger molecules such as thyroid-stimulating hormone.

“For an example of a competitive assay,” Dr. Reineks says, “we put in some kind of label on a therapeutic drug that’s in the reagent. When it’s bound to the antibody, it’s not giving off a signal, but when I put in a patient sample and it has the same unlabeled drug, the patient’s drug is going to compete with the labeled drug. The drug that’s labeled can come off the antibody and the patient drug can bind to it, so the two are competing.”

Competitive assays, because of this mechanism, tend to produce elevated values just because of the way they use biotin-streptavidin in the assay, while sandwich assays can cause falsely low values. “So it depends on the assay type and the analyte,” Dr. Valcour says. “For the same analyte, one assay might produce a falsely high value while a different assay might produce a falsely low value.”

“You can’t make assumptions about the extent of the effect, though. It could be influenced by factors such as incubation period or buffers. For some assays, there will be a large effect, while for other assays there will be a small effect even for the same principle of the assay—sandwich or competitive. The bottom line is we don’t know a lot about this because the studies haven’t been done.”

Diagnostics manufacturers confirm that they are conducting studies on biotin interference now. “But those studies will be complicated, because not only do the actual blood levels of biotin make a difference, but also the rate at which the biotin is cleared,” Dr. Valcour points out. In certain patients—those with renal function issues, for example—clearance may take longer. “So there’s just an awful lot we don’t know about the specific effect on a given assay by a given manufacturer and the specific effect of a dose on a given patient.”

Lack of clinician awareness of biotin’s potential to interfere with laboratory test results is an important aggravating factor in that interference. A professor of clinical medicine who presented at the recent International Thyroid Congress, according to the January 2016 article in *Endocrine News*, noted that most endocrinologists don’t know about this problem.

“The Endocrine Society has done quite a bit of work to educate endocrinologists on the issue of biotin interference,” Dr. Valcour says. “But quite frankly, most thyroid testing is done in primary care and not by endocrinologists, and internal medicine doctors may not know about it. What’s more, testing in other disciplines like infectious disease and gastroenterology might be affected as well, but different doctors may not tie an anomalous result to a particular dose of biotin taken by a patient.”

The FDA takes a virtually hands-off approach when it comes to regulating supplements, Dr. Reineks notes. “If the supplement manufacturers don’t make medical claims, but only wellness or health claims, I think supplements can fly under the radar unless they are saying they actually cure or treat something.” One result is that patients who are ingesting supplements may not even know that biotin, which can be marketed as vitamin H, coenzyme R, or vitamin B7, is what they are taking. So even if asked directly whether they are taking biotin, patients may answer incorrectly.

That is one of the questions manufacturers are studying as they work out how to deal with biotin interference.

“Biotin is a known interference that has been quantified for the last 20 years, and for 20 years we’ve had our immunoassay package inserts call out that patients should not take large doses of biotin for eight hours before a sample is collected,” says Alan Wright, MD, chief medical officer of Roche Diagnostics. “Biotin is just now becoming more visible to the laboratory community because of this new trend of taking high-dose biotin.”

Because megadoses of biotin are catching on as health improvement aids, Roche is actively conducting studies to determine two things: the pharmacokinetics, or how biotin is metabolized, and the prevalence of megadose biotin therapy.

“Interference has been with laboratory medicine since we started doing lab tests,” Dr. Wright says. “All the way from hemolysis and potassium measurements in the blood, which would be preanalytic interferences, or high lipids in bloods which can interfere, to medical conditions like lupus that lead to anticoagulants which can interfere with blood clotting tests. And there are also conditions that include the presence of anti-thyroid thyroglobulin which can interfere with thyroid tests. So it’s important for a laboratorian to be able to consult with clinicians and be able to explain the potential interference of any assay.”



Many diagnostics manufacturers use the biotin-streptavidin system for many assays, he confirms. “The biotin-streptavidin system is a very robust platform to bind an antibody. You have an antibody which looks like a ‘Y,’ with biotin at the base of the Y, then you have it bind to streptavidin, and that is a common technique. I would say it’s very prevalent throughout the diagnostics industry,” Dr. Wright says.

The use of megavitamins is not new, he notes, pointing out that people have consumed vitamin C in large amounts for decades. “Right now the megavitamin is biotin. We are aware of this trend and interested in understanding more about it. We’re working with our lab customers to talk with them about biotin interference.”

Whether patients are aware they are taking biotin is part of what Roche is studying. “But one of the things about this interference is you can’t get these high levels of biotin by consuming an over-the-counter multivitamin. That is not an issue. You have to specifically consume a product that is made to have super-pharmacologic doses of biotin.” At this stage, estimating patient awareness involves supposition, Dr. Wright says. “But I would think somebody who seeks out a high-dose preparation would know they are taking a high dosage of biotin.”

There’s no dispute that biotin, when taken in megadoses as a supplement, can cause interference in immunoassays that use a biotin-streptavidin architecture, says James Freeman, senior director of immunoassay development for Siemens Healthcare Diagnostics. However, he says there are differences among manufacturers in how the technologies are applied to the assays.



“The architecture of an immunoassay dictates whether an interference with biotin is observed. Heterogeneous immunoassays use a solid phase to capture the desired analyte. Several manufacturers use streptavidin immobilized on the solid phase and a biotinylated antibody to capture the analyte,” Freeman says, adding that the Advia Centaur systems use this streptavidin-biotin assay format in about one quarter of Siemens’ commercialized immunoassays. “But the biotin-streptavidin assay architecture can be either a preformed biotin-streptavidin solid phase or a non-preformed such phase. Biotin interference is typically observed only when you have a non-preformed biotin-streptavidin solid phase.”

Siemens’ immunoassays primarily use preformed solid phase, Freeman says. “Approximately six of the Siemens immunoassays on the Advia Centaur systems, which has over 75 assays available, demonstrate some level of biotin interference. We publish the level of interference in our Instructions for Use.” None of Siemens’ other immunoassay platforms demonstrates a biotin interference, to his knowledge.

Siemens is proactively conducting research to determine the extent and gravity of potential biotin interference, Freeman says. “When we use a biotin-streptavidin assay architecture, we use an experimental design to determine the exact level of biotin that would cause interference, and we publish that in our Instructions for Use.”

While biotin interference in immunoassays has been known for decades, he adds, it was not considered a serious obstacle because biotin was not used as extensively as it is today.

Now that taking megadoses of biotin has become more prevalent, there could be a lot of turmoil as manufacturers whose products have more sensitivity to biotin try to respond, Freeman predicts. “Patients taking high doses of biotin could experience ramifications with some assays. Depending on the assay manufacturer, you can have an infectious disease assay such as an HIV or HCV test where an individual is misdiagnosed.” The Siemens HCV and CHIV assays use a preformed streptavidin solid phase. “Therefore, biotin interference is not observed with these assays,” he says. “Or, for example, if I have chest pain and go to the emergency room, I could have elevated or suppressed troponin results depending on whether I took biotin or not and which assay is

used to measure the troponin levels. So those are serious clinical cases where there might be a misdiagnosis.”

Biotin happens to be useful technology for immunoassays because the biotin-streptavidin interaction has one of the highest binding constants that is known, Freeman says. “In other words, streptavidin and biotin combine very strongly and very quickly. They have a well-documented binding event that is very strong and fast, and that’s typically why manufacturers use biotin and streptavidin in immunoassay architecture.”

Freeman says it’s important for labs to understand the knowledge gap that surrounds biotin interference. “Five or 10 years ago, the only individuals who demonstrated this interference were undergoing dialysis, and doctors knew the patients were undergoing dialysis and knew they had biotin levels that might interfere, based on manufacturers’ instructions for use. Today, with people taking more and more of these supplements, the doctors don’t know and labs don’t know, unless they actually test for biotin.”

Roche always evaluates ways to remove or reduce possible interferences, including biotin, and provides clear test labeling to ensure physicians and laboratories can mitigate risk where there is interference potential, Dr. Wright says. Roche advises labs to carefully review assays’ package inserts, which fully reference biotin interference as a risk. “I’m in charge of medical and scientific affairs, and when we get inquiries about this, we refer labs back to the package insert and we have interim studies that we refer to as well,” he says. “We also work with outside labs and use our customer base to develop question-and-answer materials and conduct peer-to-peer consultation regarding the biotin issue.”

Of about 628 million thyroid tests a year supplied by Roche, Roche has had 14 biotin-related case reports so far. “So it’s important to us, but it’s important to understand the magnitude of case reports versus how many thyroid tests we do per year. We think that our assays are very high quality, very sensitive and specific.”

“In any forward-thinking analytics company—Roche and our competitors included—we always try to improve what we’re doing. In biochemistry we’re always looking for new ways to do these tests, and on an emergent basis, we’re going to continue investing to improve assay design,” Dr. Wright says.

For its part, LabCorp is hoping to increase clinician >knowledge of biotin interference and has reached out to the manufacturers to see how they are addressing the problem, Dr. Valcour says. “We are being proactive to get the issue out in the forefront. In fact, as an organization we are looking at every single test we offer to see which have exposure to this interference.” But LabCorp’s inquiry is at an early phase.

What can laboratories do? While some steps have been proposed, it’s too soon to recommend measures such as posting signs in phlebotomy units asking patients whether they are taking biotin, Dr. Valcour believes. “I would say getting clinicians to know whether patients are on biotin and to tell patients to stay off biotin before testing is probably the most efficient and most efficacious approach to preventing interference. This would help in two ways. First, it makes physicians aware of supplementation and what their patients are taking, and, second, it makes them aware, when looking at lab results, that interference is a possibility.”

An alternative would be for labs to ask at the point of collection whether the patient is taking biotin. But patients may not know when they took their last dose or exactly what they took, if they know the biotin as vitamin H or by some other name. “That puts the doctor in a very precarious position, because even if the result is perfectly normal and consistent with clinical expectations, it’s very hard to trust that result if the patient might have been taking biotin,” Dr. Valcour says. “We would have to stop them from taking biotin a day, or I would say at least two days, before they show up to be drawn—and the only one who can really do that would be the clinician.”

Laboratories should, however, push for further investigation of biotin interference, Dr. Valcour says. “At this point, working with the diagnostic manufacturers to try to understand the extent to which our assays are affected by biotin, and down the road, the effect of various disease states on clearance, would be something that laboratories should help with.”

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