EDUCATIONAL COMMENTARY – BIOTIN INTERFERENCE

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Learning Objectives

On completion of this exercise, the participant should be able to
• identify the established adequate intake for biotin;
• list indications for biotin supplementation;
• describe how biotin is used in immunoassay testing platforms;
• recognize testing that may be affected by high-dose biotin supplementation;
• discuss how biotin supplementation can influence clinical laboratory testing.

Introduction

Although most people have an adequate intake of dietary biotin, the use of high-dose biotin supplementation for hair, skin, and nail health has become common. Many tests performed in the clinical laboratory use testing platforms that involve avidin-biotin and streptavidin-biotin interaction for increased sensitivity and specificity. Although usual dietary intake of biotin does not affect tests using this methodology, high doses of biotin such as in these supplements have been found to cause erroneous laboratory results for avidin-biotin and streptavidin-biotin interaction testing platforms. Unrecognized spurious results may put patients at risk of misdiagnosis and inappropriate treatment.

Biotin

Biotin, also known as vitamin H and vitamin B7, is a small, water-soluble vitamin found in trace amounts in many foods that are part of a normal diet. The best natural sources of biotin are liver, egg yolks, soybean products, and yeast. Although there is insufficient evidence to establish a recommended dietary allowance, an adequate intake has been established at 30 µg/d providing an approximate level of biotin intake necessary to ensure nutritional adequacy. It is estimated that biotin intake in Western populations is between 35 and 70 µg/d, indicating sufficient consumption of biotin-containing foods. Although deficiency is rare and most individuals are able to obtain enough biotin from their diet, 15% to 20% of the U.S. population report taking a biotin-containing supplement. Biotin supplementation includes multivitamins, which generally contain the established adequate intake of 30 µg/d, and over-the-counter biotin supplements marketed for hair, skin, and nail health, which can contain doses as high as 5000 to 10,000 µg/day. When taken orally, biotin is completely absorbed, 100% bioavailable, and rapidly
excreted. There have been no reported adverse or toxic effects with doses of up to 300 times the adequate intake.\(^4\)

The bioavailability of biotin depends on its release from proteins in food sources by the enzyme biotinidase. Once available, biotin acts as an enzyme cofactor responsible for carbon dioxide transfer in several carboxylase reactions: pyruvate carboxylase, propionyl-coenzyme A (CoA) carboxylase, methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase.\(^1\) As such, biotin plays a key role in fatty acid and branched-chain amino acid metabolism, gluconeogenesis, and the citric acid cycle.\(^4\)

Biotin deficiency, although rare, has been noted. Deficiency may be seen with increased consumption of raw egg whites, as they contain avidin, which binds bioavailable biotin and prevents its absorption in the gastrointestinal tract. Biotin deficiency has also been known to develop in persons receiving long-term total parenteral nutrition without biotin supplementation. Multiple carboxylase deficiency (MCD), an inherited disorder of biotin metabolism in which individuals lack either holocarboxylase synthetase or biotinidase, results in biotin deficiency as well.\(^1\) Symptoms of deficiency include anorexia, nausea, hair loss, depression, changes in mental status, and dermatitis around the eyes, nose, and mouth.

Extensive research is being performed on the benefits of high-dose biotin supplementation for several disease states. Pharmacologic biotin supplementation has been used to treat MCD and biotinidase deficiency.\(^5\) Research is being performed on the use of high-dose biotin supplementation (5000 to 10,000 µg/d) for the treatment of biotin-thiamin-responsive basal ganglia disease, an autosomal recessive inherited disease that affects the nervous system as a result of an impaired ability to transport thiamin into the cells.\(^5\) In addition, promising research is being done with high-dose biotin (100,000 to 300,000 µg/d) and its effect on slowing the advancement of progressive multiple sclerosis.\(^5\)

**Biotin Use in Laboratory Testing Platforms**

Biotin is used in many immunoassays; its small size allows it to be incorporated into a number of different analytes and their respective antibodies. Avidin and streptavidin's high affinity for biotin, binding up to four molecules, make biotin ideal for amplification and increased sensitivity in avidin-biotin and streptavidin-biotin interaction immunoassays. In addition, the avidin-biotin and streptavidin-biotin interaction is very strong and resistant to denaturation by organic solvents, detergents, and extreme temperatures and pH.\(^6\) The two most common immunoassay platforms that use avidin-biotin and streptavidin-biotin interaction are competitive immunoassay platforms and noncompetitive immunoassay platforms.
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Competitive immunoassay platforms are usually used for small molecules such as free thyroxine (fT₄). They incorporate labeled analyte reagent, biotinylated antibody to the analyte of interest, and an avidin- or streptavidin-coated solid phase. The solid phase is commonly microparticles that capture antigen-antibody complexes and allow for separation of antibody-bound and free analyte when washed. The analyte of interest in the patient sample competes with the labeled analyte reagent for a limited amount of binding sites on the biotinylated antibody. These antigen-antibody complexes bind to the avidin- or streptavidin-coated solid phase. The concentration of the analyte in competitive immunoassays is inversely proportional to the signal intensity of the washed solid phase.

Noncompetitive immunoassay platforms, or sandwich immunoassays, are usually used for larger proteins such as thyroid-stimulating hormone (TSH). In noncompetitive immunoassay platforms, two types of antibodies are used, a labeled antibody and a biotinylated antibody. Both the labeled and biotinylated antibodies bind to the antigen (analyte of interest), but at different epitopes, resulting in a “sandwich.” The resulting complex binds to the avidin- or streptavidin-coated solid phase. The concentration of the analyte in noncompetitive immunoassays is directly proportional to the signal intensity of the washed solid phase.

Exogenous Biotin Interference

Although the benefits of biotin-interaction immunoassays seem apparent, these advancements in technology have not come without concern about possible exogenous biotin interference. The degree of biotin interference will vary depending on serum biotin concentration, analyte, test manufacturer, and testing platform. In competitive immunoassay testing platforms, exogenous biotin binds to the avidin- or streptavidin-coated solid phase, prevents the binding of the biotinylated antigen-antibody complexes, and decreases signal intensity, resulting in falsely elevated results. In noncompetitive immunoassay testing platforms, exogenous biotin interferes with the binding of the labeled complexes (sandwich) to the solid phase, resulting in a decrease in the signal intensity and falsely low results.

Biotin-based immunoassays that may be susceptible to exogenous biotin interference include, but are not limited to, tumor markers such as cancer antigen 19-9 (CA 19-9) and prostate-specific antigen (PSA); hormones such as TSH, fT₄, parathyroid hormone (PTH), dehydroepiandrosterone sulfate (DHEAS), testosterone, and cortisol; cardiac markers such as troponin; and tests for anemias such as vitamin B₁₂, folate, and ferritin. Although many analytes are tested using biotin-streptavidin immunoassay testing platforms, it is important to keep in mind that not all biotin-based immunoassays are affected by exogenous biotin interference. Studies have shown that normal dietary biotin intake and multivitamins do
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not cause interference in biotin-based immunoassays. Interference becomes a concern in individuals consuming more than three times the adequate intake, or 90 µg/d.³

Growing availability of biotin-interaction testing platforms and the surge in use of over-the-counter biotin supplementation has led to a number of cases of spurious laboratory results from biotin-interaction testing platforms. Some of most profound of these cases involve thyroid function testing. Thyroid-stimulating hormone is a noncompetitive immunoassay platform resulting in falsely low results and fT₄ is a competitive immunoassay platform resulting in falsely elevated results, painting a picture of hyperthyroidism and leading to unnecessary treatment.⁷ Cases have been reported for several other analytes measured using biotin-based immunoassays as well. Falsely low results for noncompetitive immunoassays such as troponin, PTH, or tumor markers such as CA 19-9 could have a significant impact on patient care.² In addition, misdiagnosis and unnecessary treatment owing to falsely elevated results has been associated with competitive immunoassay platforms for analytes such as cortisol, DHEAS, and progesterone.²

Several solutions have been proposed to exogenous biotin interference and its potential for misdiagnosis and inappropriate treatment. The first of these is to increase awareness among clinicians, laboratorians, and patients of the possibility of biotin interference from biotin supplements. Clinicians should obtain patient history on prescription medications as well as dietary supplements. Patients should be advised to refrain from taking biotin supplements for 48 hours before specimen collection.² When reviewing laboratory results, clinicians should look for inconsistent patient signs and symptoms relating to those tests that could be affected by high-dose biotin supplementation. In the laboratory, a warning of possible biotin interference could be attached to results for tests that utilize avidin-biotin and streptavidin-biotin interaction. Biotin extraction procedures could be developed for pretreatment of patient specimens prior to testing. Finally, laboratories could also consider switching to methods where the reagents are prebound with biotin in the manufacturing process rather than in the presence of the patient sample.²

Summary

The use of over-the-counter high-dose biotin supplements for healthy hair, skin, and nails continues to grow in popularity, and with it, concerns of erroneous laboratory testing. Exogenous biotin interference in biotin-interaction immunoassays is not new; it is merely becoming more frequent. Although these high doses of biotin do not pose a threat to the patient in terms of adverse or toxic effects, they can pose a health risk to patients. Spurious results may lead to misdiagnosis and inappropriate patient management. Clinicians and laboratorians must be aware of the tests affected by biotin interference to prevent harm to their patients.
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References


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