Clinica Chimica Acta 487 (2018) 293–298

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Comprehensive assessment of biotin interference in immunoassays

Jieli Li, Elizabeth A. Wagar, Qing H. Meng*

Department of Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

ARTICLE INFO

Keywords:
Biotin
Interference
Sandwich immunoassay
Competitive immunoassay

ABSTRACT

Background: Biotinylated antibodies and analogues are currently used in many immunoassays while biotin is widely used as a dietary supplement. Thus, biotin interference is an emerging issue for clinical laboratories.
Methods: Various concentrations of biotin solutions were prepared using pooled patient serum samples. All analytes were measured by sandwich or competitive immunoassay on the Roche Cobas 8000 e602 platform.
Results: Some of the sandwich immunoassay results were falsely decreased to different extents by different biotin levels, while some of the competitive immunoassay results were falsely increased. The most notable false reductions were in high-sensitivity troponin T, thyroid-stimulating hormone, and follicle-stimulating hormone results, while the most notable false increases were in triiodothyronine and vitamin D results. Other immunoassay results were also affected to some extent by biotin interference.
Conclusions: Biotin can interfere in immunoassays and result in aberrant test results. Clinicians should use caution in interpreting abnormal results in patients who ingest biotin.

1. Introduction

Biotin, also known as vitamin B7, is a water-soluble vitamin found in the normal diet, in foods such as egg yolk, pork, soybeans, and vegetables [1]. It is the coenzyme for mammalian carboxylases. Biotin deficiency generally results in dermatitis, thinning of hair with loss of color, muscle pain, atrophic glossitis, hyperesthesia, lassitude, and/or anorexia. Causes of biotin deficiency include parenteral nutrition without biotin supplementation [2] and biotinidase and biotin transporter deficiencies caused by inborn errors [3].

Approximately 15% to 20% of individuals in the U.S. consume biotin in nutritional supplements [4]. Oral biotin is completely absorbed, so the biotin is 100% bioavailable [4]. Biotin supplements at doses from 10 to 15 mg/day are used in the prophylaxis and treatment of biotin deficiency or for health benefits such as stimulating hair growth [3,5]. Somewhat higher doses are prescribed to treat inborn errors of biotin metabolism, such as holocarboxylase synthase deficiency and biotin transporter deficiency (5–20 mg/day) [6]. In the United States, use of high-dose biotin supplements has increased dramatically over the past 2 years. High-dose biotin has been reported to offer benefit in progressive multiple sclerosis [7], in malabsorption syndromes, and in persons receiving total parenteral nutrition [8]. Pharmacological doses of biotin up to 300 mg/day are well tolerated and safe. Serious biotin toxicity has never been reported in humans.

Streptavidin/biotin-based immunoassays are widely used for routine clinical laboratory tests, because this approach offers amplified signals and thus relatively high sensitivity [9]. However, exogenous biotin in specimens may interfere in the results of these immunoassays, because the biotin in the sample competes with biotinylated reagents for the binding sites on the streptavidin reagents.

The increase in exogenous biotin administration has led to emergence of the risk of clinically significant analytical errors in laboratory test results, which can result in misdiagnosis and potential inappropriate treatment [10,11]. Typical dietary intake of biotin is reported to be insufficient to affect the streptavidin/biotin-based immunoassays. However, with intake > 3 times the amount considered adequate in healthy persons, interference on immunoassays could be a concern [12]. During the past 5 y, 17 cases of biotin interference in thyroid hormone assays have been reported in the medical literature [10,13]. It was also reported that a daily 10 mg biotin supplement caused interference in several immunoassays, some of which led to misdiagnosis of thyrotoxicity or failure to identify congestive heart

Abbreviations: HCG, human chorionic gonadotropin; PCT, procalcitonin; PTH, parathyroid hormone; hs-TnT, troponin; LH, luteinizing hormone; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TSH, thyroid-stimulating hormone; CA, cancer antigen; TPSA, total prostate-specific antigen; FPSA, free prostate-specific antigen; FSH, follicle-stimulating hormone; AFP, alpha-fetoprotein; CKMB, creatine kinase-MB; HE4, human epididymis protein 4; IgE, immunoglobulin E; CEA, carcinoembryonic antigen; FT4, free T4; FT3, free T3; T3, triiodothyronine; VitD, vitamin D; VitB12, vitamin B12; T4, thyroxine

* Corresponding author at: Department of Laboratory Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030-4009, USA.
E-mail address: qhmeng@mdanderson.org (Q.H. Meng).

https://doi.org/10.1016/j.cca.2018.10.013
Received 5 July 2018; Received in revised form 16 September 2018; Accepted 4 October 2018
Available online 05 October 2018
0009-8981/

failure [5]. A recent report showed that biotin falsely increased or decreased test results in 12 different endocrine assays in a multiple sclerosis patient who was treated with a single dose of 300 mg biotin [14].

2. Materials and methods

2.1. Preparation of biotin samples

Biotin (Sigma-Aldrich) was dissolved in a phosphate-buffered saline (PBS) solution (without calcium and magnesium) as a stock solution at 10.0 μg/ml, which was stored at 4 °C. The stock solution was diluted with the same PBS solution into working solutions of different biotin concentrations that were then spiked into serum samples. The final biotin concentrations of the working solutions were 312.5, 2500, 5000, 7500, and 10,000 ng/ml. Serum samples were pooled and spiked with each of the biotin solutions. The spiked volume was 10% of the final volume in all experiments. Therefore, in all experiments, the final biotin concentrations used were 31.25, 250, 500, 750, and 1000 ng/ml. For controls, serum samples were spiked with a 10% volume of PBS solution to detect possible matrix effects.

2.2. Immunoassays

All analytes were measured on the Roche Cobas 8000 e602 system to evaluate possible biotin interference in immunoassays. In principle, two types of immunoassays are run on this instrument, immunometric sandwich assays and competitive assays. The sandwich immunoassays run for this study included AFP, CKMB, insulin, β-HCG, intact HCG, PCT, HE4, PTH, hs-TnT (Gen 5), LH, IgE, C-peptide, NT-proBNP, TSH, CEA, CA 19-9, CA-125, CA 15-3, ferritin, TPSA, FPSA, FSH, and prolactin. The competitive immunoassays run for this study comprised T4, T3, FT4, FT3, testosterone, folate, vitamin B12, and vitamin D.

For convenience and clinical relevance, tests were grouped as cardiac markers, prostate cancer, pancreatic function, ovarian cancer, pituitary function, vitamin deficiency, tumor marker, and thyroid function panels for analysis and interpretation. In these panels, the individual tests could be sandwich or competitive immunoassay.

2.3. Statistical analysis

Statistical comparison of results for biotin-spiked samples and PBS solution–spiked controls was performed using Microsoft Excel. All experiments were conducted in triplicate. Any change in a test result ≥10% from the result for the control samples was considered significant.

3. Results

3.1. Biotin interference on immunoassays

Most results of all the immunoassays after spiking of pooled serum samples with various concentrations of biotin are shown in Fig. 1. As expected, there was a strong association between the plasma biotin concentrations and the degree of interference on some assays. In the sandwich immunoassays, the reductions in results were dose-dependent. Results were falsely decreased in 13 sandwich immunoassays which were insulin, intact HCG, PCT, PTH, hs-TnT, LH, C-peptide, NT-proBNP, TSH, CA125, TPSA, FPSA and FSH. Increases in results of the competitive assays also were dose-dependent. There were 5 competitive immunoassays falsely increased, which were FT4, FT3, T3, vitamin B12, and vitamin D. Among these competitive immunoassays, T3 was the most dramatically affected.

Some laboratory tests are grouped into panels to aid in the diagnosis and treatment of disease. Results for the pancreatic function panel (insulin and C-peptide) are shown in Fig. 1A; serum insulin level was reduced by as much as 14.07% at 750 ng/ml of biotin. At 1000 ng/ml of biotin, the reduction of C-peptide was 14.26%. This result indicates that the biotin level at which interference in test results occurs varies for each test affected. Risk of Ovarian Malignancy Algorithm (ROMA) is a widely used prediction model to assess the risk of epithelial ovarian cancer in women, by utilizing the combination of HE4 and CA125 values. Therefore, the ovarian cancer panel in this study comprised HE4 and CA125 (Fig. 1B). Interestingly, biotin did not interfere in the HE4 results at biotin levels up to 1000 ng/ml, while CA125 level was significantly reduced, by 11.13%, at a biotin level of 750 ng/ml. Further reduction on CA125 levels were observed with continuous increase of biotin concentrations. Prolactin, LH, and FSH were grouped as the female pituitary function panel (Fig. 1C). Prolactin was not affected by a biotin level up to 1000 ng/ml, while LH was reduced by 16.84% by a biotin concentration of 750 ng/ml and FSH was reduced by 26.08% by a biotin concentration of 500 ng/ml. Because the most common vitamin deficiencies are folate, vitamin B12, and vitamin D, these were grouped as the vitamin deficiency panel (Fig. 1D). Folate results were not altered by biotin concentrations up to 1000 ng/ml, while vitamin B12 results were significantly increased, by 14.48%, by biotin at a concentration of 750 ng/ml and vitamin D results were increased by 16.84% by a biotin concentration of 250 ng/ml. CA19-9, CA15-3, CEA, and AFP were grouped as the tumor marker panel (Fig. 1E). None of these test results were altered by biotin levels up to 1000 ng/ml. The thyroid function panel was markedly altered by biotin (Fig. 1F). T3 result was increased and TSH result decreased by biotin at concentrations as low as 250 ng/ml, while free T4 and free T3 results both were increased by biotin at concentrations of 500 ng/ml and greater, while T4 result was not altered by biotin at concentrations up to 1000 ng/ml. Results for the cardiac and prostate cancer panels are demonstrated in Fig. 2A and C.

3.2. Biotin interference at different baseline values of analytes

To evaluate the extent of interference by biotin at various concentrations, some of the analyte concentrations in this study were set to close to their cut-off values, which are clinically significant and/or at decision point (Table 1). Notably, the magnitude (percentage) of changes in these analyte concentrations induced by the same biotin concentration were very similar irrespective of the analyte baseline level (Fig. 2). For the cardiac profile (Fig. 2A), for example, the sandwich immunoassays hs-TnT and NT-proBNP were both affected. At a biotin concentration of 250 ng/ml, the hs-TnT level of 54.14 ng/l was reduced by 13.77%. At a biotin concentration of 500 ng/ml, the two baseline levels of pro-BNP, 368.3 pg/ml and 670.5 pg/ml, were decreased by 13.05% and 10.54%, respectively. For the HCG profile (Fig. 2B), there was no interference in either β-HCG level at biotin concentrations up to 1000 ng/ml. At the biotin concentration of 750 ng/ml, however, the baseline intact HCG level of 6.16 mIU/ml was reduced by 13.97%, and the baseline intact HCG level of 29.31 mIU/ml was reduced by 14.79%. For the prostate profile (Fig. 2C), the baseline TPSA level of 3.64 ng/ml was reduced by 14.04% at a biotin concentration of 1000 ng/ml of biotin. Similarly, the baseline TPSA level of 9.43 ng/ml was reduced by 13.49% at a biotin concentration of 1000 ng/ml. For FPSA, interference was observed at lower biotin concentrations than for TPSA. At the baseline FPSA level of 0.49 ng/ml, the reduction was 17.48% at a biotin concentration of 750 ng/ml. Similarly, at the baseline FPSA level of 1.22 ng/ml, the reduction was 16.93% at the same concentration of biotin. The interference of biotin in TSH results was remarkable (Fig. 2D). Low biotin concentrations caused significant interference compared to the other immunoassays already described. At a biotin concentration of 250 ng/ml, for example, the baseline TSH level of 1.65 μIU/ml was reduced by 12.42% and the baseline level of 5.86 μIU/ml was reduced by 12.78%.