



Case report

Fake news? Biotin interference in thyroid immunoassays

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ABSTRACT

We report on a 47 year old male patient with multiple sclerosis (MS) presenting in our outpatient neurology clinic in Frankfurt/Main for therapy evaluation. Before change of treatment laboratory investigations were performed. Thyroid function tests (TFTs) with a streptavidin/biotin based immunoassay revealed severe hyperthyroidism with positive thyroid autoantibodies suggestive for Graves' disease. Clinical presentation and thyroid sonography were unremarkable. Due to the discordance between clinical presentation and TFTs, we repeated medical history, in which the patient reported taking high-doses of biotin (300 mg/day) for MS. Recent studies with patients suffering from primary and secondary progressive MS, indicated promising effects of high-dose biotin on MS-related disability. In immunoassays relying on streptavidin-biotin interaction, biotin intake can cause falsely high or low results. Two weeks after withdrawing biotin, biotin/streptavidin dependant assays showed no longer the biochemical picture of severe hyperthyroidism. Biotin intake should be paused for at least two to five days prior to the use of biotin/streptavidin dependant assays. Alternatively, non-biotin/streptavidin dependant assays (radioimmunoassay, gas chromatography–mass spectrometry/liquid chromatography–mass spectrometry) may be used.

1. Case description

We report on a 47 year old male patient with multiple sclerosis (MS) presenting for therapy evaluation in our outpatient neurology clinic in Frankfurt, Germany. He had a 4-year history of remitting-relapsing MS which subsequently evolved towards a progressive form. Treatment consisted of dimethyl fumarate. Currently, he is in preparation for treatment with ocrelizumab, a humanized monoclonal antibody that selectively depletes CD20 + B cells.

Thyroid function tests (TFTs) were performed before change of treatment. Laboratory investigations with a streptavidin/biotin based immunoassay revealed a suppressed thyroid-stimulating hormone (TSH) of 0.02 mIU/L (reference interval 0.27–4.2, test principle: one-step sandwich assay), a free thyroxine (fT4) of > 98 pmol/L (reference interval 11.6–21.9, test principle: two-step competitive assay) and free triiodothyronine (fT3) of 13.4 pmol/L (reference interval 2.9–7.9, test principle: two-step competitive assay). Thyroid-specific autoantibody levels revealed markedly increased antithyroid peroxidase autoantibodies (TPOAb) and TSH-receptor autoantibodies (TRAb) at > 600 kIU/L (reference interval < 34, test principle: inverted one-step competitive assay) and > 40 IU/L (reference interval < 1.8, test

principle: inverted modified two-step competitive assay), respectively (Table 1). Four months prior, TFTs showed euthyroid status with a TSH of 3 mIU/L, fT4 of 19.4 pmol/L and fT3 of 3 pmol/L (Table 1).

Based on laboratory findings, the patient was referred to an endocrinologist for further clinical evaluation and thyroid sonography. The patient was clinically euthyroid and thyroid sonography was unremarkable in regard of thyroid gland volume, doppler flow studies and echogenicity of the parenchyma. In order to confirm the diagnosis of hyperthyroidism, TFTs were repeated. TFTs confirmed the laboratory constellation of manifest hyperthyroidism with positive thyroid autoantibodies suggestive for Graves' disease (GD).

2. Case discussion

Hyperthyroidism along with positive thyroid autoantibodies is usually related to autoimmune thyroid disease, most likely due to GD [1]. An initial phase of hyperthyroidism can also be observed in patients with Hashimoto's thyroiditis (HT), so-called Hashitoxicosis. Patients with GD show characteristic clinical features of hyperthyroidism such as heat intolerance, sweating, palpitations, tremor, restlessness, weight loss and insomnia [2]. TFTs show low levels of TSH, elevated

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Table 1
Laboratory data.

Test	Result four months prior (without intake of biotin)	Result with intake of biotin 300 mg/day	Control of laboratory results (with intake of biotin)	Result 14 days after cessation of biotin	Reference interval of biotin
TSH, ^a mIU/L	3.02	0.03	0.02	2	0.27–4.2
fT4, ^a pmol/L	19.4	> 98	> 98	20.6	11.6–21.9
fT3, ^a pmol/L	3.02	19.1	13.4	5.7	2.9–7.9
TPOAb, ^a kIU/L		> 600	> 600	24	< 34
TRAb, ^a IU/L		> 40	> 40	2.6	< 1.8

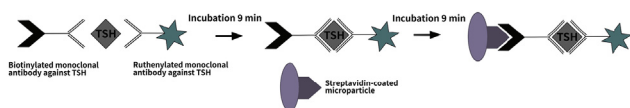
^a TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; TPOAb, antithyroid peroxidase autoantibodies; TRAb, TSH-receptor autoantibodies.

levels of fT4 and fT3, as well as positive TRAb-levels [1]. Hence, the TFTs from the patient presented above were suggestive for GD. In the absence of any clinical symptoms attributable to hyperthyroidism, no antithyroid medication was initiated. Upon further investigation, the patient revealed that four weeks prior, he started to take high-doses of biotin (Biotin USP 100 mg ApotheX® three times daily), which he considered not worth reporting on first contact. The last drug intake was one hour before the abnormal results occurred.

Biotin or Vitamin H is a ubiquitous water-soluble vitamin which holds a role as coenzyme for carboxylases in humans. The recommended daily intake for adults by the Institute of Medicine (US) is 30 µg biotin [3]. A saturable system is responsible for transporting biotin across the blood-brain barrier to be incorporated into proteins, presumably into carboxylase apoenzymes [4]. Recent studies with patients suffering from primary and secondary progressive MS indicated promising effects of high-dose biotin on MS-related disability [5, 6]. There are five biotin-dependent carboxylases which are involved in fatty acid metabolism and energy production [7]. It is suspected that two main mechanisms are responsible for the therapeutic effects of high-dose biotin in MS: it supposedly promotes remyelination through enhanced fatty acid synthesis in oligodendrocytes and it prevents demyelinated axons from degradation by increased energy production [8]. Biochemical assays from different manufacturers (e.g. Roche Elecsys®, Ortho Vitros®, Siemens Dimension®, Siemens Centaur®, Beckman Coulter®, Access®/DXI®, Abbott Architect i2000®, Diasorin Liaison XL®) [9] use biotin due to its small size of 244.3 Dalton and its high affinity and specificity to Streptavidin [10].

Depending on the immunoassay format, biotin interference can cause falsely high results or falsely low results in the performed test [11]. In competitive immunoassays, free biotin binds to streptavidin coated microparticles and prevents the binding of streptavidin to the biotinylated-analyte resulting in false-low measured signal and false-high results. In sandwich immunoassays, free biotin binds to streptavidin coated microparticles and prevents the binding of the antibody-antigen-complex resulting in false-low measured signal and false-low results (Figs. 1 and 2).

Interference of biotin intake on TFTs by enzyme immunoassay has first been described in 1996 in a newborn child [12]. Further reports in children with inherited metabolic diseases followed [13]. It is crucial to draw attention on biotin-induced interference not only on TFTs immunoassays, but also on numerous other immunoassays causing unexplainable biochemical results (Table 2) [14]. For example, dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH),



In accordance to TSH FactSheet – Roche Diagnostics. By courtesy of Roche Diagnostics.

Fig. 1. Electro-chemiluminescence immunoassay (ECLIA), test principle: one-step sandwich assay for quantitative in vitro determination of thyroid-stimulating hormone (TSH) based on the interaction of biotin with streptavidin.

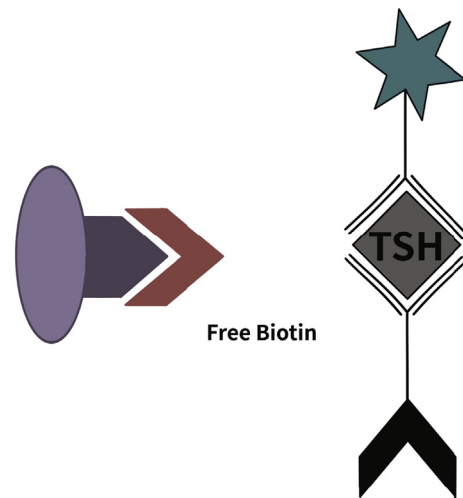


Fig. 2. Free biotin binds to streptavidin coated microparticles and prevents the binding of the antibody-antigen-complex.

Table 2
Effect of high-dose biotin intake on different immunoassays [14].

Test	Effect
Sandwich immunoassays:	↓
ACTH, ^a beta-hCG, ^a CA 15–3, ^a CA 19–9, C-peptide, BNP, ^a FSH, ^a Insulin, LH, ^a PTH, ^a SHBG, ^a Troponin T, TSH, ^a Prolactin, PSA. ^a	
Competitive immunoassays:	↑
Cortisol, DHEAS, ^b Digoxin, Folate, fT4, ^b fT3, ^b Oestradiol, Progesterone, Testosterone, TgAb, ^b TPOAb, ^b TRAb, ^b Vitamin B12.	

^a ACTH, adrenocorticotrophic hormone; beta-HCG, beta human chorionic gonadotropin; CA, cancer antigen; BNP, brain natriuretic peptide; FSH, follicle stimulating hormone; LH, luteinizing hormone; PTH, parathyroid hormone; SHBG, sex hormone binding globulin; TSH, thyroid-stimulating hormone; PSA, prostate specific antigen.

^b DHEAS, dehydroepiandrosterone sulfate; fT4, free thyroxine; fT3, free triiodothyronine; TgAb, thyroglobulin autoantibodies; TPOAb, antithyroid peroxidase autoantibodies; TRAb, TSH-receptor autoantibodies.

follicle-stimulating hormone (FSH), tumor markers such as cancer antigen (CA) 125, CA 19-9 and CA 72-4 and troponine T. The spectrum of potentially affected persons is broad, since biotin is available over the counter and is suspected to cause beneficial effects in different contexts such as hair loss and nail growth.

Follow-up of the patient with an IDK® Biotin Enzyme-linked Immunosorbent Assay (ELISA) showed a serum biotin-level of > 600 ng/L (reference interval 200–600). Assays (radioimmunoassay [RIA]) not relying on the biotin/streptavidin interaction revealed TFTs within the normal range. Two weeks after withdrawing the massive dosage of biotin, biotin/streptavidin dependant assays showed no longer the biochemical picture of severe hyperthyroidism. TSH of 2 mIU/L was within the normal range, fT4 was 20.6 pmol/L and fT3 was at 5.7 pmol/L

L. TRAb were still positive, TPOAb returned to normal levels.

Discordance between clinical presentation and biochemical results in patients taking biotin, should raise suspicion of false positive or negative results to avoid erroneous diagnoses and unnecessary treatment. Questioning for biotin supplementation should be conducted, since patients may not consider it a medication and therefore not mention it in their medication list.

Biotin intake should be paused for at least two to five days prior to the use of biotin/streptavidin dependant assays [15]. Recently, a simple procedure to overcome biotin interference in biotin/streptavidin dependant immunoassays was described [16]. Piketty et al. measured different parameters in plasmas from MS patients and healthy volunteers receiving high-dose biotin, and in biotin-unsupplemented patients, before and after a method designed to remove biotin by streptavidin-coated microparticles. After their procedure, biotin was not measurable any longer and most parameters were normal. Alternatively, non-biotin/streptavidin dependant assays (RIA, gas chromatography–mass spectrometry/liquid chromatography–mass spectrometry) may be used. However, our case showed that even a period of two weeks was not sufficient to return thyroid antibodies to normal levels.

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Conflict of interest

The authors have no potential conflicts of interest to declare.

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